NOVEL SPECTROPHOTOMETRIC ANALYTICAL METHODS FOR THE QUANTITATIVE ANALYSIS OF CITALOPRAM

[1]Mane Shiwanjali Sunil, [2]Giri Pooja Tanaji

[1]Dept. Of Pharmaceutical Quality Assurance, Dattakala College Of Pharmacy Swami-Chincholi,India..

[2]Dept. Of Pharmaceutical Quality Assurance, Dattakala College Of Pharmacy Swami-Chincholi,India..

ABSTRACT

Citalopram belongs to the class of selective serotonin reuptake inhibitors having broad spectrum of therapeutic activity against depressive disorders. Citalopram is frequently used to treat anxiety, panic disorder and body **dysmorphophobia**. It has been found to greatly reduce the symptoms of diabetic neuropathy .For the simultaneous estimation of Citalopram pharmaceuticals, Two simple, accurate, precise, and reproducible methods have been developed. For Quantitative Analysis of First Order Derivative and Area under Curve Method, on UV methodologies have been described, it was felt that a novel approach to drug analysis using methanol as a solvent was required. Citalopram has absorbance maximum at 239 nm. This drug obeys Beer's law in concentration range of 10-50µg/ml. The recovery studies confirmed the proposed method's accuracy, and the results were validated in accordance with ICH guidelines. The outcomes were found to be satisfactory and reproducible. Thus, the proposed method can be successfully applied for the quantitative analysis of Citalopram.

Key Words: Citalopram, first order derivative, ICH guidelines, Area under curve method.

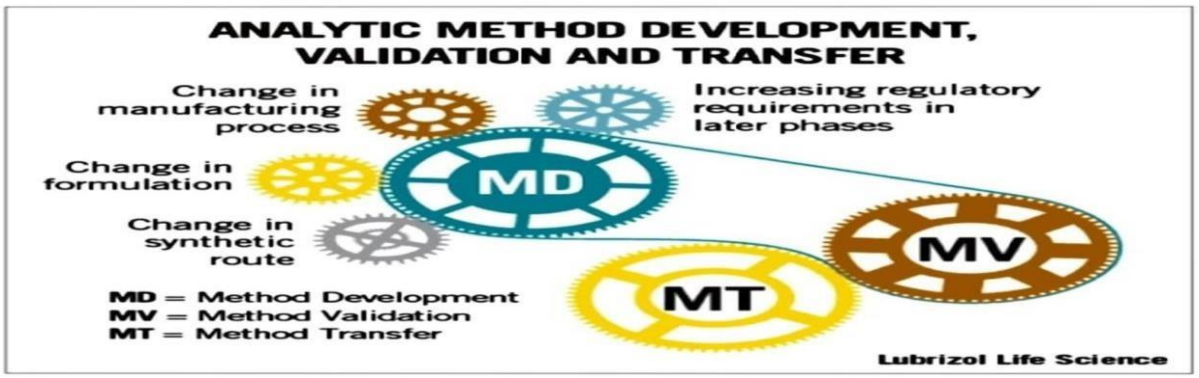
INTRODUCTION:

Geriatric depression is typically treated with selective serotonin reuptake inhibitors (SSRIs), such as citalopram.[1] In any given year, approximately 30% of the population is affected by mood disorders, and approximately 50% of the population is at risk of getting depression at some point in their lives. As an alternative to the currentlyavailable expensive procedures, it is based on the fluorescence features of SSRIs.[2] Themost common citalopram side effects include dry mouth, vomiting, increased sweating, headache, tremor, tiredness, and inability to sleep. According to a survey of the literature, few HPLC and Spectrophotometric studies have been conducted. In this study, an RP-HPLC method was developed and validated in order to provide a simple, selective, linear, precise, and accurate approach.[3] Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that regulates emotion and plays a key role in the pathophysiology of mood disorders. SSRIs (selective serotonin reuptake inhibitors) are drugs that enhance serotonin levels in the synapse and improve serotonergic neurotransmission. They're commonly used to treat mood and anxiety disorders[4]. SSRI In both adults and children, citalopram overdose can cause serotonin syndrome, QT prolongation, and convulsions[5]. The goal of this research is to develop a spectrofluorimetric method for determining citalopram hydrobromide in pharmaceutical formulations that is both optimised and validated. The suggested approach is based on the development of an ion-pair complex of the drug with eosin Y in the presence of Na2HPO4/citric acid buffer (pH 3.4) and is dichloromethane extractable. After excitation at 259 nm, the isolated compound showed fluorescence intensity at 552 nm[6].

Analytical Method Development[7]:

Analytical methods are used to determine the identity, purity, physical properties, and potency of drugs.

Figure 1



LIFE CYCLE OF ANALYTICAL METHOD[8] :

Fig1 :Life Cycle Of Analytical method

METHOD DEVELOPMENT STEPS[9]:

The common path followed in the method development are as follows:

1. Standard analyte characterization
2. Method requirements
3. Literature search
4. Selecting the method
5. Instrumental setup and preliminary study
6. Optimization of parameters
7. Documentation of analytical figure
8. Estimation of the method developmentwith the sample.
9. Determination of % recovery ofthe sample
10. Demonstration of quantitative sample analysis

ANALYTICAL PROCEDURE[10]:

The analytical procedure is not limited to, the preparation of the sample, the reference standard, and the reagents, the use of the apparatus, the generation of the calibration curve, the use of the formulae for the calculation, and so on.

VALIDATION OF ANALYTICAL PROCEDURES:

The goal of validation of an analytical procedure is to express with the intention of being appropriate for its projected reason, a tabular summary of the quality valid for detection, as well as the organization of the adulteration analysis method, is included.

VALIDATION PARAMETERS:

The following are some typical validation characteristics to consider

1. Accuracy
2. Precision
3. Specificity
4. Limit of detection
5. Quantitation limit
6. Linearity
7. Range

SPECTROPHOTOMETRY[11]:

Spectrophotometry employs photometers called spectrophotometers, which can measure the intensity investigate vast swaths of the electromagnetic spectrum, including x-ray, ultraviolet, visible, infrared, and microwave wavelengths, however they are most typically used for ultraviolet, visible, and infrared radiation.

ULTRAVIOLET-VISIBLE SPECTROSCOPY:

The term ultraviolet–visible Spectrophotometry assign to absorption or reflectance spectroscopy in the ultraviolet and adjacent visible regions of the electromagnetic spectrum.

DERIVATIVE SPECTROPHOTOMETRY[12]:

UV-Visible spectra have increasing or decreasing absorbance as a function of wavelength, A=f (λ): Zero order. The first or higher derivative of absorbance or transmittance with respect to wavelength is recorded versus the wavelength in derivative spectroscopy.

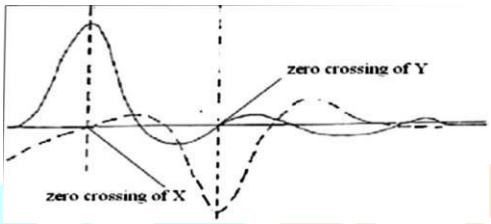


Fig2:Overlain spectra of X and Y Drug :(day/df) =f(λ): first order, (d2A/dλ2)=f(λ): second order

APPLICATIONS OF DERIVATIVE SPECTROSCOPY[13]:

- Pharmaceutical analysis
- Forensic toxicology
- Amino- acid and protein analysis
- Clinical analysis

INSTRUMENT USED:

UV-VISIBLE SPECTROPHOTOMETER UV-1800[15] :

ShimazuModel No: 1800

METHODOLOGY:

Quantitative estimation of first order for Citalopram

MATERIALS AND METHODS

a) Materials:

Chemicals and reagents: Methanol

Instrument: Estimation was performed using a Shimadzu 1800 UV spectrophotometer with a 1cm matched quartz cell.

