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A study of Analytical Method development and validation for Quantitative analysis of Paracetamol by using UV Spectroscopy

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Abstract: ***Analytical process development and validation is essential for quantitative analysis of pharmaceutical products to maintain their quality, safety and efficacy. Ultraviolet (UV) spectroscopy is a widely used technique for the analysis of pharmaceutical substances due to its simplicity, sensitivity and ability to provide rapid results .UV spectroscopy is chosen due to its ability to detect compounds or drugs based on their absorption of ultraviolet light typically within the range of 200-400nm.Additionally the method is validated for accuracy , precision, specificity and reproducibility to meet regulatory standards. All the validation parameters were chosen according to ICH guidelines for analysis of any compound. An UV spectrophotometric method has been established for the quantitative analysis of paracetamol in tablet dosage forms.***

Keywords: UV Spectroscopy, analytical process development, validation parameters, ICH guidelines ,paracetamol.

I. INTRODUCTION

UV-visible (UV-Vis) spectroscopy is a widely used analytical technique employed for the qualitative and quantitative analysis of various chemical and pharmaceutical compounds. this technique is based on the absorption of ultraviolet or visible light by molecules, offering valuable information about molecular structure, concentration, and sample purity.the international conference on harmonisation has established guidelines specifies essential parameters for method validation, including accuracy, precision, specificity, linearity, limit of detection (lod), limit of quantification (loq), and robustness . these parameters help to ensure that the method can consistently produce accurate results across different conditions and with different samples. an uv spectrophotometric method has been established for the quantitative analysis of paracetamol in tablet dosage forms. the procedure was developed and validated using a mixture of ethanol and water as diluents.

A. UV Spectroscopy:

Ultraviolet-Visible (UV-Vis) spectroscopy is a analytical technique used to measure the absorption of ultraviolet (UV) and visible light by a chemical compound .UV spectroscopy generally operates within the wavelength range of 200 to 400 nanometers (nm), while visible spectroscopy ranges from 400 to 700 nm. This technique is especially useful for examining organic compounds which contain conjugated double bonds or aromatic rings or chromophores which readily absorb UV light. [1]

1) *Beer's Law:* When a monochromatic light is move through an absorbing medium its intensity is reduce exponentially as the concentration of absorbing medium increase.
2) *Lambert's Law:* When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases

The law describes the relationship between the absorbance (A) of light and the concentration (**c**) of the absorbing species. This relationship determined by the Beer-Lambert Law:

$$A=\varepsilon.c.l$$

Where:

- A = Absorbance (unitless),
- **ε** = Molar absorptivity (or molar extinction coefficient), which is a constant that represents how robustly a substance absorbs light at a specific wavelength ($L \cdot mol^{-1} \cdot cm^{-1}$),
- c = Concentration of the solution(mol/L),
- l = cuvette path length (cm).

3) Absorbance (A):

Absorbance indicates that how much light is absorbed by the sample at a specific wavelength. It is calculated as:

$$A = -\log (I_o / I_o)$$

Where:

- I = Intensity of the transmitted light.
- I_o = Intensity of the entering light.

4) Molar Absorptivity (ε):

- The molar absorptivity, **ε**, is a constant that depends on the substance being measured and the wavelength of light used. It is a measure of how robustly the substance absorbs light at that wavelength. The substance is absorb more light when the **ε** value is higher.
- **ε** values are determined experimentally and are specific to both the substance and the wavelength of light being used.

5) Concentration (c):

The absorbance is directly influenced by the concentration of absorbing substance in the sample solution .

6) Path Length (l):

The path length refers is the distance that light passas through the sample. In most cases, it is the length of the cuvette holding the sample, and typically it's **1 cm** in standard setups. A longer path length means that light interacts with more of the sample, leading to greater absorption.[2][3]

B. Method Validation According to ICH Guidelines:[4]

The ICH Q2(R1) guideline addresses the validation of analytical methods and aim to offer recommendations for validating various types of analytical procedure. It defines the validation process for qualitative and quantitative methods, as well as specific and non-specific methods.

The guideline is applicable to various types of methods, including chromatographic, spectroscopic, and immunoassay techniques, and it is designed to ensure that the methods used to test pharmaceutical products are accurate, reliable, and consisten.

