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DEVELOPMENT AND VALIDATION OF A UV SPECTROSCOPIC METHOD FOR ANALYSIS OF TIZANIDINE HYDROCHLORIDE

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ABSTRACT

The method has been developed and validated for the assay of Tizanidine hydrochloride using distilled water. The λ_{max} (absorption maxima) of the drug was found to be 228 nm. Linear response was observed in the range of 4-24µg/ml with a regression coefficient of 0.999.Furthermore various validation parameters as per ICH Q2B guideline were tested and found accordingly. The percent purity (99.09 %) and % Recovery at 50, 100 and 150 % were found to be 99.93%, 101.77%, 100.07% respectively for marketed Tizanidine hydrochloride tablets. Developed method was a less toxic, cheap, eco-friendly but equally sensitive spectroscopic method for quantitative determination of Tizanidine hydrochloride for regular quality control purpose in laboratories.

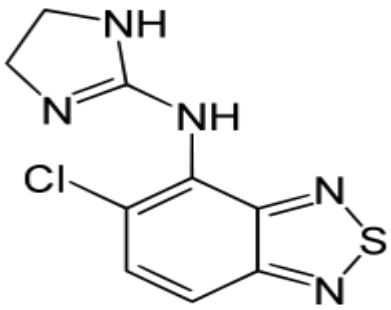
KEYWORDS: Spectrophotometry, Tizanidine hydrochloride, Accuracy, precision.

INTRODUCTION

IUPAC name of Tizanidine hydrochloride is 5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)benzo [c](1,2,5)thiadiazol-4-amine.Molecular Formula is $C_9H_8ClN_5S$ and molecular weight is 253.712 g/mol[1]. Tizanidine hydrochloride is belongs to the category of α-adrinergic agonist, and it is a white to yellowish white, crystalline powder.[2] Zanaflex (Tizanidine hydrochloride)is a central α-adrinergic agonist. Which is odorless or with a faint characteristic odoe.[3] Tizanidine is slightly soluble in water and methanol, solubility in water decreases the pH increases.[3] Tizanidine is supplied as 2 and 4mg tablets for oral administration, and in gel capsule form in dose of 2mg, 4mg and 6mg.[1] Tizanidine is used to relieve the spasms and increased muscle tone caused by multiple sclerosis. Tizanidine is in a class of medications called skeletal muscle relaxants. It works by slowing action in the brain and nervous system to allow the muscles to relax.[4] Tizanidine (Tiz-anna-deen) is a

medicine which is used in treatment of muscle spasticity associated with multiple sclerosis, treatment of muscle spasticity associated with spinal cord injury and treatment of muscle spasticity associated with spinal cord disease. The information in this medicine guide for tizanidine varies according to the condition being treated and the particular preparation used.[5]Tizanidine has been found to be as effective as other antispasmodic drugs and has superior tolerability to that of baclofen and diazepam.[6]

Method development is the setting up of an analytical procedure that will be appropriate for the analysis of a particular sample and makes the analysis simpler, sensitive and easier. Literature reveled that most of analytical work has been performed using H.P.L.C. method which is complex, time consuming and very costly. Besides in many official compendia organic solvents have been used for the analysis of Tizanidine hydrochlorine which are toxic, and costly. For this reason, in present study we tried to develop a less toxic, cheap, eco-friendly but equally sensitive spectroscopic method for quantitative determination of Tizanidine hydrochlorine for regular quality control purpose in laboratories. In line to this we used distilled water.[1] So, based on our literature survey and need of today, we have tried to develop a new method which is easier, sensitive, cost effective and ecofriendly which can be easily performed in laboratory using simple instrument like UV-spectrophotometer.



MATERIALS AND METHOS

I. Instruments

UV-Visible double beam spectrophotometer (shimadzu) with spectral bandwidth of 0.1 nm and wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used.

II. Material

The Tizanidine hydrochloride (purity 99.99%) sample was purchased by Chandra labs, kukatpally, India and used as reference standard. The commercial fixed dose formulation containing Tizanidine hydrochloride (2 mg) was purchased from local pharmacy shop.

Distilled water was used as a solvent for the preparation of stock and working standard solution in the study. All the chemical and reagents were of analytical grade.

