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RP-HPLC Method Development and Analytical Method Validation of Triclosan

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ABSTRACT:

Triclosan is a broad-spectrum antibiotic that is today used in many consumer and medical items, such as vicryl suture, surgical scrubs, and toothpaste (Bhargava and Leonard, 1996). Waters C18 (4.6 x 250 mm, 5 µm) Column Detector Wavelength: 280 nm in UV, Eluent A: acetonitrile Eluent: B 0.1% orthophosphoric acid in water as mobile phase in the ratio of (80:20). The flow rate is 0.8 ml/min with ambient temperature of column. Sample preparation with Methanol: Water of HPLC quality 80:20 ratio. The method used was verified in compliance with ICH regulations. With a correlation value of 0.999, the results showed good linearity. The retention time (Rt) was determined to be 4.6 ± 0.4 It was discovered that the technique was straightforward, specific TCS in dental formulations and for regular quality control assessments.

Keywords: TRICLOSAN, HPLC METHOD, RT

1. INTRODUCTION:

Triclosan is a broad-spectrum antibiotic that was developed more than 40 years ago and is currently used in numerous consumer and medical products, including toothpaste, surgical scrubs, and vicryl suture (Bhargava and Leonard, 1996).[1]
The antimicrobial agent *Triclosan* has been used in clinical settings for an antiseptic, disinfectant, or preservative in a range of consumer goods, including toys, plastics, cosmetics, and so on. Numerous species of non-sporulating Gram-positive and Gram-negative bacteria, Toxoplasma gondii, Plasmodium falciparum, and some fungi are among the many organisms on which it acts (Jones et al. 2000, Schweizer 2001). Additionally, research has shown that it is ecotoxic, particularly to aquatic algae (tata-raza-ko et al. 2004). Moreover, research by Waller and Kokana (2009) and Fernandes et al. (2008) has shown that it impedes the nitrogen cycle in natural systems.

Figure 1. *Triclosan* Structure [2]

Triclosan [TCS; 5-chloro-2-(2,4-dichloro-phenoxy)-phenol] is a synthetic chlorinated phenyl ether bisphenol that exhibits broad antibacterial action. This ingredient is frequently present in hygiene goods such as soaps, toothpaste, deodorants, and others at concentrations as high as 0.3%. [2]
For a long time, triclosan—a synthetic antibacterial and antifungal agent—has been a staple in consumer products. When it was first made accessible in the 1960s, it gained popularity because to how effectively it eliminated or prevented the growth of germs. Triclosan's primary mode of action involves rupturing the bacterial cell membrane and inhibiting specific enzymes that are essential for the bacteria's metabolism. [3]
Important *Triclosan* is commonly present in toothpaste, deodorants, soaps, hand sanitizers, and other personal hygiene products. It has also been added to many toys, linens, and chopping boards, among other home products, to provide antibacterial properties.
Despite being widely utilised, *Triclosan* has been the subject of regulatory scrutiny and controversy. Attention has been focused on its potential negative impacts on the environment and public health.
A survey suggests that *Triclosan* might increase antibiotic resistance, which could complicate the treatment of bacterial diseases. Although there have been questions expressed about the impact on human hormone systems, more research and discussion are need to fully understand the significance of these findings. [4]
Triclosan can enter the environment through wastewater and has been detected in water sources. Its resilience to the environment and tendency to disrupt aquatic habitats are concerning.
Owing to health and environmental concerns, regulatory authorities such as the European Union and the U.S. Food and Drug Administration (FDA) have taken measures to restrict or

completely ban the use of *Triclosan* in specified commodities. In 2016, the FDA banned *Triclosan* and several other antibacterial compounds from being used in consumer soaps.

In an effort to add antibacterial and antifungal properties to their products, numerous firms have searched for *Triclosan* substitutes. This is a result of the *Triclosan* dispute. Potential substitutes, including natural and synthetic alternatives, are being researched.[5]

Triclosan can penetrate the cell membranes of fungi and bacteria. Once inside, it damages the integrity of the cell membrane. This disruption erodes the structural integrity of the microbial cell, leading to the eventual death of the cell and discharge of its contents.

It has been demonstrated that *Triclosan* inhibits the enzyme enoyl-acyl carrier protein reductase (ENR), which is involved in the synthesis of fatty acids. Fatty acids are a crucial component of the bacterial cell membrane. *Triclosan* suppresses ENR, which stops the synthesis of fatty acids, further endangering the integrity of the cell membrane.[6]

Numerous metabolic processes in bacterial cells can be hindered by triclosan. By interfering with essential biological processes, it prevents bacteria and fungi from growing and reproducing.

Despite triclosan's demonstrated effectiveness against a variety of infections, its use has raised several issues, chief among them being antibiotic resistance. The overuse of *Triclosan* and related antimicrobials can cause treatment-resistant bacterial strains to evolve, which reduces the effectiveness of antibiotics.

