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QbD Approach for Analytical Method Development and Validation of Tryptophan by Spectroscopic Method

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ABSTRACT

According to ICH Q8 (R2) guidelines, an experimental work was planned for both spectroscopic method development and its validation. QbD approach was implemented for spectroscopic method development and its validation. This research work demonstrated that the UV is valid for the determination of assay of Serotonin. It describes the materials and methods used in experimental work. For performing experimental work analytical grade chemicals (methanol, water &ethanol) was used. The spectrophotometric method development and validated on UV spectrophotometer by using the suitable solvent (ethanol, methanol & water) and detection was performed at 218nm.QbD approach was carried out for spectroscopic method development by varying 17 parameters and critical parameters were extracted by using principal component analysis and by observation. For all the variable parameters as stated in Ishikawa diagram, the absorbance was recorded over the concentration range.

INTRODUCTION

Analytical methods play the important role supporting the implementation of QbD in process of pharmaceutical development and manufacturing. Analytical testing also plays the prominent role in pharmaceutical development, risk assessment, process monitoring and control and continuous quality assessment throughout the product. Quality-by-Design (QbD) is well-established in development and manufacture of pharmaceutical drug substance and drug product and is discussed in ICH Q8.[1] Q9 and Q2. The same QbD approach can be applied to analytical procedures as per ICH Q2. In addition, there is now a technique to definitively link the data to its intended use. These are exciting times for testing laboratories and the users of the data they produce. The knowledge obtained during development helps to justify the establishment of a design space, (process) control strategy and set point within the (regulatory approved) design space. Materials made within the design space will produce an acceptable product, and changes within the design space are regulatory acceptable. Quality by Design approach suggests looking into the quality of analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into results of an analytical process. QbD is defined as a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management‖. In alignment with the approach proposed in the draft FDA guidance for process validation, a three-stage approach can be applied to method validation.[2]

Tryptophan

As an essential amino acid, tryptophan is not synthesized from more basic substances in humans and other animals, who must ingest tryptophan or tryptophan-containing proteins. Plants and microorganisms commonly synthesize tryptophan from shikimic acid or anthranilate[1] by the following process: anthranilate condenses with phosphoribosylpyrophosphate (PRPP), generating pyrophosphate as a by-product. The ring of the ribose moiety is opened and subjected to reductive decarboxylation, producing indole-3-glycerinephosphate; this, in turn, is transformed into indole. In the last step, tryptophan synthase catalyzes the formation of tryptophan from indole and the amino acid serine.

For many organisms (including humans), tryptophan is needed to prevent illness or death, but cannot be synthesized by the organism and must be ingested; in short, it is an essential amino acid. Amino acids, including tryptophan, act as building blocks in protein biosynthesis, and proteins are required to sustain life. Tryptophan residues are among the less common amino acids found in proteins but are known to play important structural or functional roles whenever they occur. For instance, tryptophan (and tyrosine) residues play special roles in "anchoring" membrane proteins within the membrane, based on studies of membrane proteins and model transmembrane peptides. In addition, tryptophan functions as a biochemical precursor for the following compounds (see also figure to the right):

- Serotonin (a neurotransmitter), synthesized via tryptophan hydroxylase.[10][11] Serotonin, in turn, can be converted to melatonin (a neurohormone), via N-acetyltransferase and 5-hydroxyindole-O-methyltransferase activities.[2]

- Niacin, also known as vitamin B_3, is synthesized from tryptophan via kynurenine and quinolinic acids as key biosynthetic intermediates.[3]

- Auxins (a class of phytohormones) are synthesized from tryptophan.[4]

The disorder fructose malabsorption causes improper absorption of tryptophan in the intestine, reduced levels of tryptophan in the blood,[5] and depression.[6] Some studies did not find reduced tryptophan in cases of lactose mal digestion.[7]

In bacteria that synthesize tryptophan, high cellular levels of this amino acid activate a repressor protein, which binds to the top operon.[8] Binding of this repressor to the tryptophan operon prevents transcription of downstream DNA that codes for the enzymes involved in the biosynthesis of tryptophan. So high levels of tryptophan prevent tryptophan synthesis through a negative feedback loop and when the cell's tryptophan levels are reduced, transcription from the top operon resumes. The genetic organization of the trip operon thus permits tightly regulated and rapid responses to changes in the cells internal and external tryptophan levels.

QbD

Stage1. Method Design: Define method requirements and conditions and identify critical controls.

