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The Method Development and the Validation of the Ifenprodril Drug in Pure Form by Using UV-Visible Spectroscopy

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ABSTRACT:
This includes the method development and validation of the UV-Visible spectroscopy for the analysis of the Ifenprodil drug. The main aim of the proposed method of analysis of drug Ifenprodil by using UV-Visible spectroscopy to get accurate and precise results that were in the limits. The Ifenprodil drug comes under the category of Ionotropic glutamate receptor which was used as a neuroprotective agent. The ifenprodil drug shows an absorption maximum at 224nm wavelength. The instrument used for this proposed method was SHIMADZU, UV-1700 Double Beam UV-Visible Spectrophotometer with 1cm thickness quartz cell. For this proposed method ethanol was used as the suitable solvent for the analysis of the Ifenprodil drug because the drug shows more solubility in the ethanol. The drug shows a linearity Range from 2.5-75µg/ml concentrations. The LOD (µg/ml) value was 0.2594 and the LOQ (µg/ml) value was 0.7861 which was within the limits. The proposed method shows accurate and precise results. Therefore, the proposed method was satisfactorily used for the routine qualitative and quantitative analysis of pure and formulation drugs.

KEYWORDS: *UV-Visible spectroscopy, Ifenprodil, LOD, LOQ, Accuracy, Precision.*

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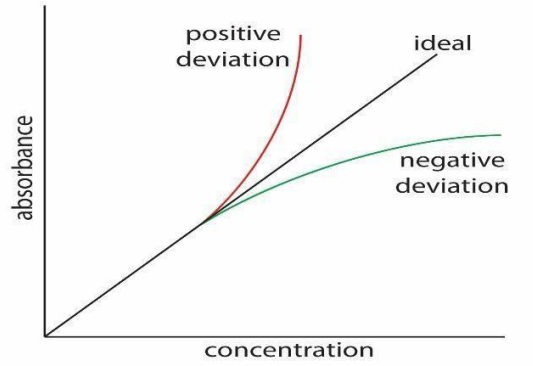
I. INTRODUCTION:

Spectroscopy defines the study of the absorption, emission, and scattering of light and other radiation by matter and mainly depends on the radiation wavelength. The ultraviolet-visible spectroscopy is a technique of spectrophotometry that involves the transition of from the ground state to the higher energy excited state. During this transition, the amount of radiation is absorbed is given as a signal in UV- visible spectrophotometry. By using UV-spectrophotometry, the number of conjugated dienes and also aromatic conjugation within the various molecules is measured. The UV region is extended from 10400nm. From 10-200nm, it is far vacuum UV region and from 200-400nm, it is near the UV region. But, the near UV- region is used more for the analysis of pharmaceutical products.

In UV-VISIBLE spectroscopy, two absorption laws are present, they are one is Lambert's law deals that the thickness of the absorbing medium is directly proportional to the intensity of the incident radiation ($I=I_0e^{-kt}$) and second one is Beer's law deals that the thickness of the absorbing solution is directly proportional to the intensity of the incident radiation as well as the concentration of the solution ($I=I_0e^{-kc}$). Even though the two absorption laws are present for UV- spectroscopy, the deviations should only explain for only Beer's law because beer's law will discuss concentration and it varies from one solution to solution but Lambert's law discusses about the thickness of the solution and it is constant for most of the marketed drugs which is in solution form.

The deviations from Beer's law can be detected by using the plot between concentration and absorbance. If the obtained curve should be linear, it indicates that there was no deviation from beer's law should present. If the obtained curve is not in linear, it indicates that there exists a deviation from beer's law. The deviations are of two types, one is positive deviation is due to the small change in concentration leads to great change in the absorbance and second one is negative deviation results due to the large change in concentration leads to the smaller change in the absorbance.



The solvent used in the UV-Visible spectroscopy should be free from impurities and the solvent wavelength should be greater than the analyte wavelength otherwise it will interfere the analyte absorption and leads to the errors in the result. The most commonly used solvents in UV-Visible spectroscopy are methanol, water, and acetonitrile.

