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Development and Validation of GCMS method for the Detection and Quantification of Potential Genotoxic Impurity Ethyl 4-bromobutyrate in Tolvaptan Tablets

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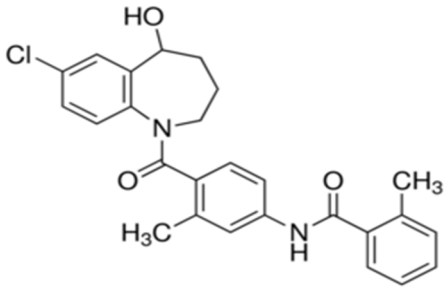
Abstract: ***A simple, sensitive rapid Gas Chromatography with mass spectrometry (GC-MS) method has been developed and validated for the determination of Ethyl 4-bromobutyrate content in Tolvaptan Tablets. Ethyl 4-bromobutyrate is a potential genotoxic impurity. The lower level of detection was achieved on fused silica capillary column Thermo Scientific™ Trace GOLD™ TG-5SilMS, 15m x 0.25mm x 0.25µm (Part number 26096-1300) coated with Low polarity phase, 5% diphenyl/95% dimethyl polysiloxane stationary phase with Electron Impact ionization (EI) in Selective Ion Monitoring (SIM) mode. GC runtime was 21.45 min employing programmed temperature with splitless mode using liquid injection technique. The developed method was validated for specificity, linearity, accuracy and precision. The detection and quantitation limits of Ethyl 4-bromobutyrate obtained were 0.03 µg/g and 0.10 µg/g respectively. The method was found to be linear in the range between 0.10 µg/g and 50.0 µg/g with correlation coefficient 0.9981. The average recovery obtained in Tolvaptan Tablets was 97.3 %. The developed method was found to be rugged for the determination of Ethyl 4-bromobutyrate in Tolvaptan Tablets. The detailed approach of experiments is discussed in the paper.***

Keywords: Ethyl 4-bromobutyrate, Tolvaptan Tablets, Gas chromatograph with mass spectrometer

I. INTRODUCTION

Solvents which are used in the manufacture of drug substances and are not completely removed by practical manufacturing techniq ues should be controlled to the extent possible as they do not provide therapeutic benefit and are harmful to human health [1,2]. Tol vaptan is chemically known as N-{4-[(5R)-7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1H-1-benzazepine-1-carbonyl]-3-methylphenyl} -2-methylbenzamide (Figure 1) having molecular weight 448.941 and chemical formula $C_{26}H_{25}ClN_2O_3$. Tolvaptan belongs to the cla ss of organic compounds known as benzanilides. These are aromatic compounds containing an anilide group in which the carboxa mide group is substituted with a benzene ring. [3].

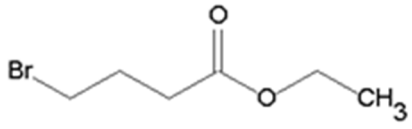
Figure. 1. Chemical structure of Tolvaptan



Tolvaptan is used to treat low blood sodium levels (hyponatremia) associated with various conditions like congestive heart failure, cirrhosis, and syndrome of inappropriate antidiuretic hormones (SIADH). Tolvaptan was FDA approved on May 19, 2009. Tolvaptan is a selective and competitive arginine vasopressin receptor 2 antagonist. Vasopressin acts on the V2 receptors found in the walls of the vasculature and luminal membranes of renal collecting ducts. By blocking V2 receptors in the renal collecting ducts, aquaporins do not insert themselves into the walls thus preventing water absorption. This action ultimately results in an increase in

urine volume, decrease urine osmolality, and increase electrolyte-free water clearance to reduce intravascular volume and an increase serum sodium levels. Tolvaptan is especially useful for heart failure patients as they have higher serum levels of vasopresin [4]. Ethyl 4-bromobutyrate (E4B) is one of the key raw materials used in the synthetic process of Tolvaptan. Ethyl 4-bromobutyrate is used as a Raw material in the manufacturing process of Tolvaptan, Hence, there is a need to prove that the levels of Ethyl 4-bromobutyrate a potentially genotoxic impurity is below 1.5 µg/day based on the maximum daily dose (MDD) of the drug. The limit for Ethyl 4-bromobutyrate potentially genotoxic impurity is found to be 25 ppm taking into account the TTC approach of the ICH guideline for genotoxic impurities and based on the maximum daily dose i.e. 60 mg/day of Tolvaptan Low levels of Ethyl 4-bromobutyrate may be present in the final drug substance as residual impurity. Ethyl 4-bromobutyrate comes under potential carcinogenic impurity as per its structure as primary alkyl halides. The chemical structure of Ethyl 4-bromobutyrate is given in Figure 2.