Stage2. Method Qualification: Confirm that the method is capable of meeting its design intent. Stage3.Continued Method Verification: Gain ongoing assurance to ensure that the method remains in a state of control during routine use. A critical function of Stage 1 is the design of an Analytical Target Profile (ATP) for the method. To design the ATP, it is necessary to determine the characteristics that will be indicators of method performance for its intended use. These are selected from the performance characteristics described in ICH Q2 as per the traditional approach. Instead of being applied in a tick box manner, they are

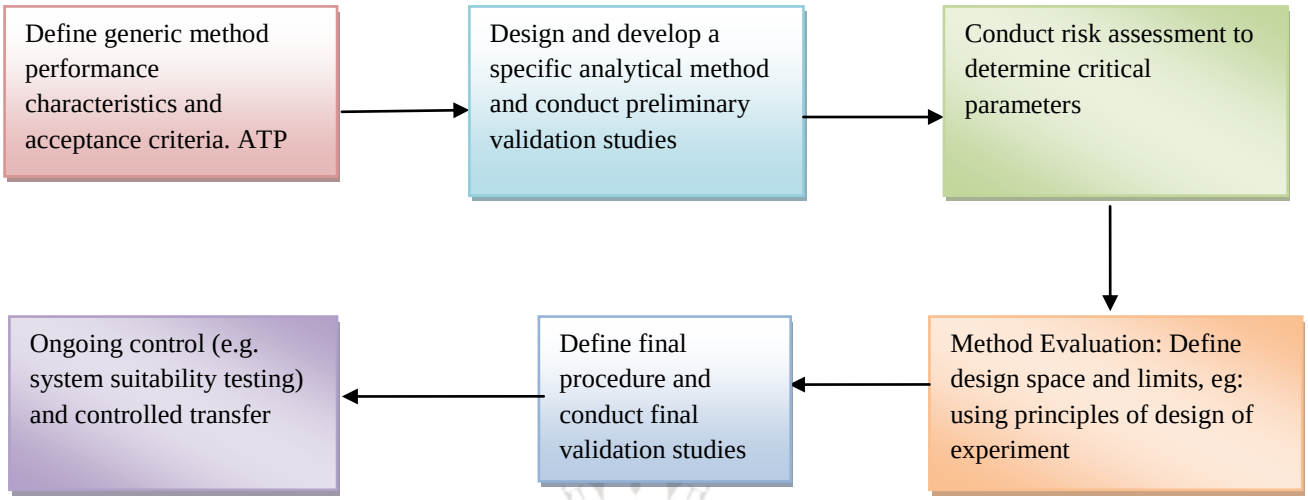

Fig 1-QbD workflow

Investigated by a risk assessment exercise as described in ICH Q9 in combination with carefully designed development studies to identify the critical method and sources of variation. Variables are then investigated by robustness and ruggedness experiments to understand the functional relationship between method input variables and each of the method performance characteristics and the results is compared to the desired outcome defined in the ATP. From this, one can identify a set of operational method controls. Also, having evaluated the critical method parameters and gained a better understanding of the method through structured experimentation Addition to validating the method characteristics as per regulatory guidance, verifying the accuracy and precision provide additional understanding of the method‘s measurement uncertainty and confirms conformance to the previously defined method performance requirements (ATP). This can be accomplished with a joint accuracy and precision. Serotonin (5-hydroxytryptamine, 5-HT) serves as a central and peripheral neurotransmitter/neuromodulator and has growth factor-like action in the developing nervous system (1,2). Serotonin mediates a range of critical behaviors (3), and it has been linked to a number of neuropsychiatric disorders including anxiety, depression,

obsessive-compulsive disorder (OCD), and autism (4-6). Serotonin and related compounds, including its precursor amino acid tryptophan (TRP) and major metabolite 5-hydroxy-tryptophan.