Selection of media: main criteria for media selection and stability, i.e., drug should be soluble as well as stable in selected media for a adequate time methanol has been chosen as the analytical medium for this work.

b) Method:

1. Solubility study of the Drug:

The drug's solubility was determined at room temperature. A small amount of the standard drug was dissolved in distilled water, acetonitrile, Nicotinamide solvent and methanol. The drug was methanol-soluble and stable.

2. Preparation of standard stock solution:

The standard stock solution was prepared by transferring 50 mg of Citalopram into a 500 ml beaker. 50ml methanol was transferred into the beaker and dissolved and transferred to the 50ml volumetric flask. The volume was made up to the mark with methanol which gives solution containing 1000 µg/ml Citalopram. from this solution 5ml was transfer to 50 ml volumetric flask to this solution 50ml methanol was added to give a solution containing 100 µg/ml of Citalopram.

3. Preparation of dilutions:

The dilutions were obtained by

pouring 2, 4, 6, 8, 10 ml of a 100 g/ml concentration into a 50 ml volumetric flask and topping up with methanol to achieve the target level. The concentration is 4, 8, 12, 16, 20 µg/ml. And continued with further concentration of [4 µg/ml,8 µg/ml, 12 µg/ml, 16 µg/ml, 20 µg/ml], [24 µg/ml, 28µg/ml, 32 µg/ml ,36 µg/ml ,40 µg/ml],[10 µg/ml ,20 µg/ml ,30 µg/ml ,40 µg/ml ,50 µg/ml] at N=5. Further concentrations of [5 µg/ml ,10 µg/ml ,15 µg/ml ,20 µg/ml ,25 µg/ml ,30 µg/ml ,35 µg/ml ,40 µg/ml ,45 µg/ml ,50 µg/ml] at N=5.The trials were carried out in order to obtain a liner graph indicating an increase absorbance.

4.Determination of wavelength of maximum absorption:

UV Spectroscopic scanning (220-260) was performed on a standard stock solution of Citalopram to determine the wavelength of maximum absorption for Citalopram. The maximum absorbance was fixed as 239nm.

5.Study of Beer-Lambert's law:

By using the standard stock solution of Citalopram various volumes 5,10,15,20 and To obtain the concentrations 10µg/ml,20µg/ml, and 30µg /ml, 40µg/ml, 50µg/ml. 25ml were transferred to five separate 50 ml volumetric flasks and volume was built up to the mark with methanol solvent. After that calibration curve was constructed.

Sr no	Concentration of citalopram	Absorbance at 220-240nm
1	10 µg/ml	0.008
2	20 µg/ml	0.014
3	30 µg/ml	0.020
4	40 µg/ml	0.026
5	50 µg/ml	0.031

Table1: Standard Calibration Table For Citalopram at 220-240nm

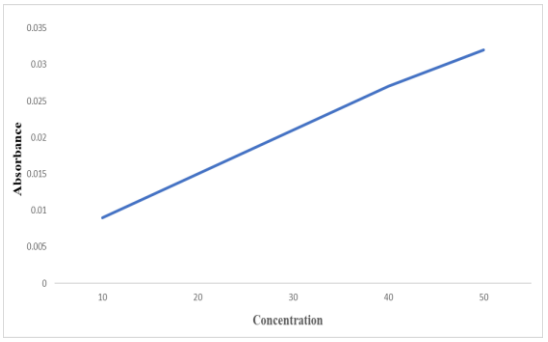


Fig3.0: The Calibration curve of Citalopram at 220-240nm

Result: Because concentration is proportional to absorbance, the obtained graph was linear, indicating an increase in solubility.

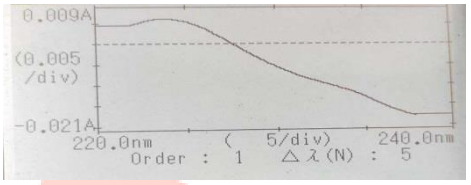


Fig 3.1: Instrumental response of First derivative spectrum of Citalopram con. 10µg/ml.

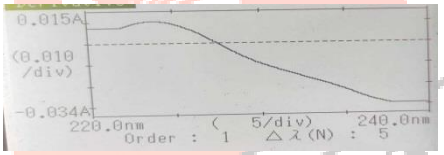


Fig3.2: instrumental response of first derivative spectrum of citalopram con.20µg/ml.

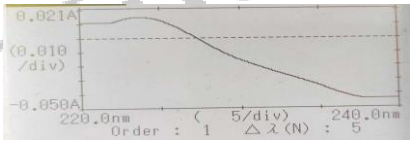


Fig3.3: instrumental response of first derivative spectrum of citalopram con. 30µg/ml.

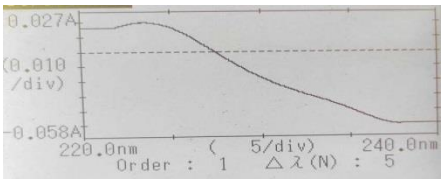


Fig3.4: instrumental response offirst derivative spectrum of citalopram con. 40µg/ml.

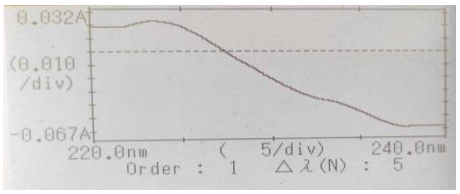


Fig3.5: Instrumental response offirst derivative spectrum of citalopram con. 50µg/ml.

Trials and errors

Trial 1:

Dilutions of 4µg/ml, 8µg/ml, 12µg/ml, 16µg/ml, 20µg/ml are made with the methanol as a solvent. In a UV Visible spectrophotometer, these quantities were tested as first order derivative in the wavelength of 220-240nm. where N=5 is used.

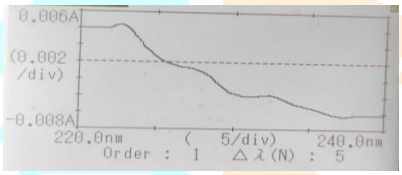


Fig4.1: Instrumental response offirst derivative spectrum of citalopram con. 4µg/ml.

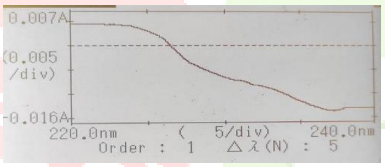


Fig4.2: Instrumental response of first derivative spectrum of citalopram con. 8µg/ml.

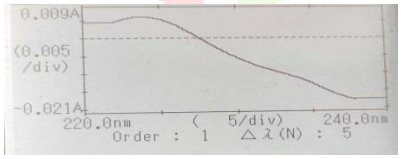


Fig4.3: Instrumental response offirst derivative spectrum of citalopram con. 12µg/ml.

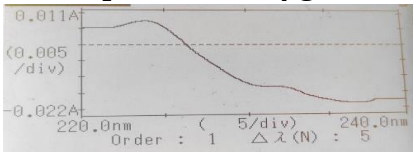


Fig4.4: Instrumental response of of first derivative spectrum of citalopram con. 16µg/ml.