III. Preparation of standard stock solutions

The Standard stock solution of pure Tizanidine hydrochloride (1mg/ml) was prepared by dissolving 10 mg Tizanidine hydrochloride in 10 ml distilled water in 10 ml volumetric flask with vigorous shaking for about 10 minutes. From this solution 1 ml was taken and diluted to 10ml with distilled water to get a stock solution containing 100 µg/ml of drug. Then solution was further diluted to get various working solutions.

IV. Determination of absorption maxima

A UV absorption maximum was determined by scanning 8µg/ml solution of Tizanidine hydrochloride in between 200-400 nm by using UV-visible spectrophotometer. Further a representative spectrum was drawn of Tizanidine hydrochloride.

V. Preparation of Calibration curve

The standard solutions for the drug having concentration 4-24µg/ml was prepared with distilled water from the stock solution. The absorbance of solutions of pure Tizanidine hydrochloride drug were measured at 228λ_{max} and a calibration curve was plotted, to get the linearity and regression equation which has shown in fig.2,4,6.

Method-A: zero order spectroscopic method

The analytical wavelength was selected by preparing a solution of concentrationm10µg/ml by dilution of standard stock solution with distilled water. The solution was scanned in the wavelength range of 200-400nm using distilled water as blank. The UV spectrum of tizanidine hydrochloride showed λmax at 228nm. The calibration curve was prepared in the concentration range of 4-24µg/ml at 228nm by measuring the absorbance of each concentration using distilled water as blank and plotted the graph by taking concentration on X-axis and absorbance on Y-axis. It was shown in Fig.1. The results were shown in table No.1.

Method-B:first order derivative spectroscopic method

For the selection of an analytical wavelength, a solution of concentration 10µg/ml of tizanidine hydrochloride was prepared and scanned in the spectrum mode in the range of 200-400nm.The absorption spectrum thus obtained was derivatized in First order. The

maximum absorbance obtained at 223nm. It was shown in Fig.2. The results were shown in table No.2.

Method-C: area under curve method [7]

The solutions having an analytical concentration range of 4-24µg/ml were scanned in the spectrum mode from the wavelength range 200-400nm and the AUC spectra were measured between wavelengths 223nm and 233nm. The calibration curve was prepared by plotting the graph by taking concentration on X-axis and α+β values on Y-axis. It was shown in Fig.3. The results were shown in table No.3.

VI. Linearity

The linearity [8] of the proposed UV spectroscopic methods were evaluated by analyzing in different concentration of standard solution of tizanidine hydrochloride and by plotting the absorbance of analyze against concentration of analyte. Beer's law [9] was obeyed for all methods in the concentration range of 4-24 µg/ml. A good linear relationship(R^2=0.999) was observed. Calibration linearity and First order linearity was shown in Fig.4 and Fig.5. The results were shown in table No.5.

VII. Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day(Intra-day) and samples run on three different days(Inter-day)[10]. To check the intra day and inter day variation of the methods, solutions containing 12, 16, 20 µg/ml concentration of tizanidine hydrochloridewere subjected to the proposed methods and the recoveries obtained were noted. The intra and inter day variations in the absorbance of drug solutions was calculated in terms of % RSD. The results were shown in table No.7.

VIII. Preparation of Sample solutions for assay

For the estimation of tizanidine hydrochloride in formulation, 10 tablets each containing 2mg of tizanidine hydrochloride were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent to 10mg of tizanidine hydrochloride was transferred into10ml volumetric flask and dissolved in sufficient quantity of distilled water and the volume made up to the mark with same solvent to obtain a stock solution of 1000µg/ml of tizanidine hydrochloride. Then the solution was filtered. Then the dilution of the stock were made with

distilled water to get required concentration of 8µg/ml. The absorbance of the solution was measured at 228nm. The results were shown in table No.4.

IX. Recovery studies

Accuracy[8] is expressed as degree of closeness of experimental value to the true value. To recovery studies were carried out by standard addition method[10]. This parameter was evaluated by percentage recovery studies at concentration levels of 50, 100, 150% . Which includes addition of known amounts of tizanidine hydrochloride working standard to formulation samples. Each of the addition was observed 6 times. The results were shown in table No.6.

RESULTS AND DISCUSSION

The absorption maximum of Tizanidine hydrochloride was found to be 228 nm. The method was further validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte. All the comparative results were shown in table 1.

Table-1: Calibration curve data for Tizanidine hydrochloride by Absorption maxima method.