EXPERIMENTAL

Standards and materials Standard materials were obtained from the following sup- pliers: N-methyl serotonin (NMS) oxalate salt and perchloric acid were from Aldrich Chemicals (Milwaukee, WI); serotonin creatinine sulfate, l-tryptophan (TRP), indole-3-propionic cid (IPA), 5-hydroxy-indole-3-acetica cid (5-HIAA), ascorbic acid, and sodium metabisulfite were from Sigma Chemicals (St. Louis, MO); and indole-3-acetic acid (IAA) was from Acros Chemicals (Pittsburgh, PA). Stock solutions of all standards were prepared by weighing the appropriate amount of the stan- dard material to obtain 10.0 mg of the free base (or acid). The free base material was dissolved in 100 mL of 0.2% ascorbic acid, resulting in stock solutions of 0.1 mg/mL. The stock solutions were further diluted in 0.2% ascorbic acid to the 440 Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission. Journal of Analytical Toxicology, Vol. 27, October 2003 appropriate concentrations for the working standards. All standards were stored at --80~ In patients with sleep disorders and altered circadian rhythms, such as occur in jet lag, night shift work, and various neuropsychiatric disorders, oral administration of melatonin can provide the necessary resynchronization of those cycles, at dosages ranging from 0.3 to 8 mg. Synthesis of melatonin from the amino acid tryptophan is decreased by exposure to magnetic fields and by the aging process. Melatonin is a potent scavenger of free radicals and exerts direct inhibition of cancer growth. Various cancer types have been shown to be responsive to oral melatonin (10-50 mg daily), including breast cancer, non-small-cell lung cancer, metastatic renal cell carcinoma, hepatocellular carcinoma, and brain metastases [5] from solid tumors. Serotonin has also been reported to lower LDL- and total cholesterol levels. Abnormally low melatonin levels have been theorized to be a factor in multiple sclerosis, coronary heart disease, epilepsy, and postmenopausal osteoporosis. These reports, while preliminary, serve to further illustrate the wide range of potential effects exerted by melatonin.[6-8] It is soluble in water; soluble in methanol, Ethanol, slightly soluble in alcohol and in chloroform; and very slightly soluble in acetone.

The present work aims at the systematic development of a simple, rapid and highly sensitive method for the analysis of Serotonin by QbD approach.

MATERIALS AND METHODS

All chemicals used in the project work were either AR. The various reagents and chemicals used during experimental work are as follows;

Table 1: chemicals and instruments

Sr. No.	Name of Chemicals	Source	Sr. No.	Name of Equipment	Source
1.	Water	D/W	1.	UV	Shimadzu, Model: UV-1800
2.	Methanol	Analytical grade	2.	Electronic weighing balance	Shimadzu BL- 220 H
3.	Ethanol	Analytical grade	3.	Sonicator	The ultrasonic's PCI Analytics Sonicator

Methods Preliminary solubility study of drug:

The solubility of the drug was determined at 26±1 ^{0}C. A small quantity of standard drug was dissolved in different solvents like distilled water, ethanol, methanol, acetonitrile, alcohol, chloroform, acetone.

Preparation of Stock solution: Preparation of standard stock solution of Tryptophan:

10 mg of Tryptophan accurately weighted by electronic balance and dissolved in 80ml of double distilled water in the 250ml conical flask. The content of flask was kept for stirring on the magnetic stirrer for 10 min and transferred in 100ml volumetric flask. Conical flask was rinsed by 20ml of double distilled water and this water was used to make up volume 100ml of volumetric flask to give conc. of 100µg/ml.

Preparation of working standard solution of Tryptophan:

The working solution of Tryptophan was prepared by further diluting the stock solution. Then pipette out 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml & 1.2ml of solution and make up to 10ml leads to 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml & 12 µg/ml concentration solution. This solution was estimated by UV spectrophotometer by using Methanol as blank at 218nm.

Fixing of wave length

After selecting the suitable solvent, the fixing of the λmax for the proposed method is very important. This can be done by scanning the drug sample (Tryptophan) solution in Methanol in the range of 400nm-200nm and the most repeated maximum absorbance with linearity and repeatability can be fixed as λmax for the drug. In addition, in the proposed method for Serotonin drug shows maximum 218 nm. With more linearity, repeatability (ruggedness) and the λmax was fixed as 218nm.

Linearity and range:

For linearity study from the working standard at different concentration 2, 4, 6, 8, 10 and 12 µg/ml of drug solution were placed in 6 different 10ml volumetric flask volume was made up to the mark with Methanol. Absorbance was measured at 225nm. Then obtained data were used for the linearity calibration plot.

Accuracy and recovery study:

This study was carried out using the stock solution (100µg/ml). Take three concentrations 8 µg/ml, 10µg/ml, and 12µg/ml. In addition, take six reading of these concentrations. Calculate the % RSD of the concentration. Results within the range of ensure an accurate method as well as indicate non-interference with the excipients of formulation. The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of Tryptophan solution of the drug to pre-analyzed tablet solutions. The resulting solutions were then reanalyzed by proposed methods.

Intra-day precision (repeatability) and inter-day precision study (intermediate precision): The standard stock solution of Tryptophan was Prepared. Prepare the three concentration of (8, 10, and 12 µg/ml), by using mobile phase methanol. Take λmax at the intraday and inter day. Calculate the % RSD. Variation of results within the day (intra-day), Variation of the result between days (inter day) were analyzed. Intraday precision was analyzing Tryptophan for three times on the same day at 218nm. Inter-day precision was determined by analyzing the drug different day for three days at 218nm. Precision data for Tryptophan at 225nm

Reproducibility:

Reproducibility is assessed by mean of an interlaboratory trial. The absorbance readings wereMeasured at 218 nm at the different laboratory using another spectrophotometer and the value obtained were evaluate using the t-test to verify their reproducibility data for Tryptophan at 218nm is recorded.