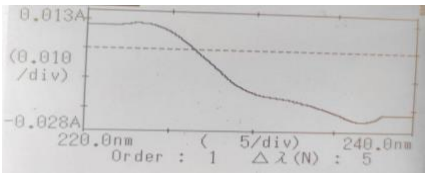


Fig4.5: Instrumental response offirst derivative spectrum of citalopram con. 20µg/ml.

Table no 2:

Standard Calibration Table For Citalopram at 220- 240nm

Sr .no	Con. of citalopram	absorbance
1	4µg/ml	0.006
2	8µg/ml	0.007
3	12µg/ml	0.008
4	16µg/ml	0.011
5	20µg/ml	0.013

Result: The graph obtained was not linear since concentration is not proportional to absorbance, science there were no increase in solubility

TRAILA2:

Making the dilution of 24µg/ml, 28µg/ml, 32µg/ml, 36µg/ml, 40µg/ml, are made with menthol as a solvent. In UV Visible spectrophotometer, these quantities were tested at first order derivative between the wavelengths of 200nm-240nm.

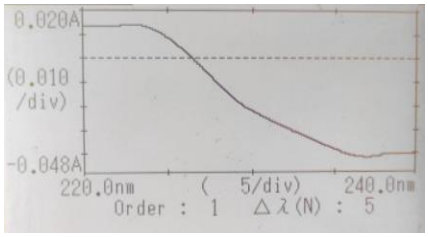


Fig5.1: Instrumental response of first derivative spectrum citalopram con.24µg/ml

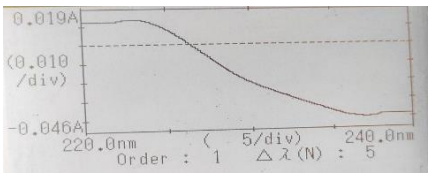


Fig5.2: Instrumental response of first derivative spectrum of citalopram con.28µg/ml

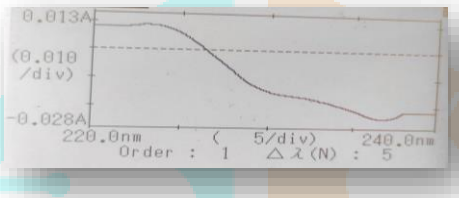


Fig5.3: Instrumental response of first derivative spectrum of citalopram con.32µg/ml

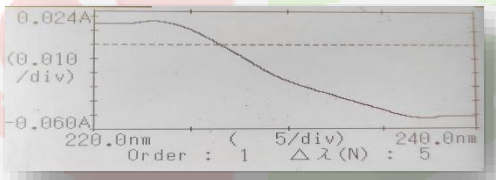


Fig5.4 :Instrumental response of first derivative spectrum of citalopram con.36µg/ml

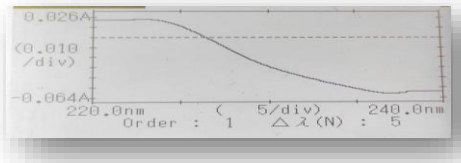


Fig5.5: Instrumental response of first derivative spectrum citalopram con.40µg/ml

Table no3:Standard calibration curve of citalopram at220-240nm

Sr. no	Con of Citalopram	Absorb-ance
1	24µg/ml	0.021
2	28µg/ml	0.018
3	32µg/ml	0.022
4	36µg/ml	0.025
5	40µg/ml	0.024

Result: Since graph obtained was not linear concentration is not proportional to absorbance hence there were no increase in solubility.

TRAIL 3:

Dilutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, are prepared using the above method. Methanol is used as a solvent. Above concentration are scanned in UV spectrophotometer for first order derivative in 220-240nm at N=5.

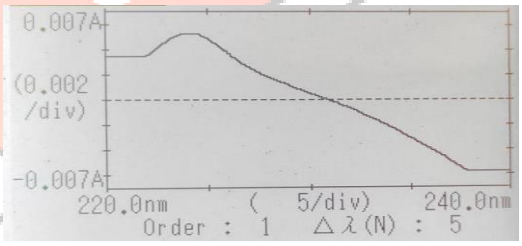


Fig6.1: :Instrumental response of first derivative spectrum of citalopram con.5µg/ml.

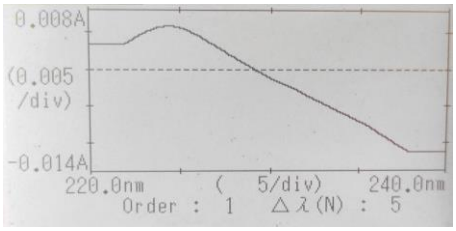


Fig6.2 :Instrumental response of first derivative spectrum of citalopram con.10µg/ml.

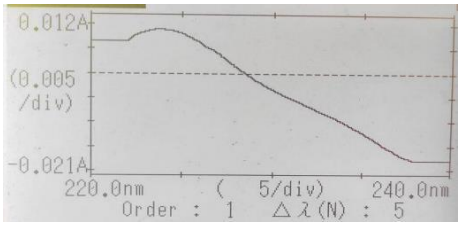


Fig6.3 :Instrumental response of first derivative spectrum of citalopram con.15µg/ml.

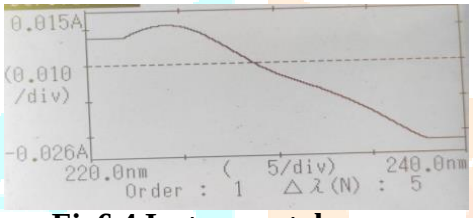
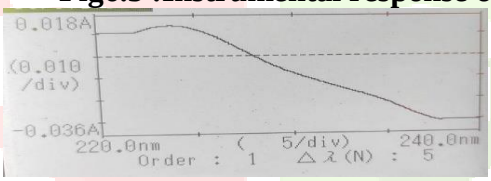


Fig6.4 Instrumental response of First derivative spectrum of citalopram con.20µg/ml.

Fig6.5 :Instrumental response of



first derivative spectrum of citalopram con.25µg/ml.

Table no 4:Standard calibration table of citalopram at 220-240nm

Sr.no	Con. Of citalopram	Absorbance at 220-240
1	5µg/ml	0.008
2	10µg/ml	0.007
3	15µg/ml	0.011
4	20µg/ml	0.012
5	25µg/ml	0.017

Result: The graph obtained was not linear since concentration is not proportional to absorbance, suggesting that there was no increase in solubility.

Trail 4:

The dilutions of 30µg/ml, 35µg/ml, 40µg/ml, 45µg/ml, 50µg/ml are prepared using methanol as a solvent. In a UV Visible spectrophotometer, these concentration were scanned in first order derivative between 220-240nm using the value N=5.

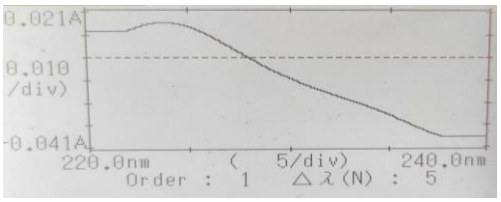


Fig7.1: Instrumental response of firstderivative spectrum of citalopram con.30µg/ml

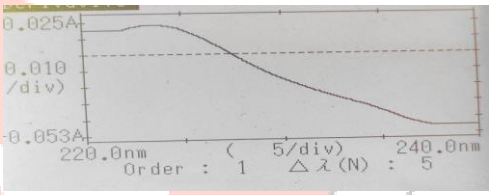


Fig 7.2: Instrumental response of firstderivative spectrum of citalopram con.35µg/ml.

