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Development and Validation of UV-Visible Spectrophotometric Method for the Analysis of Cefepime in Pharmaceutical Formulations

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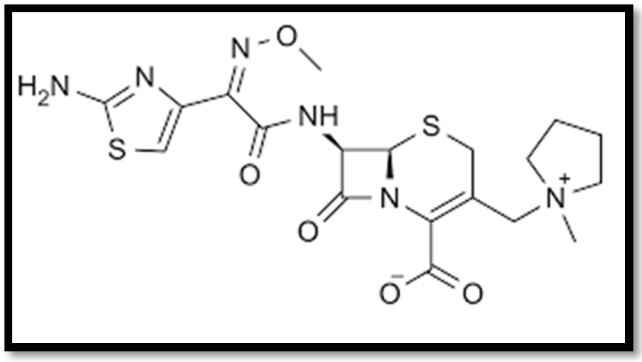
Abstract: ***A simple, sensitive, selective rapid spectrophotometric method has been developed for the determination of Cefepime in pure form and pharmaceutical formulations based on the oxidative coupling reaction with MBTH reagent, at*** P^H***–4.0 which is extractable at 610 nm. Beer's law is obeyed in the concentration ranges 5-30 µg*** ml^{-1}***. The developed method was applied directly and easily for the analysis of the Pharmaceutical formulations. R.S.D was found to be 0.2604% and Recovery 98.65% respectively. The method was completely validated and proven to be rugged. The interferences of the other ingredients and excipients were not observed. The repeatability and the performance of the proved method were established by point and internal hypothesis and through recovery studies.***

Keywords: Spectrophotometry, Cefepime, Oxidative coupling reaction, MBTH / $FeCl_3$

I. INTRODUCTION

Cefepime is chemically 6R,7R,Z)-7-(2-(2-aminothiazol-4yl(methoxyimino)acetamido)-3-((1-methylpyrrolidinium-1yl)methyle)-8-oxo-5-thia-1-aza-bicyclo(4.2.0)oct-2-ene-2-carboxylate. Cefepime is a fourth-generation cephalosporin antibiotic. Cefepime has an extended spectrum of activity against Gram-positive and Gram-negative bacteria, with greater activity against both types of organism than third-generation agents. Cefepime injection is used to treat bacterial infections in many different parts of the body. It belongs to the class of medicines known as cephalosporin antibiotics. It works by killing bacteria or preventing their growth. However, this medicine will not work for colds, flu, or other virus infections. The emperícala formula of Cefepime is $C_{19}H_{24}N_6O_5S_2$ and the molecular weight is 480.54grams.It has the following structure :

Figure1.



There is however no reported UV- Visible spectrophotometric method for the analysis of Cefepime in its technical grade and formulations. UV- Visible spectrophotometric method for the quantitative determination of Cefepime. Functional group used for color development of Cefepime was primary amine group. The results obtained in this method was based on complex formation reaction of Cefepime with Oxidative coupling reaction with MBTH .

In the present study an attempt has been made to develop simple UV-Visible spectrophotometric method for quantitative estimation of Cefepime in its technical grade, formulations and biological sample (Blood). The functional group used for the color development of Cefepime was primary amine. The result obtain in this method was based on coupling reaction formation reaction of Cefepime with MBTH/ $Fecl_3$. An attempt has been made to develop and validate to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in various gradients.

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II. MATERIALS AND METHODS

A. *Preparation Of Standard Calibration Curve Of Pure Drug*

1) Solvent: Dimethyle Sulfoxide was used as Solvent.

2) Preparation of standard stock solution: Accurately weighed 100 mg of Cefexime was dissolved in 40 ml of Dimethyle Sulfoxide l in 100 ml volumetric flask and volume was made up to the mark with Dimethyle Sulfoxide . i.e. 1000 µg ml-1 (Stock solution A) From the above stock solution A 10 ml of solution was pipette out into 100 ml volumetric flask and the volume was made up to the mark with Dimethyle Sulfoxide to obtained the final concentration of 100 µg ml-1 (Stocksolution B)

3) Preparation of Calibration curve: Fresh aliquots of Cefepime ranging from 0.5 to 3ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 5 to 30 µg ml^{-1}. To each flask 1ml of (0.01M) MBTH solution was added followed by 1ml of (0.7%) Ferric chloride solution and resulting solution was heated for 15 min and finally 1ml (0.5N) Hydrochloric acid solution was added. The solutions were cooled at room temperature and made up to mark with Dimethyle Sulfoxide. The absorbance of orange red colored chromogen was measured at 610 nm against the reagent blank. The color species was stable for 24 h. The amount of Cefepime present in the sample solution was computed from its calibration curve.