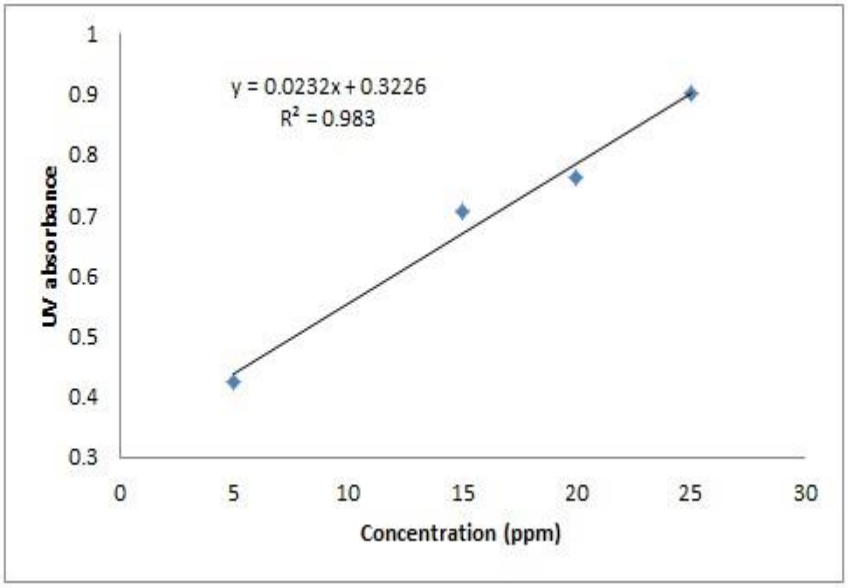
Many detectors are used in the UV-visible spectroscopy. Among them the photo multiplier tube, photo diodes and photo emissive tubes are single element detectors and solid-state array detectors are the multiple element detectors. The main function of the detectors used to detect the signal from analyte solution and convert the signal to the voltage or current flow in the readout device. The spectral sensitivity, wavelength response and gain of response time are the main important characteristics of the detectors.

QUANTITATIVE ANALYSIS OF COMPONENT SAMPLES BY USING UV- VISIBLE SPECTROSCOPY:

- QUANTITATIVE ANALYSIS OF SINGLE COMPOUNDS:
- Calibration curve method
- Regression equation method
- Single point standardization/Direct comparison method
- Double point standardization method
- Absorbance ratio method
- QUANTITATIVE ANALYSIS OF MULTIPLE COMPOUNDS:
- Simultaneous equation method
- Q analysis / absorbance ratio method
- Derivative spectroscopy
- Difference spectroscopy
- Ortho polygonal method

ANALYSIS OF SINGLE COMPOUND BY USING UV-VISIBLE SPECTROSCOPY:
CALIBRATION CURVE METHOD:

It is a plot of concentration on X-axis and absorbance of series of standard solution of known concentration on Y-axis. A straight line which may or may not passthrough origin was obtained. The straight line must coincide with maximum number points, such that the magnitude of positive and negative deviation on both sides of the line is same, this line is called "calibration curve". This method is also called as line of best fit or multiple standard method. The absorbance value is extrapolated to x axis in order to get the concentration of drug and %purity.



SINGLE POINT STANDARDISATION:

- It involves measurement of absorbance of a standard solution and sample solution. From Beer lamberts law the absorbance can be given as

$A_{std} = \square\, C_{std}\, t$

$A_{test} = \square\, C_{test}\, t$

Where

A_{std}, A_{test} = absorbance of test and standard solutions

$\square$ = molar extinction co-efficient

C_{Std}, C_{test} = concentration of standard and test solution t = path length (1 cm). By solving the above equations

$A_{std} / A_{test} = \square\, C_{std}\, t / \square\, C_{test}\, t$

C test = C Std A test / A test

From above equation concentration of sample can be determined

DOUBLE POINT STANDARDIZATION METHOD:

- Occasionally a linear but non proportional relationship between concentration and absorbance occurs, which is indicated by a significant + or – intercept in a Beer law plot. A 2point bracketing standardization is therefore required to determine concentration of sample solutions.

Concentration of standard > concentration of sample

Concentration of standard < concentration of sample

- The equation can be written as

$$Ctest = \frac{(\text{A test} - \text{Astd1})\ (\text{Cstd1} - \text{Cstd2}) + \text{Cstd1}(\text{Astd1} - \text{Astd2})}{\text{Astd1} - \text{Astd2}}$$

Std1, std 2 are more concentrated and less concentrated standard solutions

REFERENCE STANDARD METHOD:

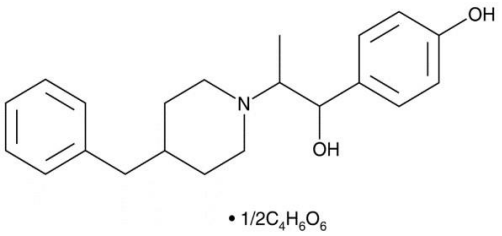
- This method involves measurement of average $A^{1\%}_{1cm}$ value, it can be determined by measuring the absorbance of different standard solutions and calculating their average. The average value can be utilized to determine the % purity using the formula:

$$percentage\ purity = \frac{\text{observed absorbance} \times 100}{\text{average A value} \times \text{concentration}}$$

DRUG PROFILE:

This review article was mainly on the IFENPRODIL drug. The Ifenprodil drug is an inhibitor of the NMDA receptor, specifically of GluN1 and GluN2B subunits. It also inhibits GIRK channels and it also interacts with the

adrenergic, serotonin, and sigma receptors. It also used as neuroprotective agent, as a cerebral vasodilator. The Ifenprodil drug is 1-Piperidineetanol, α-(4- hydroxy phenyl)-β-methyl-4-(phenylmethyl) and also category of Ionotropic glutamate receptor. The molecular structure of Ifenprodil was given below:



INSTRUMENTATION:

The absorbance was taken from the SHIMADZU, UV-1700 Double Beam UV-Visible Spectrophotometer with 1cm thickness quartz cell. The high precision balance was also used.

Reagent used:

The analytical grade ethanol was used.

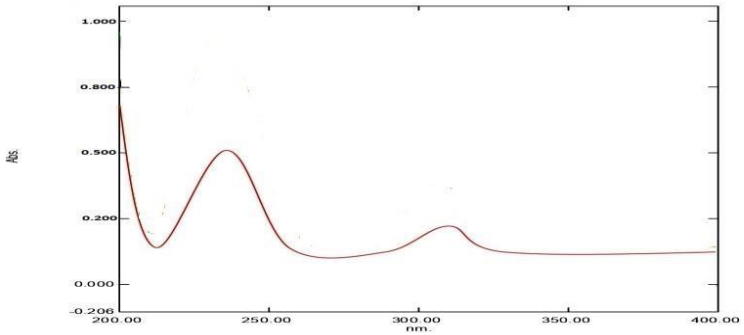
Drug used:

The ifenprodil raw material was used.

EXPERIMENTAL METHOD:

- **DETERMINATION OF WAVELENGTH OF ABSORPTION MAXIMUM (λ max):**

To determine the maximum wavelength absorption (λ max) of the drug, different concentrations of the drug (25µg/ml and 50µg/ml) in ethanol were scanned by using the UV-Visible spectrophotometer between the wavelength range of 200-400nm by using ethanol as blank. The absorption maxima of Ifenprodil were shown at the wavelength of 224nm. The absorption spectrum was presented below:



Preparation of stock solution:

The standard stock solution of Ifenprodil was prepared by accurately weigh the 10mg of Ifenprodil raw material and dissolve in 5ml ethanol in a 10ml volumetric flask and remaining was make up with ethanol to get a concentration of 1mg/ml (1000µg/ml) solution.

Preparation of standard solution and working standard solutions for the construction of standard graph:

From the above prepared stock solution pipette out 2.5ml of stock solution and dilute to 25ml with ethanol from these get 100µg/ml concentrated standard solution. From the standard concentration the working standard solutions are prepared which were utilised for the construction of standard linearity graph. For the preparation of working standard solution the required concentrations are 2.5,5,10,25,50,75 µg/ml solutions.