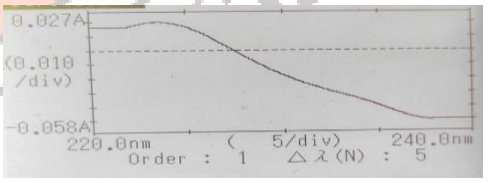


Fig7.3: Instrumental response of firstderivative spectrum of citalopram con.40µg/ml.

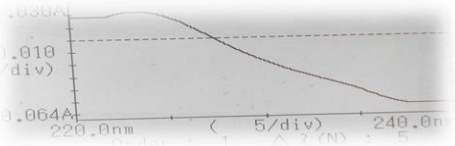


Fig7.4: Instrumental response of first derivative spectrum of citalopram con.45µg/ml.

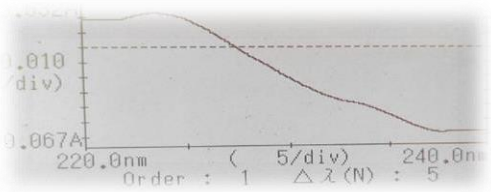


Fig7.5: Instrumental response of first derivative spectrum of citalopram con.50µg/ml.

Table no 4:Standard calibration table of citalopram at 220-240 nm

Sr.no	Con. Of citalopram	Absorbance at220-240
1	30µg/ml	0.021
2	35µg/ml	0.025
3	40µg/ml	0.027
4	45µg/ml	0.030
5	50µg/ml	0.032

Result: Science concentration is not proportional to absorbance, the graph obtained was nonlinear. Indicating therewas no increase in solubility.

Trail:5

Dilution of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml are using before mention method. Methanol is used as a solvent. Above concentration were scanned in spectrophotometer for first order derivative in 220-240nm at N=5.

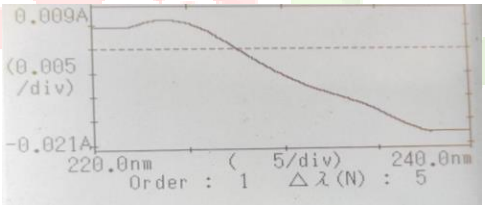


Fig8.1: Instrumental response of first derivative spectrum of citalopram con.10µg/ml.

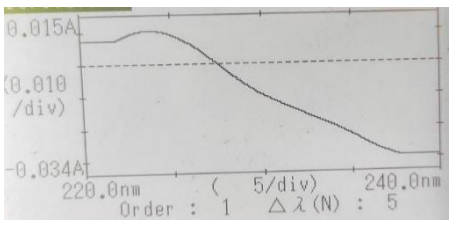


Fig8.2: Instrumental response of first derivative spectrum of citalopram con.20µg/ml.

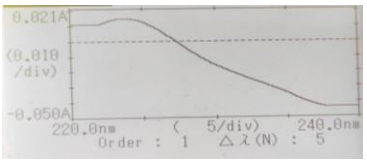


Fig8.3: Instrumental response of first derivative spectrum of citalopram con.30µg/ml.

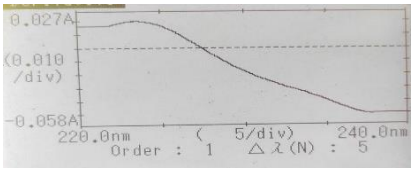


Fig8.4:Instrumental response of first derivative spectrum of citalopram con.40µg/ml.

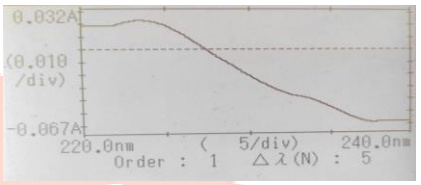


Fig8.5: Instrumental response of first derivative spectrum of citalopram con.50µg/ml.

Table no 5:Standaed calibration table of citalopram at 220-240nm.

Sr.no	Con. Of citalopram	Absorbance at 220-240
1	10µg/ml	0.008
2	20µg/ml	0.014
3	30µg/ml	0.022
4	40µg/ml	0.024
5	50µg/ml	0.032

Result: Therefore the concentration proportional to absorbance , The graph obtained was linear .Indicating there was increasing solubility.

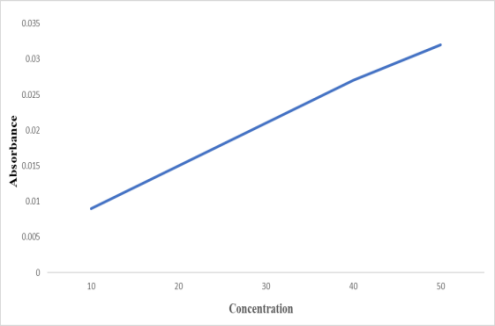


Fig10: The Calibration curve of Citalopram at 220-240nm.

Optical parameters for the calibration curve: The optical parameters are given in the table no 6.

Parameter	Citalopram
Linearity range (µg/ml)	10-50
Slope	0.00058
Intercept	0
Regression coefficient(r^2)	0.9988

Table no 6: Optical and regression parameters of the calibration curve by derivative method.

6.Determination of Citalopram in bulk:

To determine the feasibility of the proposed method for estimating Citalopram in marketed pharmaceutical formulation, the method was first tested for drug estimation in a standard bulk sample 50mg of Citalopram was accurately weighed and transferred to a 50 ml volumetric flask, where they were dissolved in methanol by vigorous shaking and The volume was adjusted to the mark transferred to a 50 ml volumetric flask and the volume was adjusted to mark with the same solvent. The solution's absorbance was measured 239nm against a blank, and the results are presented.

7.Validation of proposed method:

Application of proposed method for analysis of capsule formulation:

The commercialized two brands tablet strip of citalopram was brought in for commercial formulation analysis Determine the tablet's total weight. Then, take 10 tablet weights individually. It is necessary to crush the tablets. Prepare the 100g/ml stock solution after calculating the weight to be taken. Consider the absorbance at 239 nm. Tables 9.0 and 9.01 show the results.

Table no 7: Assay of citalopram in tablet formulation (Brand A)

Amount taken (mg)	Amount found(mg)	Amount found %
10	9.98	99.84
10	9.95	99.36
10	10.05	100.85
10	9.98	97.72
10	10.02	100.42
	Mean	100.01
	SD	0.5774
	CV	0.0059

Table no 8: Assay of Citalopram in Tablet formulation (Brand B).

Amount taken (mg)	Amount found(mg)	Amount found %
10	10.01	100.35
10	10.05	100.98
10	9.96	99.75
10	9.95	99.53
10	10.1	100.11
	Mean	100.141
	SD	0.5737
	CV	0.0044

Accuracy (Recovery Test):

Recovery experiments were used to assess the method's accuracy. The recovery experiments were carried out by introducing known amounts into the tablet The recovery was carried out at three levels: 80, 100, and 120 percent of the standard concentration of Citalopram. The recovery samples were prepared using the previously described procedure. For each level of recovery, three samples were prepared. The solutions were examined, and the percentage recoveries were determinedusing a formula.

Level Of % recovery (µg/ml)	Amount Present (µg/ml)	Amount Of stan-dard added (µg/ml)	Total Amount recov-erd (µg/ml)	% Recovery
80	10	8	18.30	100.13
80	10	8	17.98	99.657
80	10	8	18.24	100.12
100	10	10	20.02	100.17
100	10	10	19.97	99.85
100	10	10	19.92	99.95
120	10	12	21.74	99.64
120	10	12	21.45	99.72
120	10	12	21.54	99.82

%Mean recovery	SD	CV
99.968	0.2397	0.0023
99.978	0.1492	0.0014
99.733	0.083	0.0083

Table no 9: Result of accuracy Parameter of citalopram.