4) Procedure for formulations: Twenty tablets containing Cefepime were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Cefepime was dissolved in a 100 ml of Dimethyle Sulfoxide and mixed for about 5 min and then filtered. The Dimethyle Sulfoxide was evaporated to dryness. The remaining portion of solution was diluted in a 100 ml volumetric flask to the volume with Dimethyle Sulfoxide up to 100 ml to get the stock solution A. 10 ml of aliquots was pipette out into 100 ml volumetric flask and the volume was made up to the mark with Dimethyle Sulfoxide to obtained the final concentration of 100µg ml^{-1} (Stock solution). Subsequent dilutions of this solution were made with Dimethyle Sulfoxide to get concentration of 5 to 30 µg ml^{-1} and were prepared as above and analyzed at the selected wavelength, 610 nm and the results were statistically validated.

5) Procedure for Blood sample: After collection of Blood sample it will be centrifuged. For isolation of Cefepime from plasma sample, Dimethyle Sulfoxide was used for protein precipitation. Liquid- Liquid extraction was performed with plasma by alkalinization with 1M NaOH, followed by extraction with 30% dichloromethane in Hexane. The upper organic layer was evaporated to dryness, the dry residue 100 mg was dissolved in 100 ml of Dimethyle Sulfoxide (1000 µg ml^{-1}). From the above solution 10 ml is taken into a 100 ml of Volumetric flask and made up to the mark with Dimethyle Sulfoxide .(100 µg ml^{-1})

From the above solution ranging from 0.5-3 ml (5-30 µg ml^{-1}) were transferred in to 10 ml Volumetric flask and to the each flask 1ml of (0.01M%) MBTH solution was added followed by 1ml of (0.7%) Ferric chloride solution and made up to the mark with Dimethyle Sulfoxide. Then the resulting solution was heated and finally 1ml (0.5N) Hydrochloric acid solution was added. The solutions were cooled at room temperature and made up to the mark with Dimethyle Sulfoxide. The absorbance of orange red colored chromogen was measured at 610 nm against the reagent blank. The color species was stable for 24 h. The amount of Cefepime present in the sample solution was computed from its calibration curve.

Fig-1: Absorption spectrum of Cefepime With MBTH /$FeCl_3$

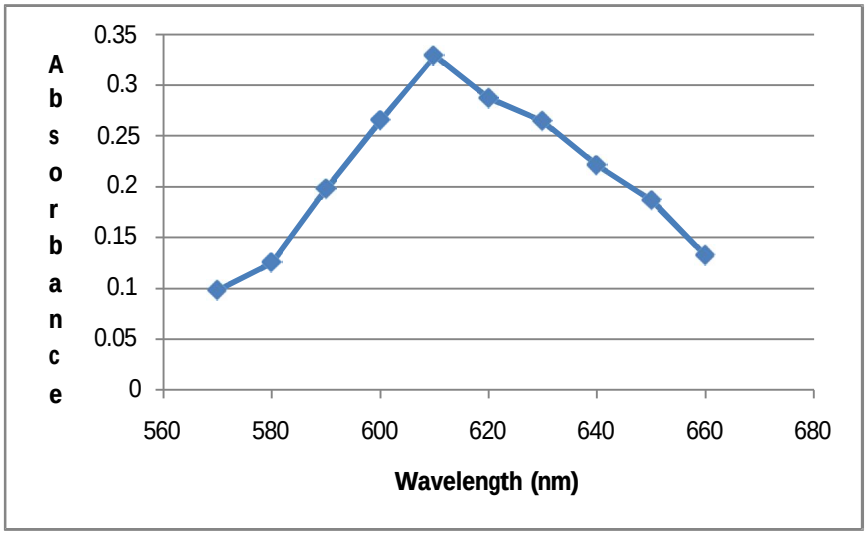

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Fig-2: Beer's law plot of Cefepime With MBTH/FeCl3