Limit of Detection & Limit of Quantitation:

The limit of detection and quantification of the drug are calculated with the standard deviation and slop.

$$LOD = \frac{3.3\sigma}{S} \quad LOQ = \frac{10\sigma}{S}$$

Where,

σ = standard deviation

S = slope of calibration curve

Stability of Sample:

Samples prepared for repeatability study were preserved for 24 hrs at room temperature and analyzed on the following day to test for short-term stability. The sample of 4µg/ml drug solution was prepared by suitable dilution with diluents and absorbance were taken at 218 nm against the blank. The stability of sample was found to be more than 10 hrs.

Acid degradation:

The preparation of 0.01N hydrochloric acid (HCl) was done by diluting 0.085 ml of conc. HCL to 100 ml of distilled water. Etoricoxib was accurately weighted and was transferred to a labeled round-bottomed flask. Reflux the sample for 2 hrs. In addition, pipette out 1ml to 10 ml volumetric flask and adjust with mobile phase. The volume of 20µl was injected into the system for chromatographic analysis and results of all chromatograms were compared to see whether degradation occurred or not. The degradation of the drug is not more than 30%.

Base degradation: The 0.01N Sodium Hydroxide (NaOH) was prepared by dissolving 0.04 gm of sodium hydroxide pellets in 100 ml of distilled water. The solution was standardized with 0.01 N HCl as per Indian Pharmacopoeia (I.P). Etoricoxib was accurately weighted and was transferred to a labeled round-bottomed flask. Reflux the sample for 2 hrs. In addition, pipette out 1ml to 10 ml volumetric flask and adjust with mobile phase. The volume of 20µl was injected into the system for chromatographic analysis and results of all chromatograms were compared to see whether degradation occurred or not. The degradation of drug sample is not more than 22%.

Neutral condition:

Weight accurately 10 mg drug and transferred in to100 ml water in round bottom flask. Reflux it for 2 hours. Pipette out 1ml into 10 ml volumetric flask and adjust with mobile phase.

Photostability study:

Photostability was performed by placing 10 mg of Tryptophan in daylight for 24 hours. The samples were diluted with methanol up to 100ml in a volumetric flask. Pipette out 1 ml sample diluted up to 10 ml by mobile phase. The volume of 20µl was injected into the system for chromatographic analysis.

Dry heat: Standard Tryptophan was placed in an oven at 60°C for 2 hours to study dry heat degradation. 10 mg drug samples were diluted with methanol up to 100ml in a volumetric flask. Pipette out 1 ml and were diluted up to 10 ml by mobile phase. The volume of 20µl was injected into the system for chromatographic analysis and results of both chromatograms were compared to see whether degradation occurred or not.

Assay Procedure -

Take the weight of 10 tablets of any brand of Serotonin tablet. Crush the tablet in the motor pestle. Accurately weigh the quantity of powder equivalent to 10mg of the drug in 100 ml volumetric flask and add ethanol to adjust the volume up to 100 ml. Pipette out the 1 ml into 10 ml volumetric flask make the volume with mobile phase to get conc. 10µg/ml and analyze the reading on HPLC. Calculate the percentagepurity of tablet.

OBSERVATIONS AND RESULTS

Preliminary solubility study of drug:

The solubility of the drug was determined at 28±1 ^{0}C. A small quantity of standard drug was dissolved in different solvents. Implementation of QbD approach for the Spectrophotometric method development as per ICH Q8(R2) guidelines for estimation of Tryptophan by varying various parameters and these variable parameters were designed as per Ishikawa. Critical parameters for the development of zero order Spectrophotometric method are considered as various solvent, sample preparation of tablet, wavelength at 220 & 222 nm, slit width as 1, scan speed and sampling interval (0.05, 0.1, 0.2, 0.5, 1.0, and 2.0)

Determination of Variable Parameters

According to QbD approach, the first step is to determine the variable parameters for the respective method. Thus, the variable parameters for both the spectrophotometric methods were designed as Ishikawa diagram (Figures 3). For all the variable parameters as stated in Ishikawa diagram, the absorbance's were recorded over the concentration range according to respective method. Working solution was scanned from 400 to 200nm and three peaks were observed at wavelengths225nm, 227nm. These three wavelengths were used as variable parameters. In addition, the solubility was studied in various solvents including distilled water, and methanol. The sharpness of spectra was compared for selection of critical parameter. Scan speed was varied as fast, medium, slow, and very slow over the range 400–200nm, while slit width and sampling interval were varied in particular ranges of 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0nm and 0.05,0.1,0.2,0.5,1.0,and2.0nm,respectively. For the estimation of melatonin, two types of sample preparations were selected and evaluated. Tablets were formulated as per the master formula and were used in method development. Average weight of tablets was noted and tablets were triturated. Tablet powder equivalent to average weight was taken for study and evaluated for the method development. Recovery study was carried out at three levels 80%, 100%, and 120%.