Formula for calculation of % recovery:

$$\%\text{recovery} = \frac{\text{Observe amount of compound in sample.}}{\text{Amount of all compound present in sample.}} \times 100$$

Level Of % Recovery (µg/ml)	Amount Present (µg/ml)	Amount of standard added (µg/ml)	Total Amount Recoverd (µg/ml)	% recovery
80	20	16	36.4	100.12
80	20	16	35.92	99.88
80	20	16	36.01	100.02
100	20	20	39.96	99.63
100	20	20	40.10	100.145
100	20	20	40.14	100.26
120	20	24	44.06	100.12
120	20	24	44.03	100.02
120	20	24	44.05	100.13

% Mean recovery	SD	CV
100.04	0.1814	0.0018
100.005	0.3304	0.0033
100.03	0.05507	0.0007

Table no 10: Result of accuracy parameter of Citalopram.

Precision: Three independent assays of Citalopram test sample were used to assess method precision. The method's intermediate precision was assessed using four different analyst and system in the same laboratory. Table 11.0 summarizes the assay val ue abstained by four analysts.

Sample no.	Assay of Citalopram as % of labeled amount			
	Analyst 1	Analyst 2	Analyst 3	Analyst 4
1	100.15	99.69	100.92	100.99
2	99.39	99.50	99.51	99.93
3	99.89	100.71	99.68	99.47
4	99.42	99.43	100.31	99.73
5	100.17	99.20	99.84	100.14
6	99.72	99.81	99.50	99.87
Mean	99.79	99.81	99.95	100.02
SD	0.3423	0.5277	0.6067	0.5218

Table no 11: Determination of precision of citalopram for the first derivative method.

1.AREA UNDER T CURVE METHOD ESTIMATION OF CITALOPRAM:

Material and method:

a) Material:

I. Chemicals and reagents: Methanol

II. **Instrument:** Estimation was performed using a Shimadzu 1800 UV spectrophotometer with a 1cm matched quartz cell.

III .Selection of media: main criteria for media selection and stability, i.e., drug should be soluble as well as stable in selected media for a sufficient period of time methanol has been chosen as the analytical medium for this work.

b) Method:

I.Solubility study of the Drug: The drug's solubility was determined at room temperature. A small amount of the standard drug was dissolved in distilled water, acetonitrile, Nicotinamide solvent and methanol. The drug was methanol-soluble and stable

II. Preparation of standard stock solution:

The standard stock solution was prepared by transferring 50 mg of Citalopram into a 500 ml beaker. 50ml methanol was transferred into the beaker and dissolved and transferred to the 50ml volumetric flask. The volume was made up to the mark with methanol which gives solution containing 1000 µg/ml Citalopram From this solution 5ml was transfer to 50 ml volumetric flask to this solution 50ml methanol was added to give a solution containing 100 µg/ml of Citalopram.

III.Preparation of calibration curve:

TRAIAL 1:

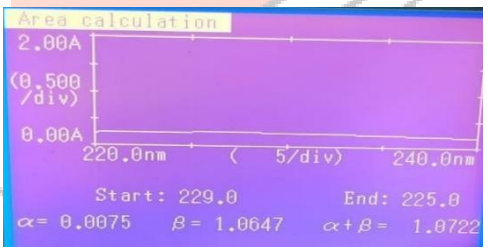


Fig11.1: Instrumental response of area under the curve of citalopram con. 2µg/ml.

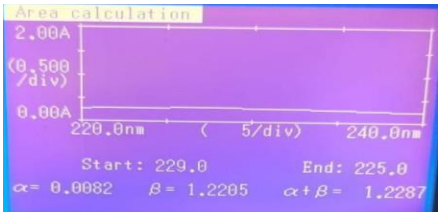


Fig11.2: Instrumental response of area under the curve of citalopram con. 4µg/ml.

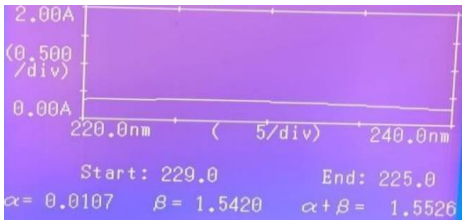


Fig11.3: Instrumental response of area under the curve of citalopram con. 6µg/ml.

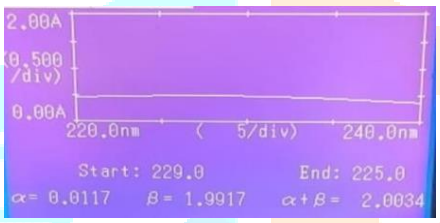


Fig11.4: Instrumental response of area under the curve of citalopram con. 8µg/ml.

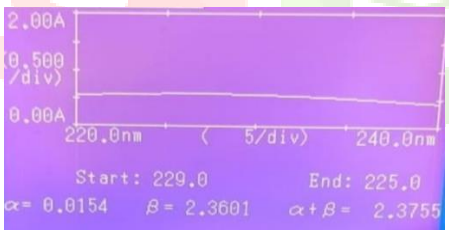


Fig11.5: Instrumental response of area under the curve of citalopram con. 10µg/ml.

Table no 12:Calibration table of AUC of citalopram of(2-10µg/ml)

Sr. No.	Concentration µg/ml	α	β	α + β
1	2µg/ml	0.0075	1.064	1.0722
2	4µg/ml	0.0082	1.2205	1.2287
3	6µg/ml	0.0107	1.5420	1.5526
4	8µg/ml	0.0117	1.9917	2.0034
5	10µg/ml	0.0154	2.3601	2.3755

Result:

Science concentration is not proportional to absorbance, the graph obtained was not linear. Indicating there was no increase in solubility.

TRAIAL 2:

Pipette off aliquots of 6,7,8,9,10 from the aforementioned working standard stock solution (100g/ml) and transfer to a series of 50ml volumetric flasks, where the final volume is built up to the mark with methanol to generate solutions of 12µg/ml, 14µg/ml, 16µg/ml, 18µg/ml, and 20µg/ml. These solutions were scanned in a range of 220-240nm against the methanol as blank. The absorbance maximum was found to be 239nm Citalopram. The range was selected from 220-240nm.

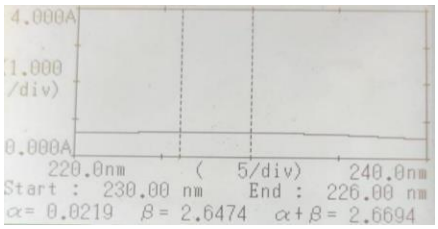


Fig12.1:Instrumental response of area under the curve of citalopram con.12µg/ml.

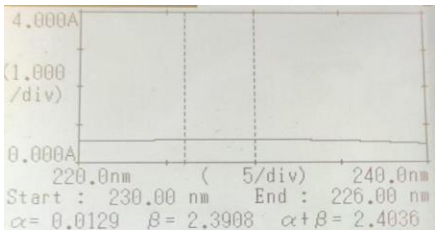


Fig12.2: Instrumental response of area under the curve of citalopram con.14µg/ml.

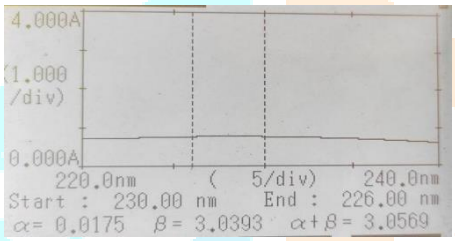


Fig12.3: Instrumental response of area under the curve of citalopramcon.16µg/ml.