S.No	Concentration(µg/ml)	Absorbance
1	4	0.173
2	8	0.321
3	12	0.472
4	16	0.621
5	20	0.752
6	24	0.905

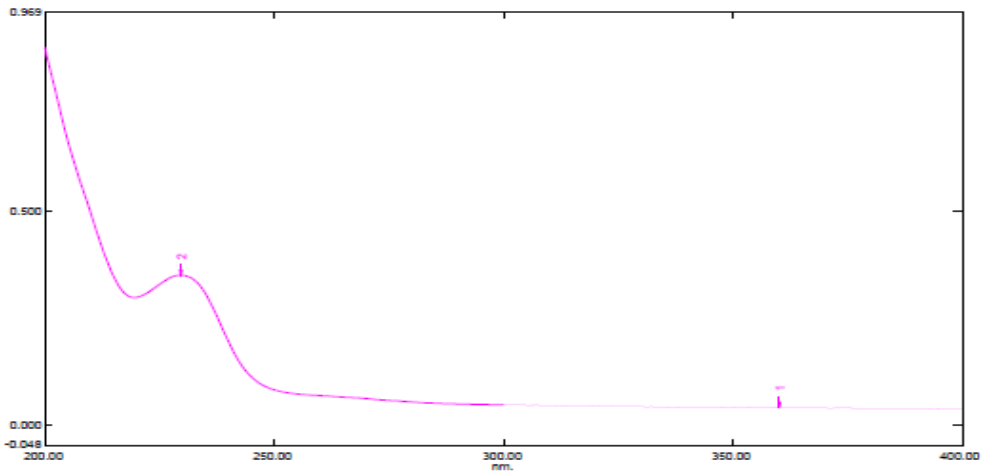


Figure-1:Maximum absorbance spectrum of Tizanidine hydrochloride.

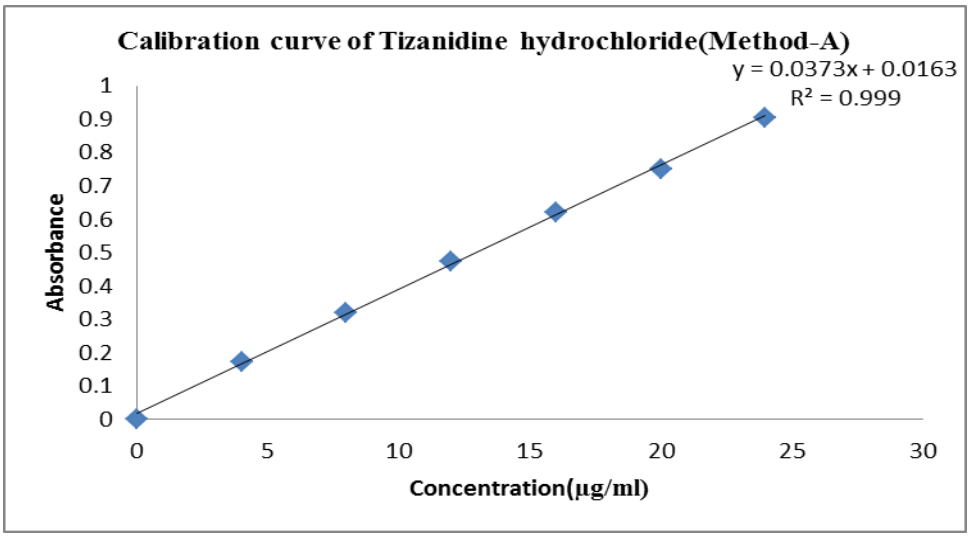


Figure-2:Linearity curve of Tizanidine hydrochloride by Method-A

Table-2: Calibration curve data for Tizanidine hydrochloride by First order derivative method.

S.No	Concentration(µg/ml)	Absorbance difference(dA/dλ)
1	4	0.0035
2	8	0.0080
3	12	0.0120
4	16	0.0160
5	20	0.0198
6	24	0.0238