Figure 2. Chemical structure of Ethyl 4-bromobutyrate



Most of the countries have their own specific guidelines for testing of pharmaceuticals for genotoxicity. As per regulatory requirements, the carcinogenicity and mutagenicity testing of a molecule is required, when the compound or its metabolite is structurally related to a known carcinogen or when the nature and action of the drug suggest a mutagenic/carcinogenic potential [5]. Ethyl 4-bromobutyrate (E4B) is reasonably anticipated to be as human carcinogen based on sufficient evidences on its potential carcinogenicity. Tolvaptan tablets are for oral administration. Tolvaptan tablets are supplied in two dosage strengths containing Tolvaptan equivalent to 15 mg and 30 mg. Tolvaptan is generally considered safe and its maximum daily dose is 60 mg. [6]. The impurity Ethyl 4-bromobutyrate should not be present in drug substance or should be less than 25 µg/g as per TTC approach based on daily dosage of drug substance [7]. Therefore, keeping the view of the impurity level, we have chosen GCMS instead of GC. Review of some of the analytical methods already available in literature for the determination of Ethyl 4-bromobutyrate. To the best of our knowledge, no method has been reported for the determination of Ethyl 4-bromobutyrate in the Tolvaptan tablets by GCMS in literature till date. This paper describes the development, optimization and validation of GCMS method in accordance with ICH guideline Q2(R1) and United States Pharmacopeia.

II. MATERIALS AND METHODS

A. Chemicals, reagents and samples

The Investigated samples of Tolvaptan tablets were procured from Lupin. (Resodim 15 mg tablets). Analytical reagent GCHS Methanol was procured from Thermo Fischer, Ethyl 4-bromobutyrate procured from Sigma Aldrich.

B. Equipment

The gas chromatograph system and mass spectrometer was a Thermo Fischer TRACE™ 1310 Gas Chromatograph with ISQ LT Single Quadrupole Mass Spectrometer equipped with Thermo Fischer Tri Plus RSH Autosampler (Make: Thermo Fischer) was used. The data handling system Xcalibur™ Software was used to monitor the output signals and for processing. Capillary GC column Thermo Scientific™ Trace GOLD™ TG- 5SilMS, 15m x 0.25mm x 0.25µm (Part number 26096-1300) was used in this study.

C. GCMS Chromatographic Conditions

The analysis was carried out on a Thermo Scientific™ Trace GOLD™ TG-5SilMS, 15m x 0.25mm x 0.25µm (Part number 26096-1300) GC Column. Helium gas was used as carrier gas, maintaining Column flow at 1.0 mL/min, constant flow in a splitless mode. The temperature of the capillary injector was set as 300°C and column oven temperature was programmed as initially 70°C maintained for 1.0 min, then raised to 300°C at a rate of 15°C per minute held for 5.0 min.

MS transfer line temperature	:	260 °C
Ion source temperature	:	250 °C
Ionization mode	:	EI

Oven Method		
Initial temperature	:	70.0 °C
Initial hold time	:	1.00 min
Number of ramps:	:	1
Ramp 01 rate	:	15.0 °C/min
Ramp 01 final temperature	:	300.0 °C
Ramp 01 hold time	:	5.00 min
S/SL - Front Method		
S/SL mode	:	Split
Temperature	:	300 °C
Split flow	:	5.0 mL/min
Purge flow	:	5.0 mL/min
Carrier flow	:	1.000 mL/min
Sampling		
Sample volume (µl)	:	1.00

The mass parameters were set as follows.