Method validation assure the accuracy, reliability, and consistency of an analytical method for its desired purpose. It plays a vital role in confirming that the measurement procedure generates valid results and meets the requirements of good analytical practice. Validation is required before implementing a method into routine analysis, when there are significant changes in instrumentation, sample matrix, or when modifications are made beyond the original method scope. Some organizations like ASTM, ICH, FDA, FAO, CITAC, CCMAS, CEN, and EA provide standardized guidelines for method validation. Among them, the ICH Q2(R1) guideline is widely adopted, outlining principles for validating analytical procedures, which include sample preparation, reagent use, instrumentation, calibration, and calculation steps.

VALIDATION PARAMETERS ACCORDING TO ICH GUIDELINES :

- ACCURACY
- PRECISION
- LINEARITY
- Intermediate precision
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- SPECIFICITY
- Range
- Repeatability
- Robustness
- System suitability

C. Paracetamol

Paracetamol is also referred as acetaminophen. It is one of the most commonly used medication for relieving pain and reducing fever. It is widely available over-the-counter (OTC) and is commonly used to treat mild to moderate pain, such as headaches, toothaches, menstrual cramps, muscle aches, and minor aches and pains associated with the common cold or flu.Paracetamol is also frequently used to reduce fever in both adults and children.

It is often considered a first-line treatment for pain and fever due to its wide availability, ease of use, and favorable safety profile when used at recommended dosages. [5]

The onset of analgesia is approximately 11 minutes after oral administration of paracetamol,and its half-life is 1–4 hours. Although paracetamol is typically safe for use at recommended doses (upto 1000mg per dose and maximum 4000mg per day for adults),taking too much in short period can lead to serious liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. **[6]**

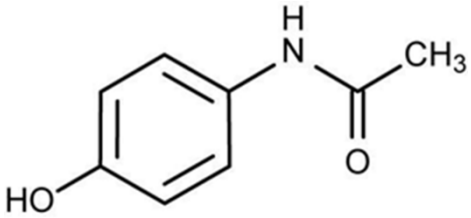


Figure1: Paracetamol ,*N*-(4-HYDROXYPHENYL)ACETAMIDE[7]

Chemical Structure and Properties : **[9][10][11]**

Chemical name of paracetamol is N-acetyl-p-aminophenol, and its molecular formula is $C_8H_9NO_2$.Paracetamol is a aniline group of compounds, and in their structure present one benzene ring with a hydroxyl group (-OH) and an amide group (-NHCOCH3) attached.

Molecular Weight: 151.16 g/mol

Solubility: It is slightly soluble in water but more soluble in organic solvents like alcohol or acetone.

Mechanism of action: The accurate mechanism of action of paracetamol is not completely known. Paracetamol is mainly considered as an analgesic and antipyretic rather than an anti-inflammatory agent. Inhibition of Cyclooxygenase (COX): Paracetamol acts primarily in the central nervous system (CNS) by inhibiting the COX enzyme, particularly COX-2. Recent research suggests that paracetamol may also interact with the endocannabinoid system in the brain, potentially enhancing its pain-relieving effects. Some studies have proposed that paracetamol may act on serotonergic pathways in the CNS, modulating pain transmission, though this effect is still under investigation. [8]

Pharmacokinetics of Paracetamol [6]

1) *Absorption:* Paracetamol is quickly absorbed through the gastrointestinal tract, reaching its highest concentration in the blood approximately 30-60 minutes after oral administration.
2) *Distribution:* It is widely distributed throughout the body, including the brain, and is capable of crossing the blood-brain barrier to exert its effects centrally.
3) *Metabolism:* Paracetamol is metabolized primarily in the liver, where it is converted into both non-toxic and toxic metabolites. The majority of the drug undergoes conjugation with sulfate and glucuronide, producing non-toxic metabolites. It is adhered that Glucuronidation is responsible for 40% metabolism of paracetamol , while sulfate conjugation may give approximately 20–40% . A small portion is metabolized by cytochrome P450 enzymes into a highly reactive intermediate metabolite, forming a minor yet significant alkylating metabolite known as NAPQI (N-acetyl-p-benzo-quinone imine).
4) *Excretion:* Paracetamol and its metabolites are mainly excreted through urine.

II. METHODOLOGY

A. *Aim:*

This method is helps to developed suitable spectrometric method for quantitative analysis of paracetamol tablet and perform the validation procedure .

B. *Materials And Methods:*

1) *Materials:* API paracetamol were provided by Gluconate Health Ltd. (label claim, paracetamol 650mg). Distilled water and ethanol were used as diluent. A 90:10 v/v mixture of water and methanol was used as the mobile phase for sample preparation and dilution.
2) *Diluent Preparation:* Water and Ethanol (90:10, v/v) used as a diluents.
3) *Preparation of Standard Stock Solutions*

10 mg of paracetamol was accurately weighed and then transferred to 100 ml volumetric flask. Small volume of mixture of Methanol: water(10:90) was added to dissolve the drug.The solution was then volume adjusted with same solvent mixture, which yielded stock solution with a concentration 100ppm.