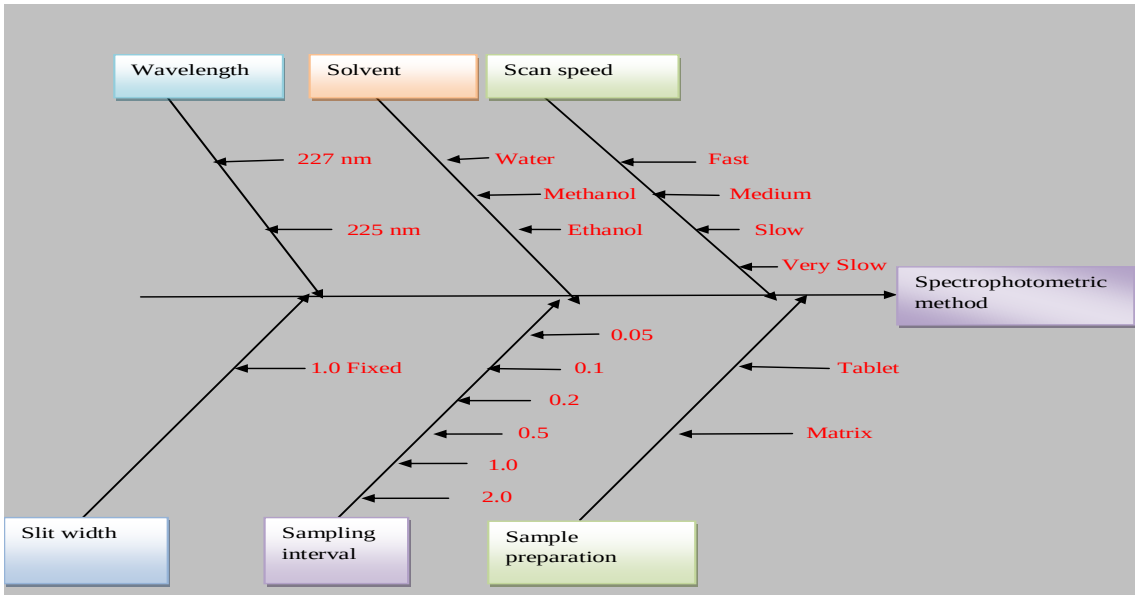

Figure 3: Ishikawa diagram

Table 2: Solubility study in different Solvent by UV-absorbance

Solvent	Concentration	Absorbance	Solvent	Concentration	Absorbance
Water	2	0.047	**Methanol**	2	0.205
	4	0.072		4	0.334
	6	0.095		6	0.597
	8	0.102		8	0.667
	10	0.188		10	0.764
	12	0.215		12	0.896
Ethanol	2	0.0047	**Methanol (With stirring)**	2	0.313
	4	0.010		4	0.533
	6	0.013		6	0.739
	8	0.064		8	0.970
	10	0.092		10	1.248
	12	0.105		12	1.474

Table 3: Effect of Stirring on absorbance at (ʎmax-218nm) As per Stirring time

Solvent	Conc. µg/ ml	Effect of Stirring on absorbance at (ʎmax-218) As per Stirring time							
		0 min		2 min		4 min		6 min	
		225nm	227nm	225nm	227nm	225nm	227nm	225nm	227nm
Methanol (With Stirring)	2	0.311	0.313	0.327	0.329	0.339	0.341	0.354	0.357
	4	0.531	0.533	0.545	0.547	0.556	0.558	0.570	0.572
	6	0.737	0.739	0.0.751	0.753	0.765	0.767	0.779	0.781
	8	0.767	0.970	0.982	0.984	0.996	0.999	1.110	1.112
	10	1.246	1.248	1.260	1.262	1.274	1.276	1.880	1.882
	12	1.471	1.474	1.481	1.483	1.495	1.497	1.509	1.511
Solvent	Conc. µg/ ml	**Effect of Stirring on absorbance at (ʎmax-218) As per Stirring time**							
		8 min		**10 min**		**12 min**		**14 min**	
		225nm	227nm	225nm	227nm	225nm	227nm	225nm	227nm
Methanol (With Stirring)	2	0.365	0.368	0.373	0.375	0.386	0.388	0.394	0.397
	4	0.584	0.586	0.598	0.610	0.622	0.624	0.636	0.638
	6	0.793	0.795	0.807	0.809	0.821	0.823	0.835	0.837
	8	0.124	0.126	0.138	0.140	0.152	0.154	0.166	0.168
	10	0.194	0.196	0.208	0.210	0.222	0.224	0.236	0.238
	12	1.523	1.525	1.537	1.539	1.541	1.543	1.555	1.557