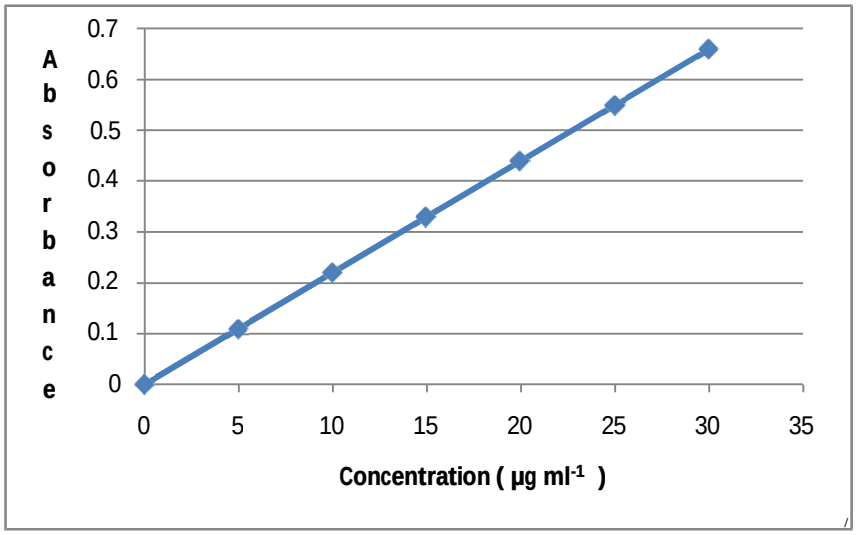

Fig-3: Beer's law plot for MBTH in Blood sample

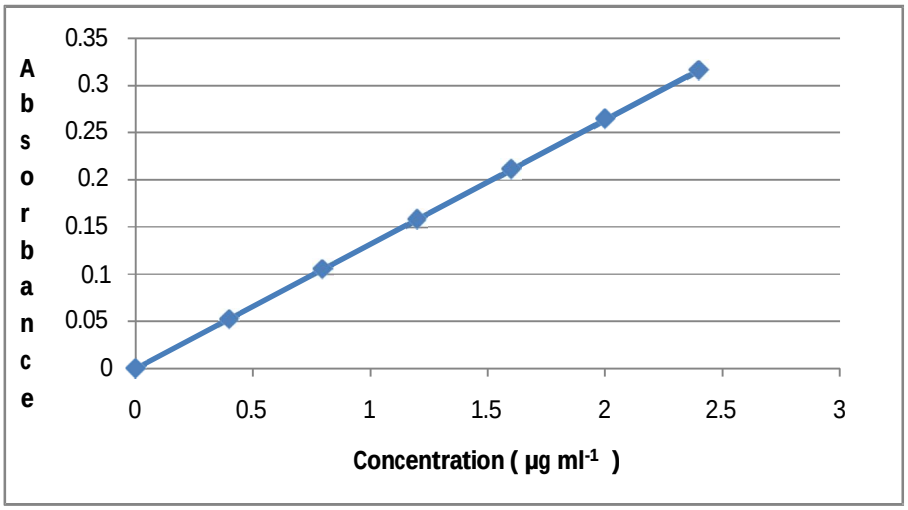

Fig-4: A Schematic reaction Mechanism of Cefepime With MBTH

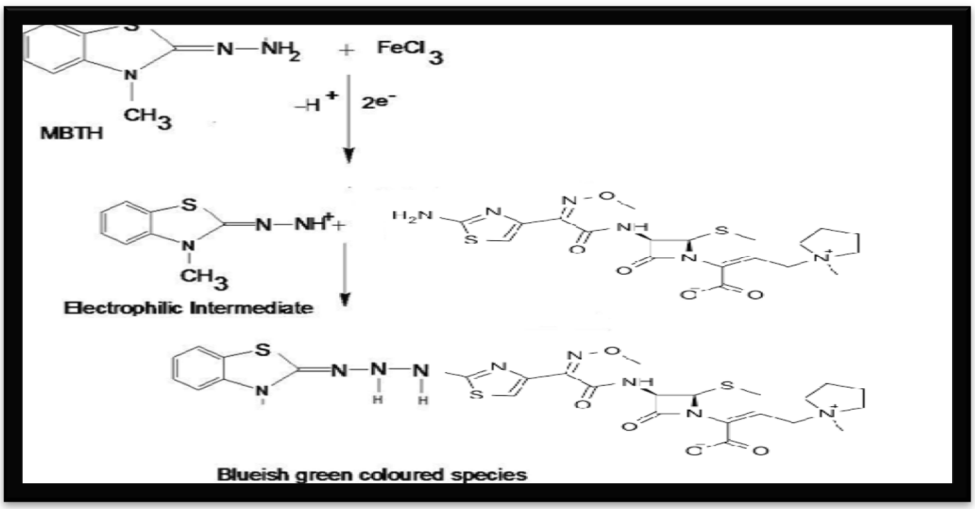

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Table-1.1: Optical characteristics and precision by MBTH

Parameter	**Visible method**
Color	Green
Absorption maxima (nm)	610
Beer's law limits (µg ml^{-1})	5-30
Molar absorptivity (l $mol^{-1}cm^{-1}$)	5.263×10^{4}
Sandell's Sensitivity (µg cm^{-2})	0.0452
Regression equation (Y*)	
Slope (b)	0.022
Intercept(a)	0.001
Standard deviation(SD)	0.001
Correlation coefficient (r^2)	0.9999
%RSD (Relative Standard deviation)	0.2604
Range of errors	
Confidence limits with 0.05 level	0.0008
Confidence limits with 0.01 level	0.0010
Limits of detection (LOD)(µg ml^{-1})	0.1363
Limits of quantification (LOQ) (µg ml^{-1})	0.4545

RSD of 6 independent determinations

Table-1.2: Assay results of Cefepime in formulations by visible Method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method (mg)	% Recovery
CEFIXIME	250	249.56 t=0.0029 F=8.5897	246.25	98.65
MAXIPIME	250	249.98 t=0.0028 F=8.5796	245.5	98.17

t and F- values refer to comparison of the proposed method with reference method.
Theoretical values at 95% confidence limits t= 0.00297 and F= 7.6177

Table-1.3: Determination of accuracy of Cefepime

Amount of CEF in formulation (mg)	Amount of Standard CEF added (mg)	Total amount Found (mg)	% Recovery
249.16	200	448.48	99.66
247.91	200	446.23	99.16
248.64	200	447.55	99.45
248.16	250	496.32	99.26
249.30	250	498.6	99.72