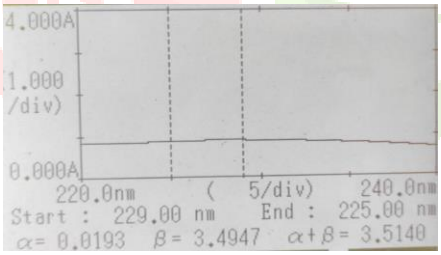


Fig12.4: Instrumental response of area under the curve of citalopram con.18µg/ml.

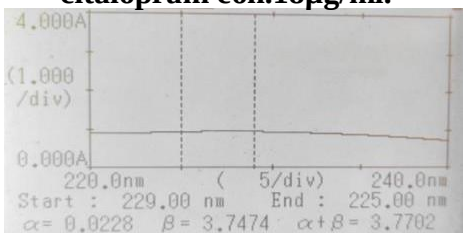


Fig12.5: Instrumental response of area under the curve of citalopram con. 20µg/ml.

Table no 13:

Calibration data of AUC of citalopram(12-20 µg/ml).

Sr. No.	Concentration µg/ml	α	B	α + β
1	12µg/ml	0.0219	2.6474	2.6694
2	14µg/ml	0.0129	2.3908	2.4036
3	16µg/ml	0.0175	3.0393	3.0569
4	18µg/ml	0.0193	3.4947	3.5140
5	20µg/ml	0.0228	3.7474	3.7702

Result:

Science concentration is not proportional to absorbance, the graph obtained was not linear. Indicating there was no increase in solubility.

TRAIAL 3:

Pipette out aliquots of 11,12,13,14,15 from the aforesaid working standard stock solution (100g/ml) and transfer to a succession of 50ml volumetric flasks, where the final volume is built up to the mark with methanol to generate solutions of 22µg/ml, 24µg/ml, 26µg/ml, 28µg/ml, 30µg/ml. The methanol was used as a blank and these solutions were scanned in the region of 220-240nm. Citalopram had the highest absorbance at 239nm. The wavelength range was chosen to be

220-240nm.

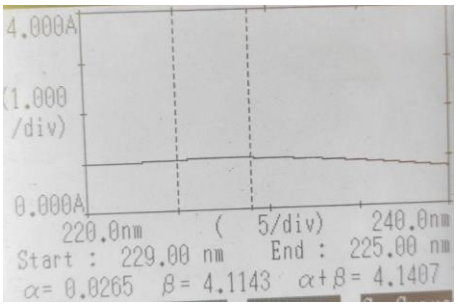


Fig13.1: Instrumental response of Area under the cover of Citalopram at con.22µg/ml.

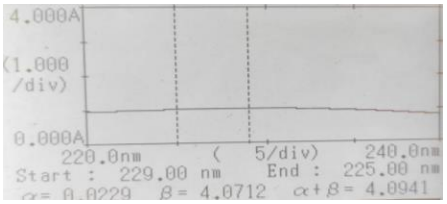


Fig13.2: Instrumental response of Area under the cover of Citalopram at con.24µg/ml.

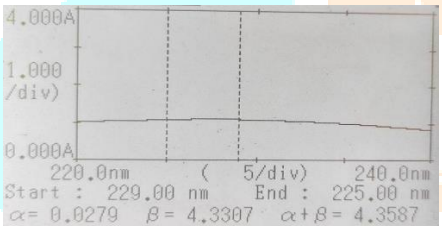


Fig13.3: Instrumental response of Area under the cover of Citalopram at con.26µg/ml.

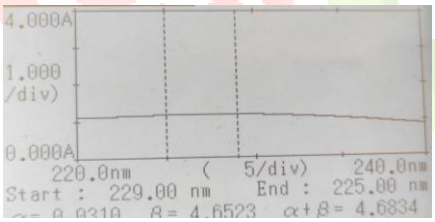


Fig13.4: Instrumental response of Area under the cover of Citalopram at con.28µg/ml.

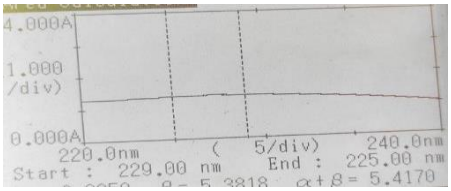


Fig13.5: Instrumental response of Area under the cover of Citalopram at con.30µg/ml.

Table no 14:Calibration data of AUC of citalopram(22-30 µg/ml).

Sr no	Conc.µg/ml	α	β	α + β
1	22µg/ml	0.0256	4.1143	4.1407
2	24µg/ml	0.0229	4.0712	4.0941
3	26µg/ml	0.0279	4.3307	4.3587
4	28µg/ml	0.0310	4.6523	4.6834
5	30µg/ml	0.0352	5.3818	5.4170

Result: Science concentration is not proportional to absorbance, the graph obtained was not linear. Indicating there was no increase in solubility.

TRAIAL 4:

From above working standard stock solution (100µg/ml), Pipette out aliquots of 16,17,18,19,20 and transfer to a succession of 50ml volumetric flasks, making up the final volume with methanol to make solutions of 32µg/ml, 34µg/ml, 36µg/ml, 38µg/ml, 40µg/ml.

The methanol was used as a blank and these solutions were scanned in the region of 220-240nm. 239nm Citalopram was determined to have the highest absorption. The wavelength range was chosen to be 220- 240nm.

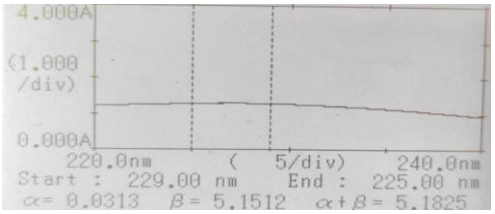


Fig14.1: Instrumental response of Area under the cover of Citalopram at con.32µg/ml.

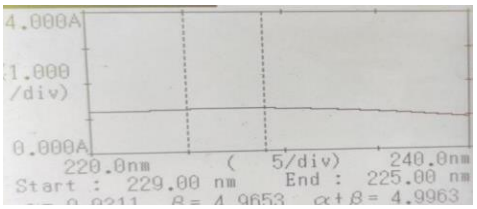


Fig14.2: Instrumental response of Area under the cover of Citalopram at con.34µg/ml.

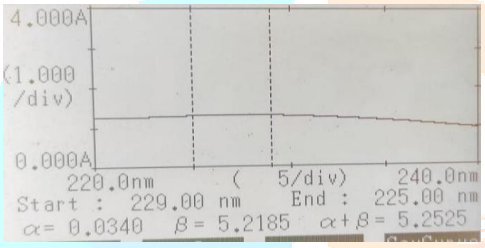
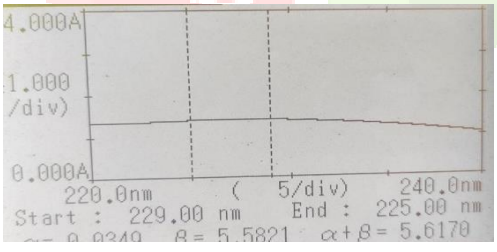


Fig14.3: Instrumental response of Area under the cover of Citalopram at con.36µg/ml.

Fig14.4: Instrumental response of



Area under the cover of Citalopram at con.38µg/ml.