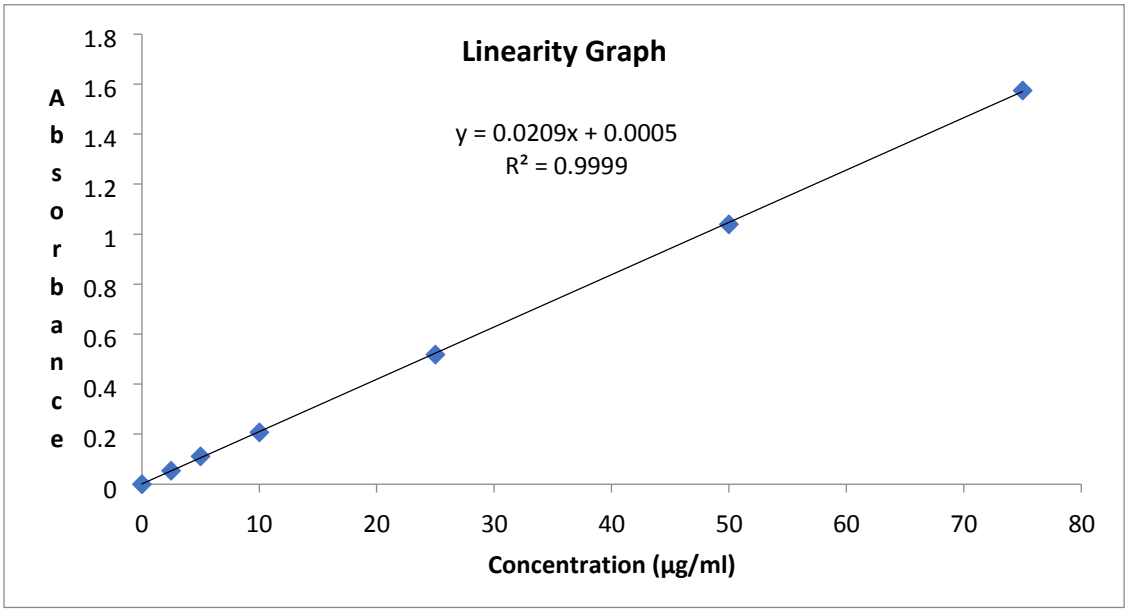
The working standard concentrations are diluted to 10ml of ethanol and absorbance of each working standard solution was measured at λ max at 224nm by using ethanol as blank solution the results were given in the below table:

Linearity of Ifenprodril. (Pure drug)

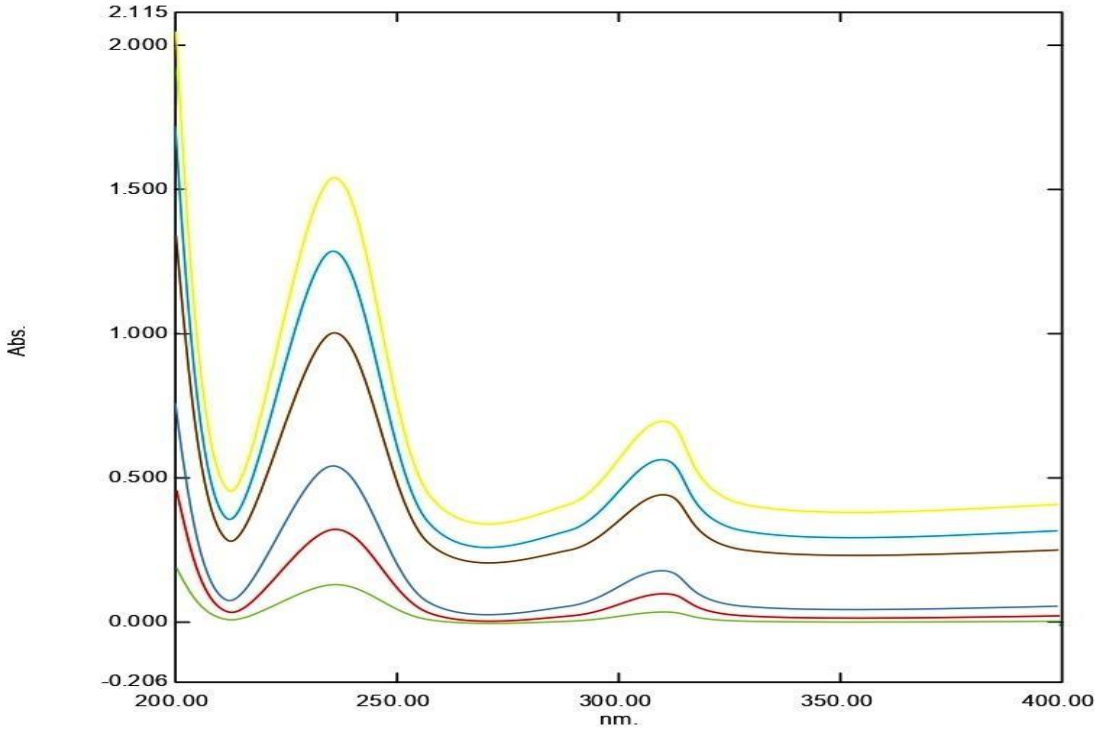
Concentration (µg/ml)	Absorbance
0	0
2.5	0.054
5	0.112
10	0.208
25	0.519
50	1.041
75	1.576

The standard linearity curve of Ifenprodril drug was drawn by taking concentration of drug on x-axis and absorbance on y-axis for the values given in above table. The drug obeys Beer's law within the linear concentration range of 2.5 to 75 µg/ml.