Group & Events						
Start time	End time	Avg. Mode	Event time (sec)	Channel (m/z)	Channel (m/z)	Channel (m/z)
6 min	24 min	SIM	0.30	88 amu	151 amu	121 amu

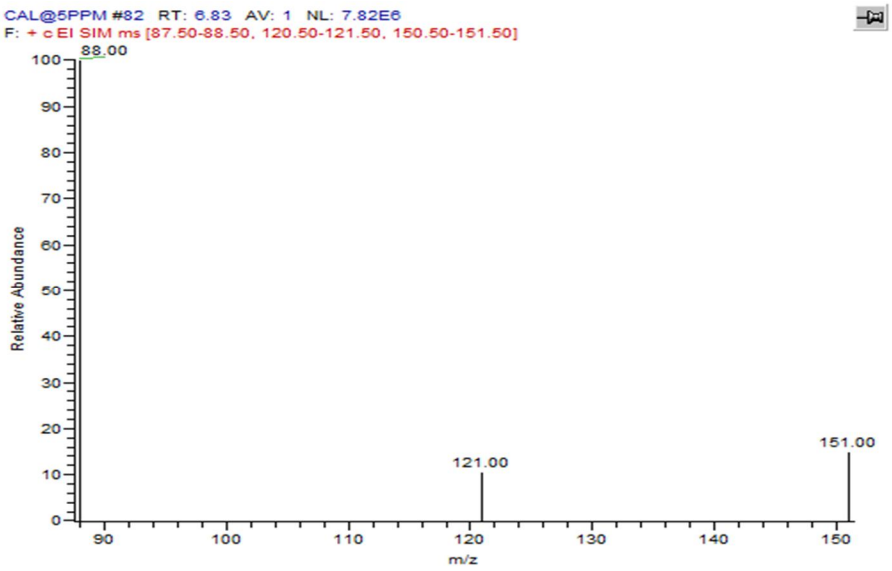
Ion source temperature & Interface temperature - 250°C, Threshold-200 and detector voltage is relative to the tuning result.
The liquid autosampler sample injection volume was 1.0 µl.
Preparation of solutions Blank solution Methanol GCHS Grade Standard stock solution Accurately weigh and transfer about 100 mg of Ethyl 4-bromobutyrate into a 100 mL clean, dry volumetric flask containing about 50 ml of Methanol, mix and make up to volume with Methanol. Dilute 1.0 ml of this solution to 100 ml with Methanol. Standard solutionFurther dilute 5.0 ml of this solution to 100 ml with Methanol. Sample solution Accurately weigh and transfer about 200 mg of crushed tablet powder into a 10 mL clean, dry volumetric flask. Add 5.0 ml of Methanol mix and make up to volume with Methanol.

III. RESULTS AND DISCUSSION

A. Method Development And Optimization

The challenge was to achieve the detection and quantitation at low level using the Gas Chromatography Mass Spectrometry (GC-MS) to acquire the good separation with desired sensitivity. In this study, Thermo Scientific™ Trace GOLD™ TG-5SilMS, 15m x 0.25mm x 0.25µm (Part number 26096-1300) GC Column was employed for the GC-MS analysis. This column was chosen because of low polar stationary phase, which is suitable for retaining Ethyl 4-bromobutyrate and resolving other analytes from Ethyl 4-bromobutyrate. Electron impact ionization (EI) mode was selected because EI is generally more robust and easy to transfer when compared to other GC-MS ionization techniques/mode. Development trials were initiated on autosampler technique. Standard solution (0.5µg/g) of Ethyl 4-bromobutyrate was prepared in Methanol with respect to sample concentration (20 mg/ml) and standard solution was transferred in to a autosampler vial and sealed the vial by screwing on the screw cap. The standard was injected through Tri Plus RSH Autosampler auto injector in to GC-MS (in scan mode). After completion of acquisition, the retention time of Ethyl 4-bromobutyrate was extracted with the help of National Institute of Standards and Technology (NIST) library target analyte channels [mass-to-charge ratio(m/z)]. The spectrum of Ethyl 4-bromobutyrate extracted from NIST library is given in Figure 3.

Figure 3. Spectrum of Ethyl 4-bromobutyrate (Extracted from NIST library)



There was no interference observed between sample matrix and analyte peak.