248.32	250	496.64	99.32
248.47	300	546.63	99.38
248.83	300	547.42	99.53
247.91	300	545.40	99.16

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Table-1.4: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
248.57	0.6279	0.2526
248.59	0.6171	0.2482
248.40	0.4636	0.1866

The results are the mean of three readings at each level of recovery.

Table-1.5: Repeatability data for CEF at 610 nm

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%)RSD
5	0.108	0.109	0.105	0.107	0.0002	0.1869
10	0.219	0.218	0.212	0.216	0.0003	0.1388
15	0.329	0.326	0.327	0.327	0.0001	0.0305
20	0.439	0.437	0.439	0.438	0.0001	0.0228
25	0.549	0.546	0.543	0.546	0.0003	0.0549
30	0.659	0.654	0.653	0.655	0.0003	0.0458

Average of six determinations.

Table-1.6: Color stability data for MBTH Method

Conc. in µg ml^{-1}	Time in Hours							
20	4	8	12	16	20	24	28	32
	0.439	0.439	0.439	0.440	0.441	0.441	0.398	0.295

Table-1.7: Assay results of Cefepime in Blood sample

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method in (mg)	Amount found by the reference method (mg)	% of Recovery
CEFIXIME	5	2.9 t=0.0029 F=0.0004	2.85	95.78
MAXIPIME	5	2.99 t=0.0027 F=0.00039	2.86	95.45

tand F values refer to comparison of the proposed method with reference method.
Theoretical values at 95% confidence limits t=0.00796 and F=0.0029.

Table-1.8: Determination of accuracy of Cefepime

Name of the Formulation in (mg)	Amount of Drug in Blood sample (mg)	Amount of Standard Drug added in (mg)	Total amount found (mg)	% Recovery
5	2.99	5	5.98	59.8
5	2.98	5	5.96	59.6

The results are the mean of two readings at each level of recovery.