Linearity graph of Ifenprodil



Linearity graph spectrums of Ifenprodril



Optical characteristics of proposed method.

Parameter	Ifenprodil
λmax (nm)	224
Beer's Law limit (µg/ml)	2.5-75
Sandells sensitivity (µ12g/cm^2/0.001absorbance unit)	0.4798
Regression equation (Y)	0.0209x + 0.0005
Slope (a)	0.0209
Intercept (b)	0.0005
Correlation co-efficient	0.9999
LOD (µg/ml)	0.2594
LOQ (µg/ml)	0.7861

Y= aX+b, where 'X' is concentration in µg/ml and Y is absorbance unit.

VALIDATION

Precision:

The precision was calculated by taking six replicates of fixed concentration of drug which were in the range of Beer's law and find the absorbances by the optimized method. From these absorbances calculate mean, standard deviation and %R.S.D. The results were given below tables:

Precision data (inter day)

Sl. No.	Concentration (µg/ml)	Absorbances At 224nm	Statistical Analysis
1	25	0.52	Mean = 0.5175
2	25	0.517	S.D. = 0.001643
3	25	0.516	%R.S.D. = 0.03169

4	25	0.516	
5	25	0.519	
6	25	0.517	

Precision data (intraday)

Sl. No.	Concentration (µg/ml)	Absorbances At 224nm	Statistical Analysis
1	25	0.511	Mean =0.511333 S.D. = 0.001366 %R.S.D. = 0.2659
2	25	0.509	
3	25	0.512	
4	25	0.511	
5	25	0.513	
6	25	0.512	

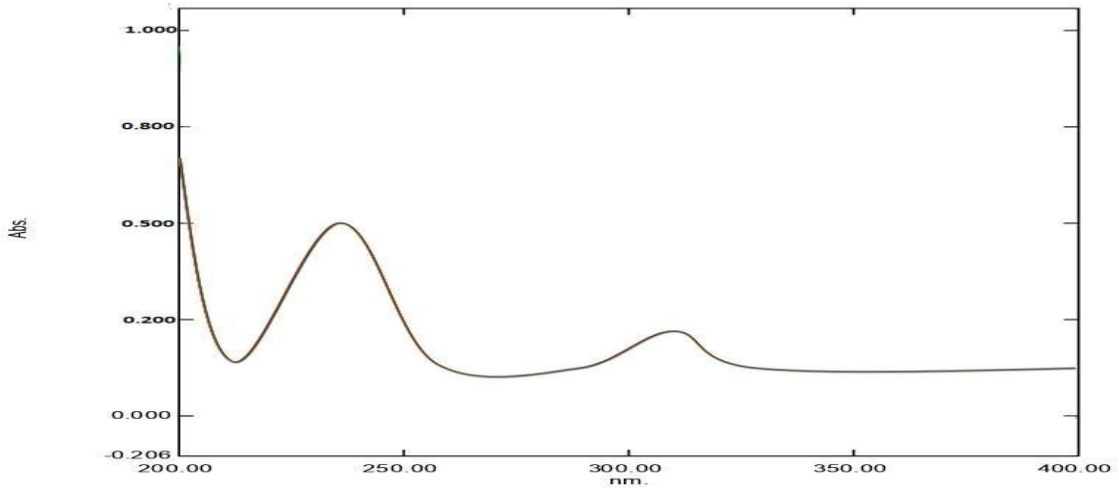
Precision data (Analyst-1)

Sl. No.	Concentration (µg/ml)	Absorbances At 224 nm	Statistical Analysis
1	25	0.52	Mean = 0.5185 S.D. = 0.001517 %R.S.D. = 0.2925
2	25	0.52	
3	25	0.519	
4	25	0.518	
5	25	0.516	
6	25	0.518	

Precision data (Analyst-2)

Sl. No.	Concentration (µg/ml)	Absorbances At 224nm	Statistical Analysis
1	25	0.52	Mean = 0.520667 S.D. = 0.001751 %R.S.D. = 0.3361
2	25	0.523	
3	25	0.52	
4	25	0.521	
5	25	0.522	
6	25	0.518	