The effect of initial column temperature on the separation of Ethyl 4-bromobutyrate with other analytes was investigated. The initial column temperature was varied from 40°C to 70°C in the splitless mode. The results show that the peak shape and separation of each analytes was satisfactory when the initial column temperature was kept as 70°C comparatively to other temperatures. The optimized method was validated as per ICH guideline Q2(R1) [8].

B. Validation

Specificity As per ICH, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Injected Blank, standard Solution, Sample solution and Specificity mixture solution into GCMS and monitored the responses in SIM mode. Recorded the retention times and peak responses of all peaks. Checked the interference of Blank and Ethyl 4-bromobutyrate. The typical chromatogram of Standard solution is given in Figure 4.

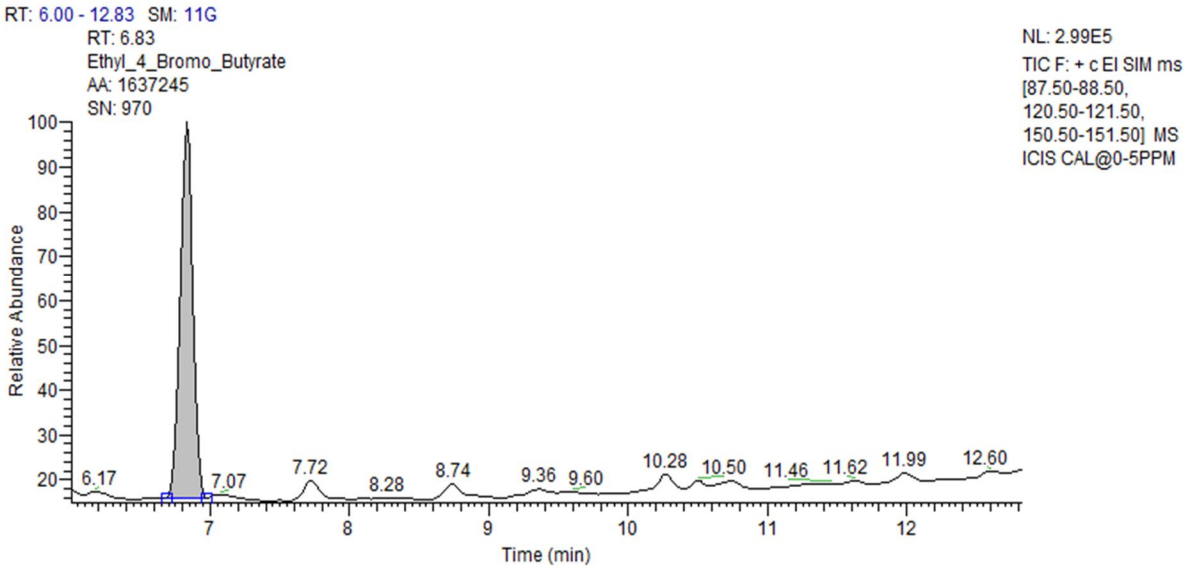


Figure 4. Typical GCMS total ion chromatogram of a) Standard solution,

Limit of detection and Limit of quantitation For determining LOD and LOQ, standard solution with increasing concentrations was prepared and injected into the system and determined the areas. Graph of concentration vs. area is plotted and limit of detection and limit of quantitation are calculated based on following formula.

$$LOD = \frac{STEYX \times 3.3}{Slope} \qquad LOQ = \frac{STEYX \times 10}{Slope}$$

The predicted concentrations of LOD and LOQ of Ethyl 4-bromobutyrate was verified for precision by preparing the solutions containing at about predicted concentrations and injected each six times into GCMS and calculating the %RSD of peak areas. The LOD/LOQ values are given in Table 1.

C. Linearity

A series of solutions were prepared using Ethyl 4-bromobutyrate at concentration levels from LOQ to 50 (µg/g) and each solution was injected. Calculated the statistical values like Correlation coefficient from linearity plot drawn for concentration versus area. The statistical data is given in Table 1.