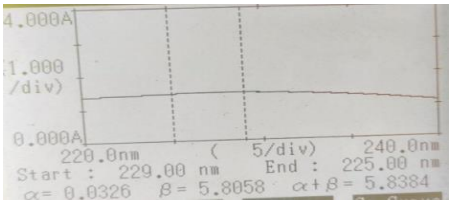


Fig14.5: Instrumental response of Area under the cover of Citalopram at con.40µg/ml

Table no 15: Calibration data of AUC of citalopram(32-40 µg/ml).

Sr no	Conc.µg/ml	α	β	α + β
1	32µg/ml	0.0313	5.1512	5.1825
2	34µg/ml	0.0311	4.9653	4.9963
3	36µg/ml	0.0340	5.2185	5.2525
4	38µg/ml	0.0349	5.5821	5.6170
5	40µg/ml	0.0326	5.8058	5.8384

Result:

Science concentration is not proportional to absorbance, the graph obtained was not linear. Indicating there was no increase in solubility.

TRAIAL 5:

From the out aliquots of 5,10,15,20,25,30 and transferred to series of 50ml volumetric flask and final volume made up to the mark with methanol to form solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µgmll, 60µg/ml. The methanol was used as a blank and these solutions were scanned in the region of 220-240nm. 239nm Citalopram was determined to have the

highest absorption. The wavelength range was chosen to be 220- 240nm.

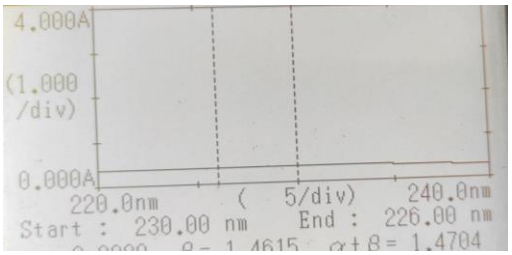


Fig15.1: Instrumental response of Area under the cover of Citalopram at con.10µg/ml.

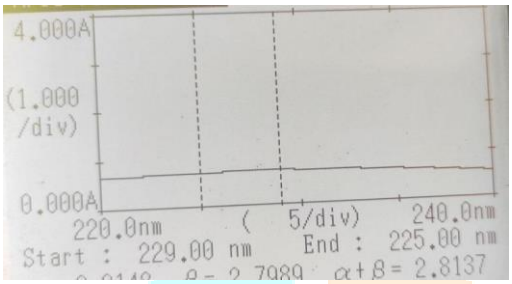


Fig15.2: Instrumental response of Area under the cover of Citalopram at con.20µg/ml.

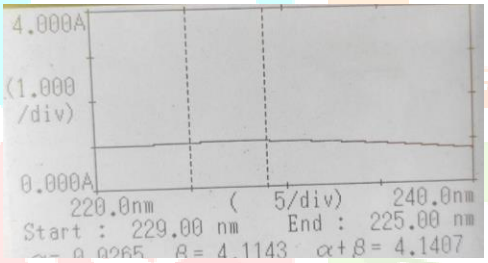


Fig15.3: Instrumental response of Area under the cover of Citalopram at con.30µg/ml.

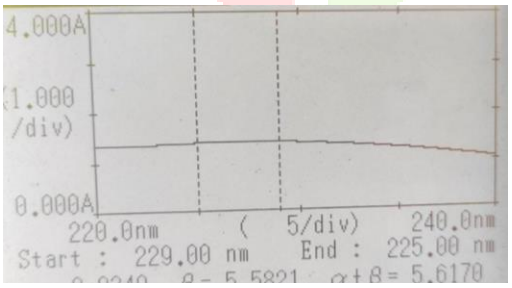


Fig15.4: Instrumental response of Area under the cover of Citalopram at con.40µg/ml

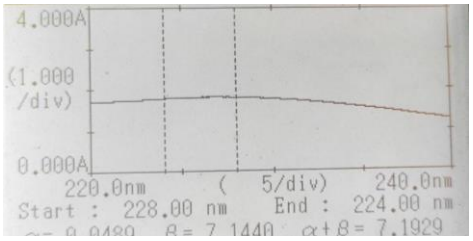


Fig15.5: Instrumental response of Area under the cover of Citalopram at con.50µg/ml.

Sr no	Conc.µg/ml	α	B	α + β
1	10µg/ml	0.0089	1.4615	1.4704
2	20µg/ml	0.0148	2.7989	2.8137
3	30µg/ml	0.0265	4.1143	4.1407
4	40µg/ml	0.0349	5.5821	5.6170
5	50µg/ml	0.0480	7.1440	7.1929

Table no 16: calibration data of AUC of citalopram (10-50µg/ml).

Result: Therefore, the concentration is proportional, the obtained graph was linear. Indicating an increase in solubility.

Visual parameters for the calibration curve:

The optical parameters are given in the table

Table 17.0: Optical and regression parameters of the calibration curve by Area under Cure method.

Parameter	Citalopram
Linearity(**µg/ml.**)	10-50
Slope	0.142483
Intercept	0
Regression coeffcients(r^2)	0.9986

iv. Validation of proposed method:

Application of recommend method for analysis of capsule formulation:

The commercialized two brands tablet strip of Citalopram was brought in for commercial formulation analysis. Determine the tablet's total weight. Then, take 10 tablet weights individually. It is necessary to crush the tablets. Prepare the 100µg/ml stock solution after calculating the weight to be taken. Consider the absorbance at 239 nm. Table 18.0 displays the results.

Table no 18:Absorbance of assay mixtures.

Amount taken(mg/tab)	Amount found(mg/tab)	Amount found%
10	10.5	100.35
10	9.01	99.29
10	9.06	99.77
10	11.2	100.81
10	10.02	100.05
	Mean	100.054
	SD	0.57522
	CV	0.0057

Linearity:

From the above standard stock solution(100µg/ml) pipet out aliquot the 5,10,15,20,25,30 ml and transferred to series of 50ml volumetric flask and final volume made up to the mark with methanol to form solution of 10µg/ml,20µg/ml,30µg/ml,40 µg/ml,50µg/ml,60µg/ml. The solution were scanned in arrange of 220-240nm against the methanol as blank. then calibration pick was plotted as absorbance vs. concentration to check the linear relationship between absorbance and concentration of Citalopram.

Table no 19:Linearity of citalopram.

Sr. no	Con. µg/ml	α	β	α +β
1	10µg/ml	0.0089	1.4615	1.4704
2	20µg/ml	0.00148	2.7989	2.8137
3	30µg/ml	0.0265	4.1143	4.1407
4	40µg/ml	0.0349	5.5821	5.6170
5	50µg/ml	0.0454	6.2397	7.1929

Accuracy (Recovery Test):

Recovery experiments used to assess the method's accuracy. The recovery experiments were carried out by introducing known amounts into the tablet. The recovery was carried out at three levels: 80, 100, and 120 percent of the standard concentration of Citalopram. The recovery samples were prepared using the previously described procedure. For each level of recovery, three samples were prepared. The solutions were examined, and the percentage recoveries were determined using a formula.

$$\%\text{recovery} = \frac{\text{Observed amount of Compound found in sample}}{\text{All amount of compound present in sample}*100}$$

Table no 20:Recovery study data of citalopram by AUC method.