Precision spectrum of Ifenprodil

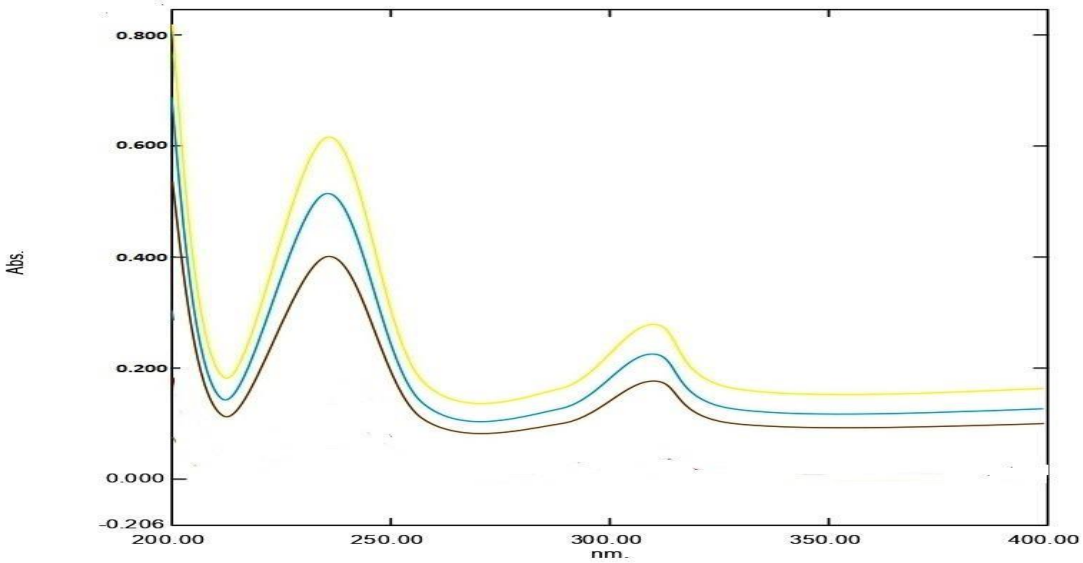


Accuracy:

For the calculation of accuracy for the above optimized method the recovery studies were carried out by adding different amounts of bulk samples (80%,100% and 120%) of Ifenprodil were taken. From this % recovery values were calculated.

According to ICH guidelines the accuracy was calculated by taking any one concentration as 100% of the test concentration. In this method the accuracy was calculated by taking 25 µg/ml concentrated solution as an 100% of the test concentration solution and from this 100% solution prepare 80% (20 µg/ml) test concentrated solution and 120% (30 µg/ml) test concentrated solution.

Accuracy spectrum of Ifenprodil



Accuracy data

Sample ID	Concentration (µg/ml)	%Recovery of Pure drug	Statistical Analysis
	Pure drug		
S1 : 80 %	20	99.4%	Mean = 99% SD = 0.366606 %RSD = 0.3703
S2 : 80 %	20	98.92%	
S3 : 80 %	20	98.68%	
S4 : 100 %	25	100%	Mean= 99.74%
S5 : 100 %	25	99.81%	

S6 : 100 %	25	99.42%	S.D. = 0.2957 % R.S.D.= 0.2963
S7 : 120 %	30	99.76%	Mean= 100.076% S.D. = 0.3150 % R.S.D. = 0.3147
S8 : 120 %	30	100.08%	
S9 : 120 %	30	100.39%	

II. RESULTS:

As per the literature survey, ethanol was selected as a solvent in which the drug Ifenprodril had shown good solubility rather than other polar and non-polar solvents.

The above-proposed method shows results within the limits. First, the wavelength of the maximum absorption (λ max) can be detected by using the 25µg/ml concentration of drug solution by using ethanol as a blank. The resulting spectrum of the Ifenprodril drug shows absorption maxima at 224nm. The linearity from the proposed method should be observed for the concentration range from 2.5-75µg/ml which was mentioned in the optical characteristics of the proposed method and from these characteristics the molar extinction coefficient, the Sandell's sensitivity, correlation coefficient, slope, intercept, LOD, and LOQ were observed.

From the results of the precision, the %RSD values of the proposed method were within the limits, it indicates that the proposed optimized method produce

reproducibility. Results of the accuracy should tells us that the % recovery values of pure drug was within the limits. It means that the above results should reveal that there was no interfering results in the proposed values.

III. CONCLUSION:

From the above results, it would conclude that the above-proposed method of UV-Visible spectroscopy for the analysis of ifenprodil drug in pure form should produce accurate and precise results which were within the limits and the proposed method should validate statistically. The optical characteristics like Beer's law limit, correlation coefficient, sandell's sensitivity, slope, intercept, LOD, and LOQ were calculated.

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