Figure 5. Linearity plot for Ethyl 4-bromobutyrate

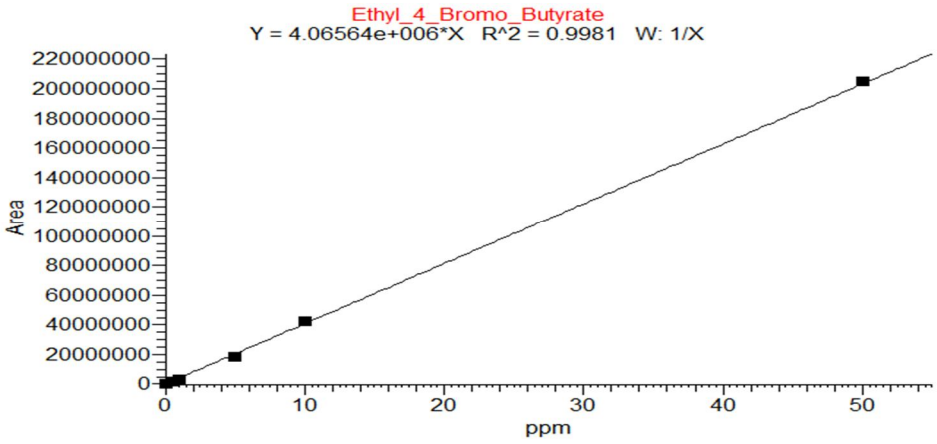


Table 1. Statistical data of linearity, LOD/LOQ for Ethyl 4-bromobutyrate

Statistical parameters	Results
Correlation coefficient	0.9981
Concentration range (µg/g)	0.1 to 50 (µg/g)
Limit of detection (µg/g)	0.03
Limit of quantification (µg/g)	0.10
Precision for Limit of Detection (%RSD	2.82 %
Precision for Limit of Quantification (%RSD)	1.72 %
Calibration points	5

D. Precision

The Precision (system precision) was evaluated by injecting six injections of standard solution of Ethyl 4-bromobutyrate in to GCMS and calculating the % relative standard deviation (RSD). The method precision was checked by injecting six individual preparations of Tolvaptan Tablets spiked with Ethyl 4-bromobutyrate at specification level and calculated the % RSD of Ethyl 4-bromobutyrate content. The achieved precision experiment results are reported in Table 2.

Table 2. Statistical data of Precision Experiments

Tolvaptan Tablets		
Injection	System precision a	Method precision b
		(µg/g)
1	1239336	25.4
2	1223097	26.1
3	1205731	25.8
4	1123637	25.3
5	1203812	24.8
6	1137245	25.2
Mean	1188810	25.4
SD	47215.0918	0.4590
% RSD	0.04	1.81

a: area of Ethyl 4-bromobutyrate. b: content of Ethyl 4-bromobutyrate

Accuracy The accuracy study was carried out by preparing Tolvaptan Tablets sample solutions in triplicate by spiking Ethyl 4-bromobutyrate at LOQ level, 50%, 100% and 150% of specification level (25 µg/g) and calculated the percentage recovery. The accuracy experiment results are reported in Table 3.

Table 3. Accuracy data of Ethyl 4-bromobutyrate in Tolvaptan Tablets

Accuracy (Average of 3 replicates)	Ethyl 4-bromobutyrate			
	Level-I	Level-II	Level-III	Level-IV
Added (µg/g)	0.10	0.25	0.50	0.75
Recovered (µg/g)	0.09	0.26	0.49	0.73
Recovery (%)	90.0	104.0	98.0	97.3
R.S.D(%)	0.15	2.32	1.15	3.64
Overall recovery (%)	97.3			

IV. CONCLUSION

Method validation data demonstrated that the developed GC-MS method is sensitive, specific and as well as accurate for the estimation of Ethyl 4-bromobutyrate in Tolvaptan Tablets. Hence, the validated GC-MS method can be employed for routine analysis.