- the stock solutions is preapred by using the $C_1V_1 = C_2V_2$ formula:

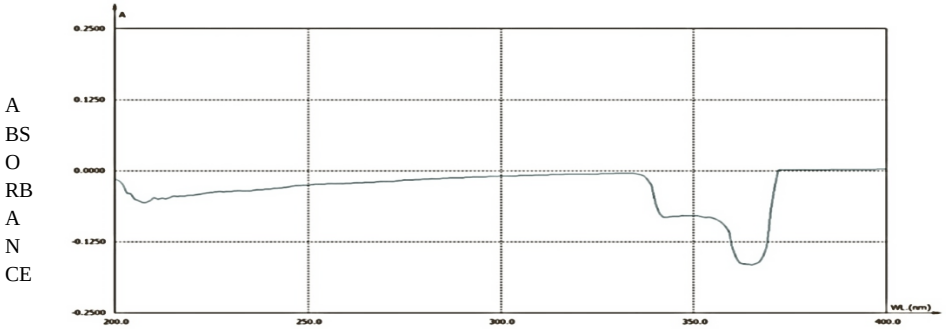
$$C_1V_1=C_2V_2$$

Where:

- C_1= Stock concentration (1000 µg/ml)
- V_1 = Volume of stock solution required
- C_2 = Desired final concentration
- V_2 = Final total volume

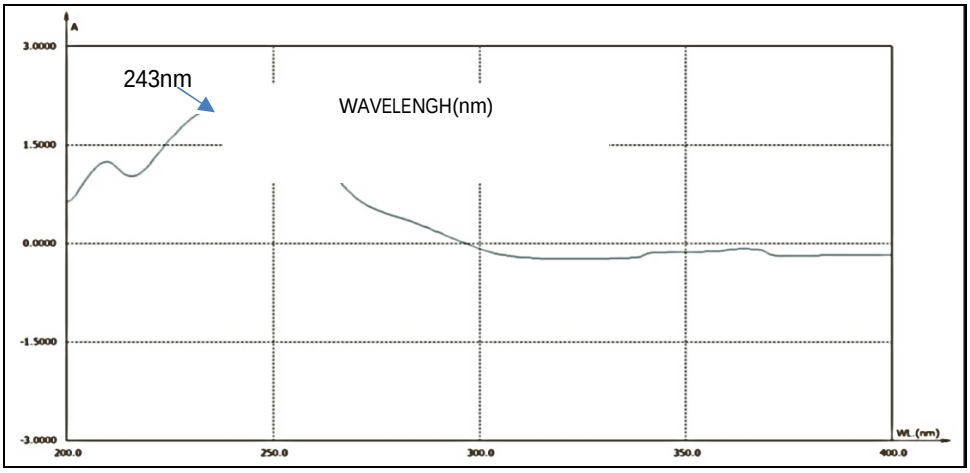
Various concentrations ranging from 50 µg/ml to 150 µg/ml were prepared for paracetamol using the 90:10 water: ethanol mixture as diluent. These solutions were used to develop calibration curves and assess method linearity and sensitivity.

4) *Test Preparation: At first* 20 tablets of paracetamol were weighed and finely powdered by using mortar and pestle. Powdered tablet equivalent to 100mg of paracetamol was weighed and taken into 100ml volumetric flask then add 10ml of ethanol and shaked well .After that 90ml of water was added to adjust the volume upto 100ml .1ml of solution was withdrawn from the prepared solution and take it in 100ml volumetric flask and make up the volume up to 100ml.
5) *Instruments and Apparatus:* A UV-Visible spectrophotometer (LABINDIA UV 2000U) was used for absorbance measurement, operating with a spectral bandwidth of 0.1 nm, wavelength accuracy of ±0.5 nm, and automatic wavelength correction. Additional equipment included a digital analytical balance,volumetric flasks (100 ml), measuring cylinders,Whatman filter paper ,glass rod, beaker.
6) *Selection of wavelength*: The standard solution was scanned between 200 to 400 nm, first using only diluent as a blank. Paracetamol shows it shows maximum absorbance at 243 nm.