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Table-1.9: Repeatability data for Cefepime at 610nm

Concentration in µg ml^{-1}	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
0.4	0.052	0.051	0.052	0.051	0.0005	0.9803
0.8	0.105	0.104	0.102	0.103	0.0015	0.9708
1.2	0.158	0.156	0.156	0.156	0.0011	0.7051
1.6	0.211	0.213	0.219	0.214	0.0004	0.1869
2.0	0.264	0.267	0.263	0.264	0.0020	0.7575
2.4	0.316	0.319	0.317	0.317	0.0015	0.4731

Average of six determinations

III. RESULTS AND DISCUSSION

A. Optical Parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV spectrophotometric method and of the colored species formed in each so the visible spetrophotometric method, specified amount of Cefepime in solution 5-30 µg ml^{-1} were taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 380-800 nm against corresponding reagent blank. The regent blank absorption spectrum of each method was also recorded against distilled water / Dimethyle Sulfoxide. The results are graphically represented in (fig- 1).

B. Parameters fixation

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development MBTH Method reproducibility and reasonable period of stability of final colored species formed. The following studies were conducted.
Method: The results obtained in this method were based on oxidation followed by coupling reaction of Cefepime with MBTH, Ferric chloride and Orthophosphoric acid to form an green colored chromogen that exhibited maximum absorption at 610 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of Cefepime with MBTH reagent was shown in (fig-4). The effect of various parameters such as concentration and volume of MBTH and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameters at a time.

C. Optical Characteristics

The reference method adhere to beer's law the absorbance at appropriate wave length of a set of solutions contains different amounts of Cefepime and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank. The beers law plot of the system illustrated graphically (fig: 2) least square regression analysis was carried out for the slope. Intercept and Correlation Coefficient. Beer's law limits, Molar absorptivity & Sandells sensitivity for Cefepime with each of mentioned reagents was calculated. The optical characteristics were present in the table- 1.1. In order to test whether the colored species formed in the method adhere the beer's law the absorbance at appropriate wavelength of a set of solutions contain different amounts of Cefepime and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The beers law plots of the system illustrated graphically (fig -2 & 3) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity Sandells sensitivity for Cefepime with each of mentioned reagents were calculated. The optical characteristics are presented in the table – 1.1.

D. Precision

The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtain by actual determination of a fixed amount of Cefepime n (10, 5 µg ml^{-1} respectively) in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and presented in tables – 1.1.

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E. Analysis of formulations

Commercial formulations of Cefepime were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the t and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in table-1.2. The proposed methods also applied for Biological Samples (Blood) for good recoveries are obtained which were recorded in table-1.7.

F. Accuracy

Recovery studies were carried by applying the Standard addition method to Drugs sample present in formulations for the known amount of Cefepime the recovery studies were carried .By applying the same method to Biological sample (Blood) to which known amount of Cefepime correspond to 2 mg Formulations taken by the patient. By the follow of Standard addition method 2 mg of label claim was added. After the addition of these standards the contents were transferred to 100 ml volumetric flash and dissolved in solvent. Finally the volume was made up to the mark with solvent. The solution was filtered through Whitman No. 41filter paper. The mixed sample solutions were analyzed and their absorbance value was determined. At each level of recovery five determinations were performed and present in Table – 1.3. The results obtain were compared with expected results and were statistically validated in Table – 1.4.

G. Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in sample with in a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

H. Specificity and Selectivity

Specificity is a procedure to detect quantitatively the analyze in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyze qualitatively in presence of components that may be expected to present in the sample matrix. The excipient in formulations was spiked in a pre-weighed quantity of Drugs and then absorbance was measured and calculations were done to determine the quantity of the Drugs.

I. Repeatability

Standard solutions of Cefepime were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure six times and standard deviation was calculated and presented in table -1.5.

J. Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Cefepime under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in formulations.

K. Stability of the color for MBTH Method

The color was developed using 5 µg/ml of Cefepime solution and 1ml of (0.01M) MBTH solution was added followed by 1ml of (0.2%) Ferric chloride solution and resulting solution was heated at 100 ^{0} c for 15 min. The solutions were cooled to room temperature and added 1ml (0.2M) of orthophosphoric acid solution and made up to mark with distilled water in 10 ml volumetric flask. The absorbance of Bluish green colored species was measured at 610 nm against the reagent blank.