Table 4: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 2ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		218 nm	220 nm	218 nm	220 nm	218 nm	220 nm	218 nm	220 nm
0.05	Abs.	0.311	0.312	0.310	0.311	0.310	0.311	0.309	0.310
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	0.311	0.312	0.310	0.311	0.309	0.310	0.309	0.310
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	0.312	0.313	0.310	0.311	0.309	0.310	0.309	0.310
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	0.311	0.312	0.310	0.311	0.309	0.310	0.309	0.310
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	Abs.	0.312	0.313	0.310	0.311	0.309	0.310	0.309	0.310
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	0.312	0.313	0.309	0.310	0.309	0.310	0.309	0.310
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 5: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 4ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		218 nm	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**
0.05	Abs.	0.531	0.532	0.530	0.531	0.530	0.531	0.529	0.530
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	0.531	0.532		0.531	0.529	0.530	0.529	0.530
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	0.532	0.533	0.530	0.531	0.529	0.530	0.529	0.530
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	0.531	0.532	0.530	0.531	0.529	0.530	0.529	0.530
	Time Req.	0.45 min		1.23 min		3.2 min		8 min	
1.0	Abs.	0.532	0.533	0.530	0.531	0.529	0.530	0.529	0.530
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	0.532	0.533	0.529	0.530	0.529	0.530	0.529	0.530
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 6: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 6ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		218 nm	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**
0.05	Abs.	0.738	0.739	0.736	0.737	0.740	0.741	0.742	0.743
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	0.738	0.739	0.736	0.738	0.740	0.741	0.742	0.743
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	0.737	0.738	0.738	0.739	0.740	0.741	0.742	0.743
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	0.738	0.739	0.739	0.740	0.740	0.741	0.742	0.743
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	Abs.	0.738	0.739	0.740	0.741	0.741	0.742	0.742	0.743
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	0.738	0.739	0.740	0.741	0.742	0.743	0.742	0.743
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 7: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 8ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		218 nm	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**
0.05	Abs.	0.969	0.970	0.973	0.974	0.977	0.978	0.980	0.981
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	0.969	0.970	0.973	0.974	0.977	0.978	0.980	0.981
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	0.969	0.970	0.973	0.974	0.977	0.978	0.980	0.981
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	0.969	0.970	0.973	0.973	0.977	0.978	0.980	0.981
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	Abs.	0.969	0.970	0.973	0.974	0.977	0.978	0.980	0.981
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	0.970	0.970	0.973	0.974	0.977	0.978	0.980	0.981
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 8: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 10ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		218 nm	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**
0.05	Abs.	1.247	1.248	1.251	1.252	1.255	1.256	1.258	1.259
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	1.247	1.248	1.250	1.252	1.254	1.257	1.258	1.259
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	1.247	1.248	1.251	1.252	1.255	1.256	1.258	1.259
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	1.253	1.248	1.251	1.252	1.255	1.256	1.258	1.259
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	Abs.	1.247	1.248	1.251	1.252	1.255	1.256	1.258	1.259
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	1.247	1.248	1.251	1.251	1.255	1.256	1.257	1.259
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 9: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 12ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		218 nm	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**	**218 nm**	**220 nm**
0.05	Abs.	1.473	1.474	1.477	1.478	1.480	1.481	1.484	1.485
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	1.473	1.474	1.477	1.478	1.480	1.481	1.484	1.485
	Time Req.	1.40 min	-	6 min	-	12 min.	-	21 min	-
0.2	Abs.	1.473	1.474	1.477	1.478	1.481	1.482	1.484	1.485
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	1.473	1.474	1.477	1.478	1.480	1.481	1.484	1.486
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	Abs.	1.473	1.474	1.477	1.478	1.480	1.481	1.484	1.485
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	1.472	1.474	1.477	1.478	1.480	1.481	1.484	1.485
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