ABSORBANCE

FIGURE 2: UV SPECTRUM OF BLANK



ABSORBANCE

WAVELENGTH(nm)

FIGURE 3: UV SPECTRUM OF PARACETAMOL IN SAMPLE SOLUTION

III. RESULT AND DISCUSSION

Validation of UV method for analysis of paracetamol:

1) *Accuracy:* Three levels of standard addition method are used to assess the accuracy .50%, 100% , 125% , target concentration should be added to the sample . The recoveries ranging from 98.64% to 99.11%, these results showed that the procedure is accurate .Table 2 displays the accuracy study .

% Recovery Level	% Recovery		Mean % Recovery		SD		%RSD	
75%	98.68		98.64		0		0	
	98.65				0.0057735		0.005854	
	98.60				0.00816497		0.008279	
100%	98.55		98.55		0.00693889		0.007041	
	98.53				0.00942809		0.009566	
	98.58				0.1503083		0.015251	
150%	99.10		99.11		0.00707107		0.007134	
	99.11				0.00707107		0.007134	
	99.14				0		0	

TABLE 1: Evaluation data for Accuracy study

2) *Precision:*The study of precision examines how closely values in a set of measurements taken from multiple samplings of the sa me homogeneous sample match up. To assess the precision repeatability and intermediate precision were used.

The relative standard deviation (%RSD) was calculated for each concentration and, the intra-day and inter-day %RSD values were within acceptable limits (<2%), indicating high reproducibility of the method.

REPEATABILITY:

Sample No.	% Assay
1	101.7
2	101.3
3	101.2
4	101.6
5	100.4
6	99.12
mean	101.1
SD	0.99
%RSD	0.97

TABLE 2: Evaluation data for repeatability study

INTERMEDIATE PRECISION:

Sample No.	% Assay	
SET	Intraday	Interday
1	101.7	99.4
2	101.3	98.5
3	101.2	99.0
4	101.6	99.1
5	100.4	98.2
6	99.12	100.2
mean	101.1	99.2
SD	0.99	0.77
%RSD	0.97	0.78

TABLE 3: Evaluation data for intermediate Precision study

3) Linearity: For this study, the linearity of paracetamol was assessed by preparing a series of working standard solutions at concentrations ranging from 50 to 150 µg/mL. The regression equation was found to be y = 0.2158x - 0.2215, with a correlation coefficient (R^2) of 0.9998, indicating good linearity

The high correlation coefficients signify that the method is linear across the selected concentration range for both drugs and can reliably quantify varying concentrations in formulations.

Concentration (ppm)	Absorbance
0	0
50	0.145
75	0.283
100	0.418
125	0.550
150	0.684

TABLE 4: Concentration vs. Absorbance for linearity study

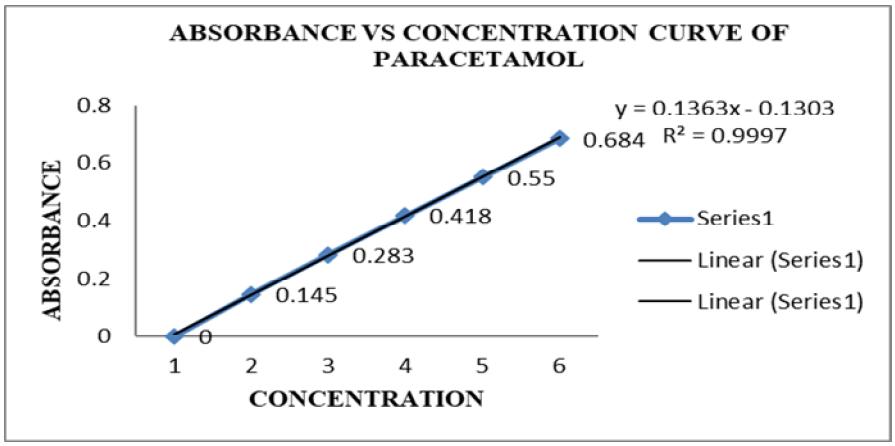


FIGURE 4: Linearity curve for paracetamol

C.Robustness: Robustness shows the reliability of the process with respect to deliberate changes in the parameters of the method. Precautionary steps should be included in the procedure if the findings are susceptible to various analytical condition.

Here robustness is calculated by deliberately changing the absorption wavelength for the sample.

SL NO.	242nm	243nm	244nm
1	0.65	0.653	0.657
2	0.65	0.653	0.656
3	0.651	0.653	0.656
4	0.651	0.653	0.657
5	0.651	0.654	0.657
6	0.652	0.654	0.657
Mean	0.65083	0.653333	0.656667
SD	0.00073	0.000514	0.000514
%RSD	0.115663	0.079045	0.078633

TABLE 5: Evaluation data for Robustness study

IV. RESULTS

The result of validation parameters like linearity, accuracy, precision and robustness of UV method was found with in limit . Percent recovery of paracetamol was found 99.42% ± 0.31 . % RSD of all precision study were less than 2%. So the proposed UV spectroscopic method is accurate, precise and robust.