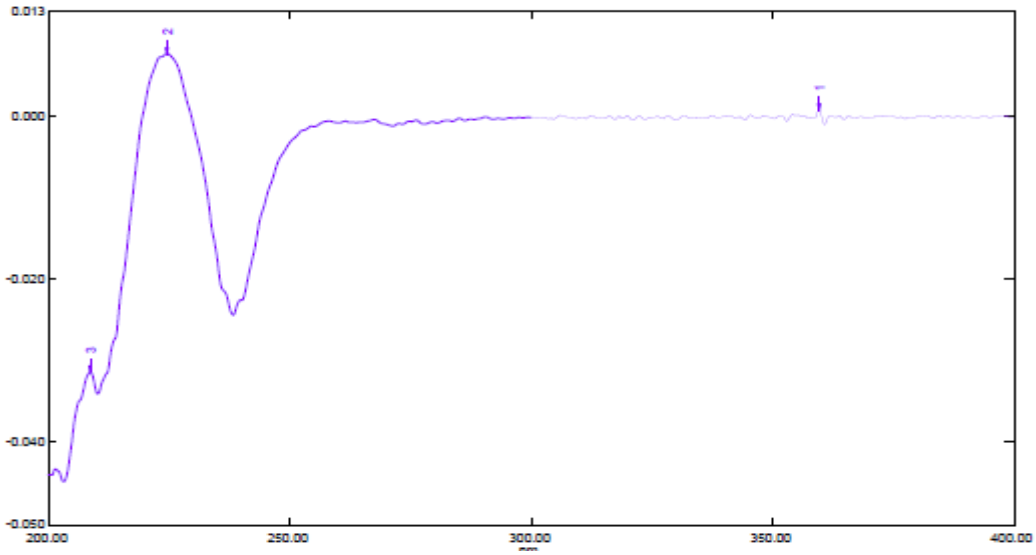


Figure-3: First order derivative spectrum of Tizanidine hydrochloride.

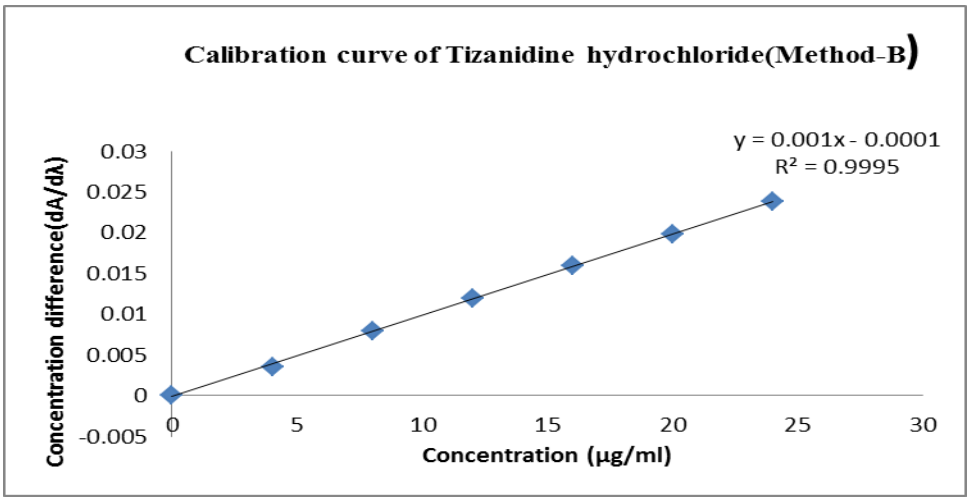


Figure-4: Linearity curve of Tizanidine hydrochloride by Method-B

Table-3: Calibration curve data for Tizanidine hydrochloride by Area under curv method

S.No	Concentration(µg/ml)	α	β	α +β
1	4	0.07832	1.3811	1.4593
2	8	0.1493	2.6036	2.7529
3	12	0.2346	3.8988	4.1334
4	16	0.3155	5.0359	5.3514
5	20	0.3866	6.1241	6.5107
6	24	0.4611	7.3889	7.8500