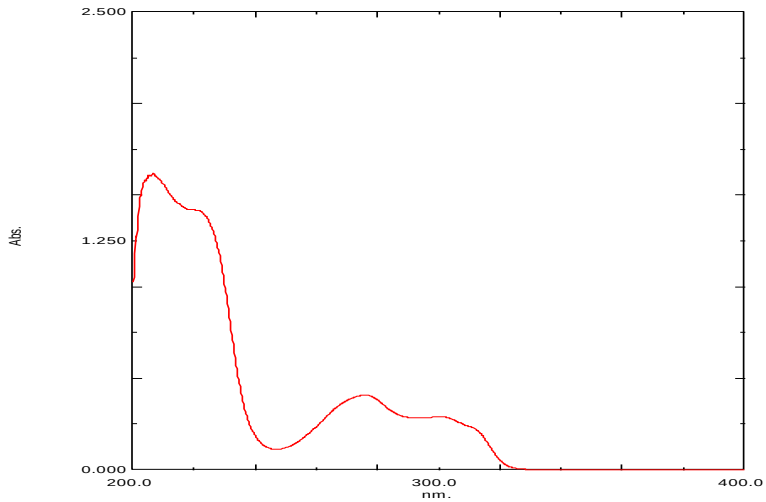

Figure 4: Fixing of wavelength for Tryptophan

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2 to 12 mg/ml was linear with a correlation coefficient (R2) 0.997.

Table 10: Linearity and range for Tryptophan at 218nm

Sr. No.	Concentration (µg/ml)	Absorbance's
1.	2	0.311
2.	4	0.555
3.	6	0.751
4.	8	0.92
5.	10	1.22
6.	12	1.474

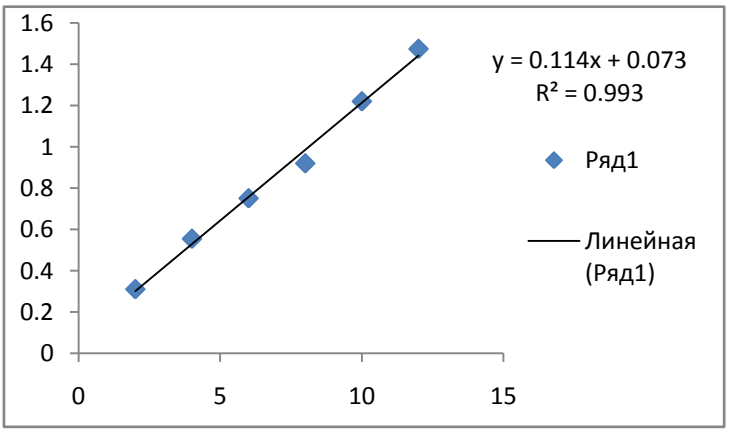

Figure 5: Linearity and range for Tryptophan

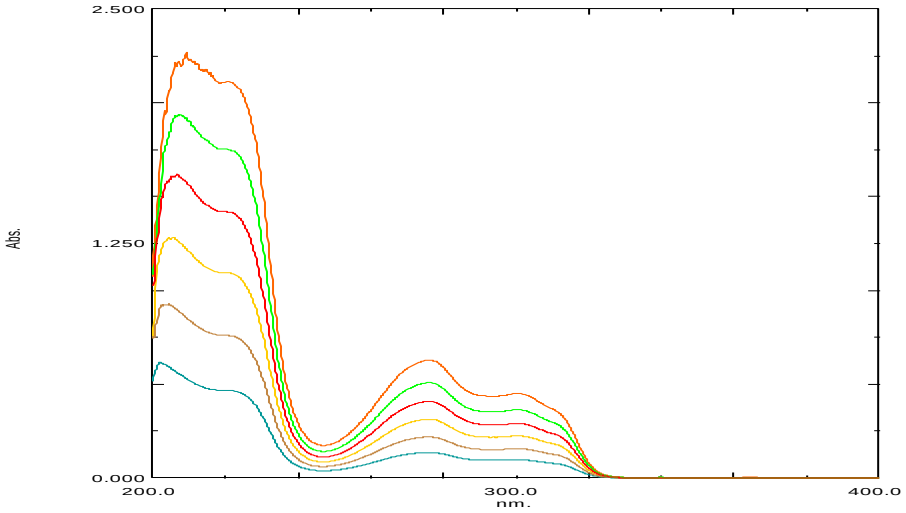

Figure 6: Accuracy sample overlay Tryptophan

Table 11: Linearity Parameter

Parameter	Data
Range	2 µg/ml to 12 µg/ml
Correlation coefficient	0.993
Slope	0.116
Intercept	0.0614

Intra-day precision (repeatability) and inter-day precision study (intermediate precision):

Table 12: Precision data for Tryptophan 218nm (Intra-Day)