V. CONCLUSION

For the assay of paracetamol , this validation method is developed . By using UV spectroscopy this method was successfully validated for accuracy, precision, linearity, and robustness as per ICH guidelines. The method demonstrated strong linearity over the selected concentration range, excellent precision with %RSD < 2%, and high accuracy with recovery rates close to 100% and rodust.So concluded that this analytical method can be used for intended purpose .

Conflict of Interest: Nil

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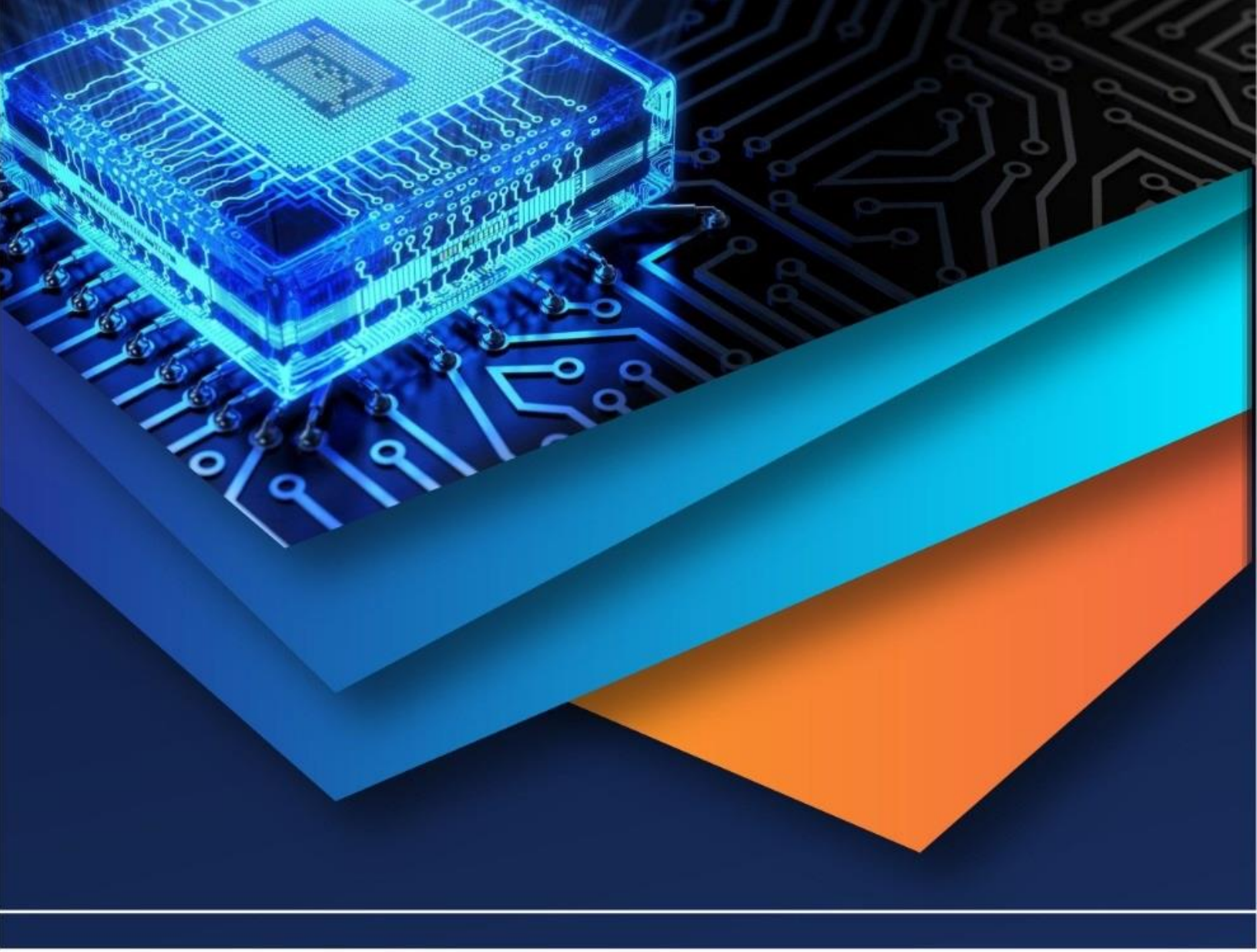

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