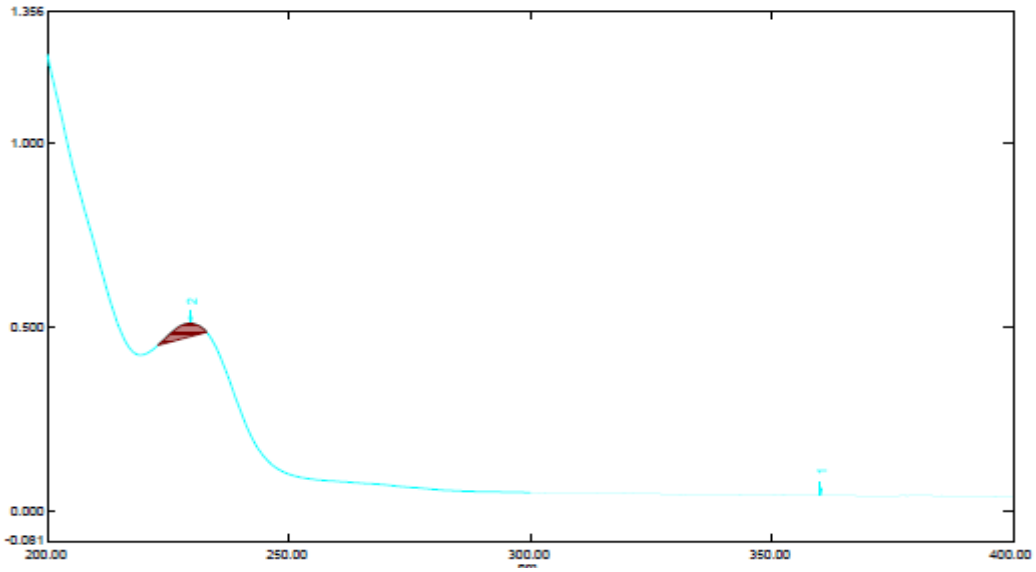


Figure-5:UV-spectrum of Tizanidine hydrochloride AUC method

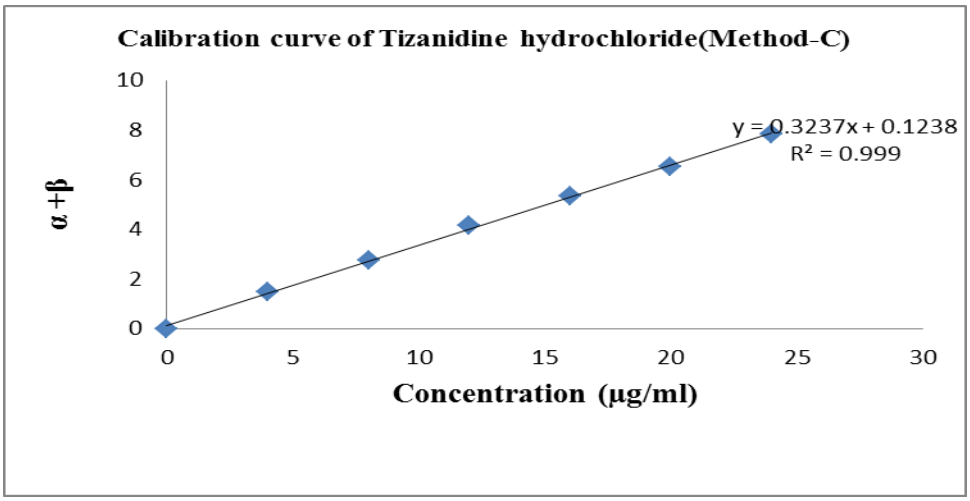


Figure-6:Linearity curve of Tizanidine hydrochloride by Method-A

Table-4:Assay results of Tizanidine hydrochloride formulation by 3 methods(8µg)

Analysis method	Lable claim(mg/tab)	Mean amount(mg) found(n=6)	% Amount found	% RSD
A	2mg	8.39	104.87	0.0644
B	2mg	9	112.5	0.2336
C	2mg	7.92	99.07	0.049

Table-5: linearity studies of tizanidine hydrochloride by proposed methods

S.No	PARAMETER	METHOD-A	METHOD-B	METHOD-C
1	Linearity(µg/ml)	4-24	4-24	4-24
2	Slope	0.0373	0.001	0.3237
3	Intercept	0.0163	0.0002	0.1242
4	Correlation oefficient	0.999	0.9996	0.999