Conc. (µg/m)	Absorbance						AVG	SD	% RSD
8	0.412	0.413	0.414	0.415	0.416	0.417	0.414	0.0016	0.394
10	0.512	0.512	0.513	0.513	0.513	0.514	0.512	0.00075	0.147
12	0.637	0.637	0.639	0.639	0.639	0.638	0.638	0.00098	0.016

Table 13: Precision data for Tryptophan at 218nm (Inter-Day)

Conc. (µg/m)	Absorbance						AVG	SD	% RSD
8	0.375	0.385	0.385	0.386	0.386	0.387	0.384	0.0044	1.163
10	0.469	0.470	0.470	0.471	0.471	0.471	0.470	0.0081	0.172
12	0.594	0.594	0.595	0.596	0.596	0.596	0.595	0.0009	0.0163

Limit of Detection & Limit of Quantization:

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where,

σ = standard deviation

S = slope of calibration curve

Table 14: Limit of Detection & Limit of Quantization

LOD	LOQ
13.43	31.67

Stability of Sample:

Table 15: Stability of Sample

Sr. No	Conc. (µg/ml)	Time (min)	Absorbance at 222nm
1.	4	0	0.521
2.	4	30	0.525
3.	4	60	0.525
4.	4	90	0.526
5.	4	120	0.525
6.	4	150	0.526
7.	4	180	0.526
8.	4	210	0.527
9.	4	240	0.526
10.	4	360	0.524
11.	4	480	0.525
12.	4	600	0.523

Table 16: Degradation Studies

Stress condition	% Degradation Observed	Remarks
0.01N NaOH	90	Unstable
0.01N HCl	7.5	Stable
Oven	9	Stable
Water	23	Unstable
Oxidation	73	Unstable

CONCLUSION

The proposed methods were found to be accurate, precise, and economical and can be used for routine quality control analysis of Tryptophan in pharmaceutical dosage form. QbD implementation which such a use for a save time & money value.

REFERENCES:

1. Bhusnure O.G., FasmaleR.N.,Gandge N.V' Gholve S.B., Giram P.S., QbD Approach for Analytical Method Development and Validation of Serotonin by spectroscopic Method, International Journal of pharma and bio sciences 2017,11(5),Ref M.S.No: 9333.
2. Bhusnure O.G.*, Gandge N.V., Gholve S.B., Giram P.S., QbD Approach for Analytical Method Development and Validation of Melatonin by spectroscopic Method, Journal of Pharmacy Research 2017,11(5),464-471.
3. M. M. Rapport, A. A. Green, H. Irvine Page. J. Biol. Chem. 176 (1948) 1243-1251.
4. M. Maurice Rapport. J. Biol. Chem. 180 (1949) 96.
5. E. Garelis, Adv. Exp. Med. Biol., 133 (1981) 717. [5]. J. Barchas, E. Usdin, Eds, in "Serotonin and Behaviour", Academic Press, New York, (1973) 192-194
6. Nichols DE, Nichols CD (2008) Serotonin receptors. Chem Rev 108:1614–1641
7. Krishnan V, Nestler EJ (2010) Linking molecules to mood: new insight into the biology of depression. Am J Psychiatry 167:1305– 1320
8. Cook IA (2008) Biomarkers in psychiatry: potentials pitfalls and pragmatics. Prim Psychiatr 15:54–59
9. Yao JC, Yan-hui QU, Zhao XY, Hu L, Zhu R, Li H, Ding J. Determination of Zolmitriptan in Human Plasma byHigh-performance Liquid Chromatography-Electrospray Mass Spectrometry and Study on Its Pharmacokinetics. J. Chinese Pharm. Sci. 2005, 14, 25-28.
10. Srinivasu MK, Rao BM, Sridhar G, Chandrasekhar KB and Kumar PR. A validated chiral LC method for the enantiomeric separation of Serotonin key intermediate, ZTR-5. J. Pharm. Biomed. Anal. 2005, 39, 796-800.
11. Srinivasu MK, Rao BM, Sridhar G, Kumar PR, Chandrasekhar KB and Islam A. A validated chiral LC method for the determination of serotonin and its potential impurities. J. Pharm. Biomed. Anal. 2005, 37, 453-460.
12. Yu L, Yao T, Ni S, Zeng S. Determination of Serotonin enantiomers in rat liver microsomes by chiral high-performance liquid chromatography with fluorescence detection. Biomed. Chromatogram. 2005, 19, 191–195.
13. Chen J, Jiang X, Jiang W, Mei N, Gao X and Zhang Q. High-performance liquid chromatographic analysis of serotonin in human plasma using fluorescence detection. J. Pharm. Biomed. Anal. 2004, 3, 639-645.