Level of % recovery	Amount Present (µg/ml)	Amount Of Standard Added(µg/ml)	Total Amount Recovered µg/ml	% Recovery
80	10	8	18.30	100.12
80	10	8	17.97	99.71
80	10	8	17.94	99.54
100	10	10	20.02	100.09
100	10	10	20.05	100.12
100	10	10	19.97	99.62
120	10	12	22.04	100.03
120	10	12	22.03	99.96
120	10	12	21.98	100.12

% Mean recovery	SD	CV
99.79	0.2982	0.003
99.94	0.2804	0.0028
100.03	0.0802	0.0008

Precision:

Repeatability (intraday precision) and interlay precision are two types of precision studies. The same concentration of Citalopram was tested three times on the same day and three times on three distinct days. (10µg/ml) was estimated. The precision study's findings were expressed as a percent relative standard deviation.

Table no 21: result of Intraday precision study.

Con. (µg/ml)	AUC	AUC	AC	Average %RSD
	morning	afternon	Evening	
10	1.4707	1.2413	1.5231	
10	1.3321	1.2391	1.4321	
10	1.2120	1.2134	1.3451	
10	1.1239	1.3121	1.4231	0.1036
10	1.4531	1.3210	1.2312	
RSD %	0.1512	0.0508	0.1095	

Table no 22.1.:Analysis of Intradayprecision study.

Con.(µg/ml)	AUC	AUC	AUC
	Day1	Day2	Day3
10	1.4615	1.2450	1.2162
10	1.2110	1.3211	1.4423
10	1.3212	1.5232	1.6542
10	1.4210	1.5222	1.6142
10	1.2221	1.6212	1.4421
RSD%	0.1135	0.15689	0.1735
avg % RSD	0.1479		

LOD AND LOQ:

The lowest concentration of analyte that can be detected is defined as the limit of detection (LOD), whereas the lowest concentration of analyte that can be quantitated is defined as the limit of Quantitation. With the necessary precision and linearity, the following formulas can be used to compute LOD and LOQ.

LOQ = 10 * r/ S, LOD = 3.3* r/ S

Where r is the standard deviation of the regression line's y-intercept and S is the pitch of calibration curve.

Table no 22: Result of LOD&LOQ.

Drug	LOD (µg/ml)	LOQ (µg/ml)
Citalopram	2.17626	6.667756

RESULT AND DISCUSSION

A. ESTIMATION OF FIRST ORDER DERIVATIVE CITALOPRAM IN PHARMACEUTICAL PREPARATION:

The derivative spectra of Citalopram in methanol (10g/ml) were taken at N=5using a Shimazu 1800 spintronic UV-Visible spectrophotometer after scanning 220 nm to 240 nm at first order. The wavelength maximum was discovered to be at 239 nm. The calibration curve of Citalopram was found to be at 239nm. Figure 5.0. Beer's law obeys in the concentration range of 10-50µg/ml. Figure 5.1, 5.2, 5.3, 5.4 & 5.5.

The new method was validated in accordance with international guidelines and parameters. The novel method for the quantitative analysis of Citalopram was tested for selectivity and specificity in the presence of formulation additives and excipient, linearity and range at various concentration levels, as well as calibration standards where the detection range was optimized, accuracy was demonstrated by recovery trials at various concentration levels, and precision was verified by various analyst investigations.

Initially, the approach was tested on bulk pharmaceuticals in a synthetic mixture sample, to determine the applicability of the established technique for evaluating commercially available pharmaceutical formulation brands, and concentrations were estimated. The technique was then tested on tablets in marketed dosage forms, and based on the label claim for Citalopram, good results were obtained within acceptable ranges. Tables 10.0 & 10.1

The approach was tested for specificity, linearity, accuracy, and precision-repeatability using ICH recommendations and the findings were determined to be satisfactory, Citalopram in bulk and dose forms has lower standard deviation and coefficient of variation values that are within acceptable norms, i.e., commercialized tablet formulations for UV-Spectrophotometric First Derivatization Estimation. Because there was no interference from the tablet formulation additives, the method demonstrated specificity in the presence of formulation additives. The method was also accurate, as evidenced by successful recovery studies at various levels of confidence. Different analysts conducted investigations of intermediate precision, and the results were found to be satisfactory, demonstrating that the process was reproducible. Because the data obtained were reproducible in varied temperature conditions used at the time of detection of these pharmacological compounds with extremely small variances under the conditions used, the scheme was not

vulnerable to changes in the technique parameters. Table 11.0. The described method provides precise and accurate results for the Quantitation of Citalopram in their bulk drugs and tablet formulations and can be used for routine determinations with ease. The procedure is also simple, quick, and cost-effective, with repeatable results regardless of the instrument employed. The reported method is a low-cost method that uses only 1 N Methanol as a solvent and does not require the use of expensive reagents. This proposed approach is capable of being used for the Quantitation of Citalopram medicines in bulk and tablet dose forms without the interference of additives and it has a considerable and comparable correctness and exactness to the methods previously published. This newly developed method has an advantage over previously described methods in that it is more cost effective.

The proposed approach gives acceptable fluctuation of Citalopram, as shown by the percentage standard deviation values. The proposed technique's standard deviation percentages are within acceptable limits for Citalopram, demonstrating the technique's ability to remain unchanged by minute and purposeful changes in system restrictions and ensuring its consistency in regular routine application.

B.ESTIMATION OF AREA UNDER CURVE OF CITALOPRAM IN PHARMACEUTICAL PREPARATIONS:

Method development and optimization:

The current study Novel spectrometer analytical methods for the quantitative analysis of Citalopram in pharmaceutical preparation Solubility studies revealed that Citalopram has a higher solubility in methanol than in other solvents, the mix of Citalopram was found to be 239nm..In comparison to other described systems, the current UV Spectrophotometric methods can be favored for small scale companies because they are cost-effective and require less maintenance.

Linearity: The linearity of Std. Citalopram at five different concentrations was tested. Within the conc. range of 10-50µg/ml, Citalopram was found to be linear with a regression coefficient of 0.9986. The regression analysis results are summarized in Table 17.0.

Precision:

Repeatability is measured using percentage relative standard deviations (percent RSD) (intra-day precision). For Citalopram at a concentration of 10 g/ml, the average percent RSD value of intra-day precision was 0.1036, whereas the average % RSD value of intraday precision was 0.1479.

The intraday and interday precision percent RSD levels were all less than 2%, indicating that there were no significant variations in the analysis of Citalopram at the concentrations and that the proposed method was precise.

Accuracy:

The accuracy of the standard addition method was evaluated using three replicate determinations of three separate solutions containing 80, 100, and 120 percent Citalopram. For three different concentrations, the average percent recovery was determined to be 99.92.

LOD and LOQ:

The detection limit was determined to be 2.1762µg/ml, and the quantification limit was determined to be 6.6677.

DISSCUSSION

As a result, they claim that the approach demonstrates linearity with sufficient precision in the used range. The lack of any unknown chemicals is referred to as chemical purity. Complete purity is practically hard to attain, but if enough care is used during the manufacturing process, it

can be achieved as narrowly as required. However, the high expenses connected with achieving the greatest purity standards may make the technique useless; therefore it's vital to strike a balance in practice to find a product that's sufficiently pure for all medical uses at a reasonable price.

The key value of the above work is its simplicity, as the instrument presented is easy to use. It can also be utilized for routine analysis for a wide range of investigations, including dissolution studies, rate determination studies, release studies, pharmacokinetic studies, **bioavailability studies, and other common** day-to-day evaluations. Methanol is the only solvent employed in the operation, and no further reagents are needed. While chromatographic processes need the use of expensive reagents, solvents, and chemicals, the methodology just requires a few pieces of equipment, such as ordinary laboratory glassware for dilutions.

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