IV. CONCLUSION

The proposed method can be used for determination of Cefepime in Formulations. The method is rapid, simple and has great sensitivity and accuracy. Proposed method makes use of simple reagents, which an ordinary analytical laboratory can afford. Method is sufficiently sensitive to permit determination even down to 10µg ml^{-1} . The proposed method is suitable for routine determination of Cefepime in its formulation and Blood. The commonly used additives such as Starch, Lactose, Titanium dioxide, and Magnesium state do not interfere with the assay procedures.

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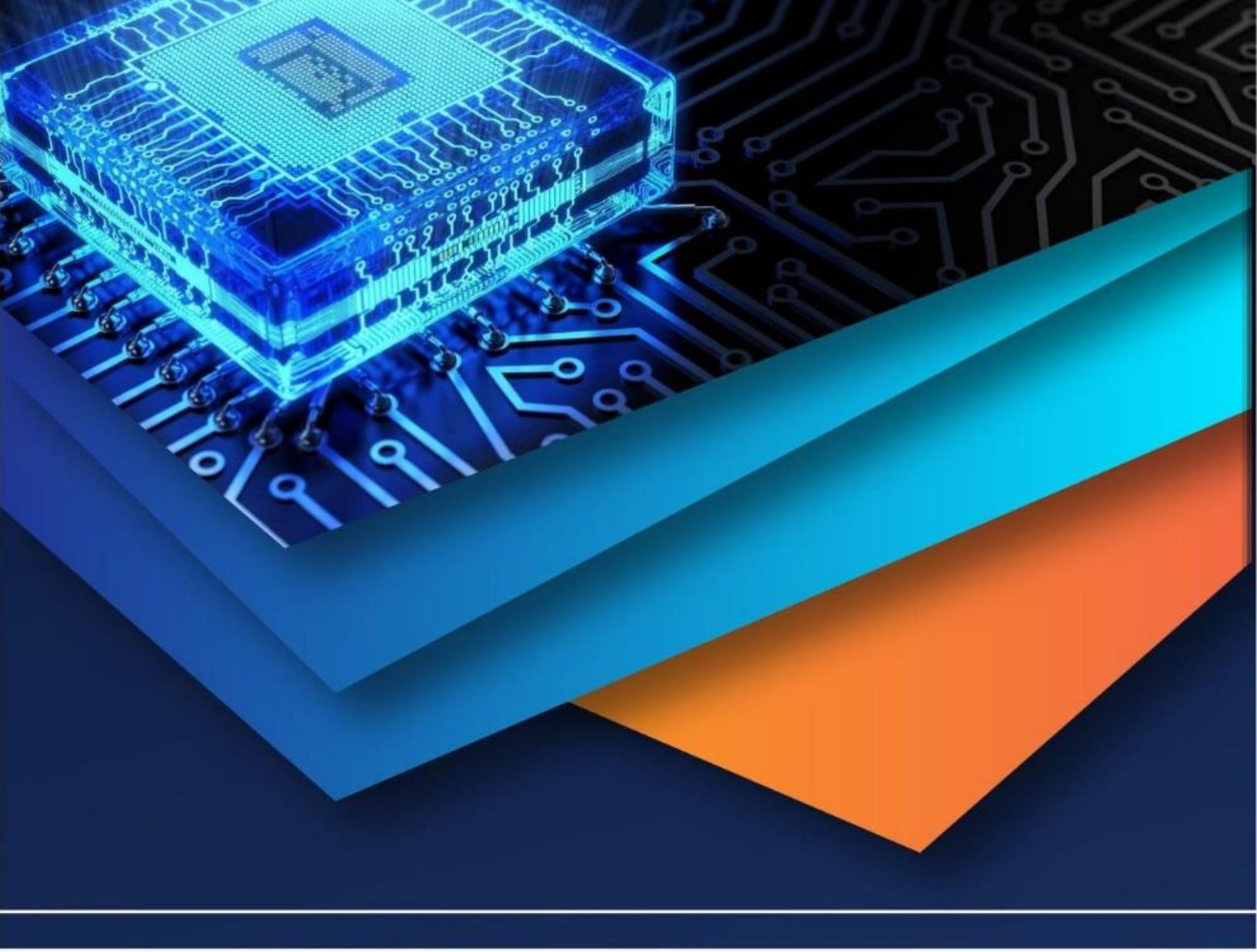

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