V. ACKNOWLEDGEMENTS

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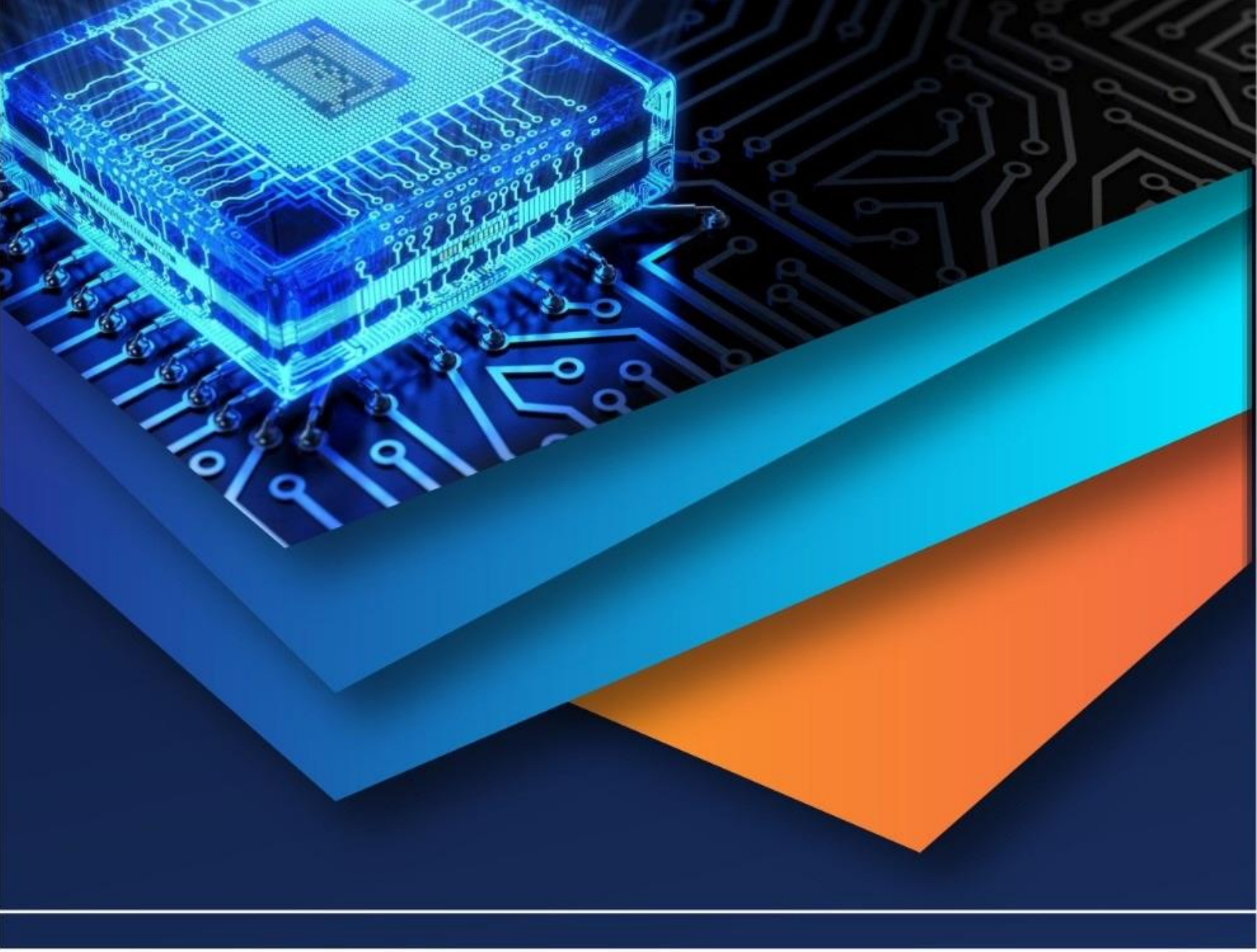
REFERENCES

[1] United states pharmacopoeia, general chapter ⟨467⟩, Residual Solvents, USP39–NF34, 2016, 339.
[2] International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Impurities: Guideline for Residual Solvents Q3C(R5), 2011.
[3] https://www.drugbank.ca/drugs/DB06212
[4] https://www.drugbank.ca/drugs/DB06212
[5] L. Muller, R. J. Mauthe, C. M. Riley, M. M. Andino, D. D. Antonis, C. Beels, J. Degeorge etal, Regul. Toxicol. Pharmacol., 2006, 44, 198-211.
[6] https://www.drugs.com/dosage/tolvaptan.html
[7] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf
[8] International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical procedures: Text and methodology Q2(R1), Step 4, 2005.



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