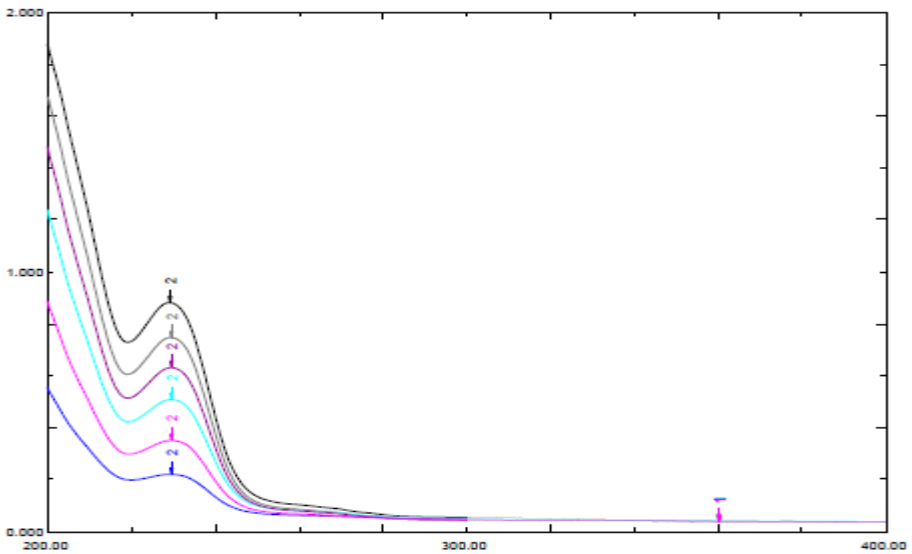


Figure-7:Linearity overlay spectrum of Tizanidine hydrochlorides(zero order).

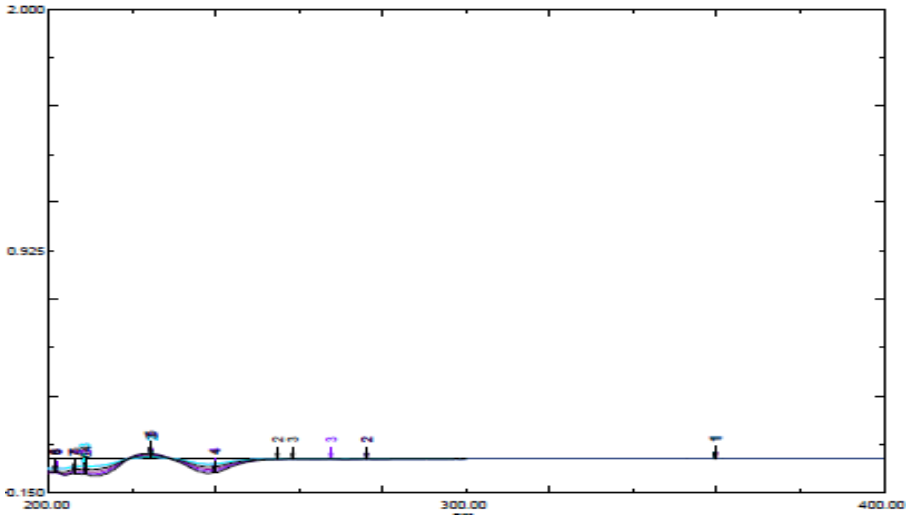


Figure-7: Linearity overlay spectrum of Tizanidine hydrochloride(first order).

Table-6: Accuracy studies of Tizanidine hydrochloride by proposed methods.

Concentration taken(µg/ml)	Spiked level(%)	Amount added(µg/ml)	Amount found(n=6)			%Recovery		
			A	B	C	A	B	C
8	50%	4	11.99	12	11.59	99.93	100	96.65
8	100%	8	16.28	16.01	15.82	101.77	100.62	98.87
8	150%	12	20.01	20.20	20.21	100.09	101.01	101.09

A=Method-A, B=Method-B, C=Method-C

Table-7: Precision studies of Tizanidine hydrochloride.

Concentration taken(µg/ml)	Intra day precision		Inter day precision	
	Mean±SD	%RSD	Mean±SD	%RSD
12	0.4717±0.0002	0.0424	0.4803±0.0002	0.0416
16	0.6203±0.0002	0.0284	0.6320±0.0003	0.0431
20	0.7527±0.0007	0.0991	0.7590±0.0003	0.0359

SD =Standard deviation , RSD=Relative Standard deviation

Table-8: Validation parameters.

S.No	PARAMETER	METHOD-A	METHOD-B	METHOD-C
1	λ_{max}(nm)	228	223	223-233
2	Beer´s limit(µg/ml)	4-24	4-24	4-24
3	Linearity indicated by correlation	0.999	0.9996	0.999
4	coefficient Accuracy indicated by %Recovery	100.58%	100.50%	98.84%

SD =Standard deviation, RSD=Relative Standard deviation

CONCLUSION

From the above nresults and data, it may be concluded that the proposed new method is simple, sensitive**,** eco-friendly, precise, and cost effective. Besides this developed method has suitability for daily laboratory practice.

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