Concerns about potential harm to human health and the environment have prompted regulatory bodies in certain countries to restrict or outright ban the use of *Triclosan* in a number of consumer items. As a result, producers are looking into other antibacterial compounds and mixtures to replace *Triclosan* in a variety of products. [7]

Experimental:

Instruments:

High Performance Liquid Chromatography method development was conduct using Shimadzu HPLC Waters 2489 Waters C18 (4.6 x 250 mm, 5 µm) Column system with waters pump 515 model from Singapore and with Empower software, with Shimadzu UV detection for separate, identify, and quantify specific components in mixtures.

Chemical:

Pure drug of *Triclosan* (Central Drug House (p). Ltd) was obtained from KIET school of pharmacy, Uttar Pradesh, India.

HPLC Grade Methanol, Acetonitrile & Water, Ortho phosphoric acid for dilutions and mobile phase the toothpaste gel of *Triclosan* (Hydent-k, batch no. CLH0130mfg lic no: KD/ 565 EXP date 10/2024).

Preparation of standard solution:

30mg *Triclosan* was accurately weighed and transferred into a 100 ml volumetric flask and made up to the mark with methanol and water (80:20), Standard solutions were prepared in the concentration of 300 ppm.

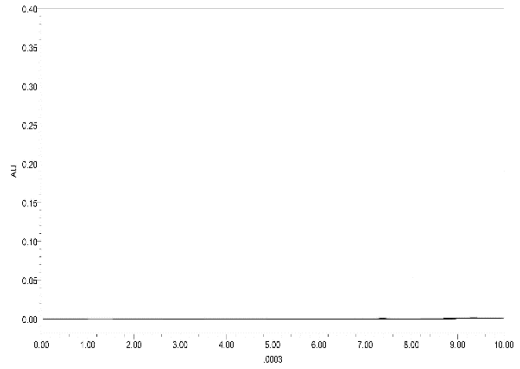
Preparation of *Triclosan* toothpaste sample solution:

Weighed *Triclosan* toothpaste equivalent to 30mg and transfer into mortar and triturate with 50 ml of methanol and sonicate the Solution for 3-5 minute then take solution into centrifugal tube fill up to the mark and centrifuge at 6000rpm for 10 min for Prepared The stock solution of 600 ppm then take 5ml of solution in 10 ml volumetric flask and make the volume up to the mark with methanol and water with ratio 80:20 and prepared the standard solution of 300ppm.

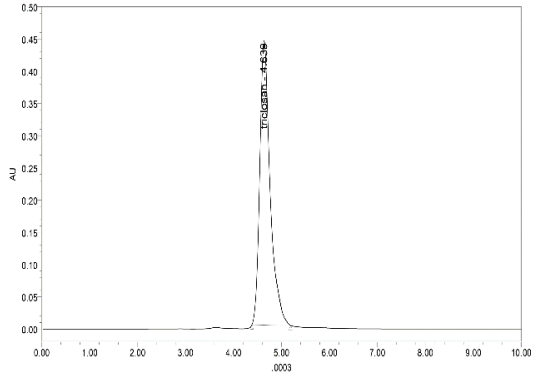
2. METHOD DEVELOPMENT

Selection of wavelength:
Scanning of 10ppm solution of *Triclosan* with UV-visible spectrophotometer from 200nm to 400nm using different solvent like Ethanol, Methanol, Diethyl-ether, Acetonitrile and Methanol: Water with ratio of 80:20 v/v.
The wavelength and solubility at which absorption takes place in UV detector is selected for further analysis or preparation of dilutions.

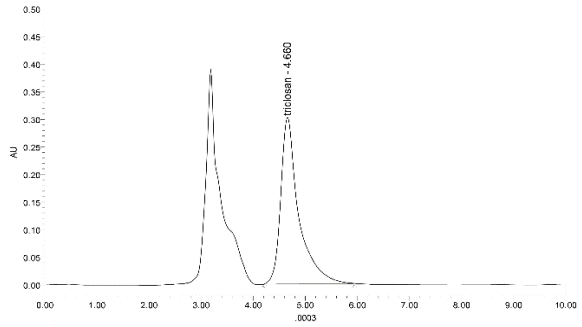
METHOD VALIDATION
Selectivity/Specificity:
A comparison of the chromatograms of *Triclosan* in hydent-k toothpaste and *Triclosan*standard and blank was used to assess the specificity of the suggested approach. A significant correlation was observed between the retention times of the sample and the standard, and there was no evidence of interference.



a. Figure 2. Blank



b. Figure 3. Standard



c. Figure 4. sample

System suitability:
The particular and selective aspect of the chosen approach ensures that measures like peak retention time, peak area, area under curve, peak height, limit of detection, limit of quantitation, and amount determined can all be ensured.[8]
The system suitability circumstances are necessary to confirm that the analytical system is operating correctly and producing precise and accurate data. The chromatographic system's repeatability is confirmed by system suitability testing. On newly made standard solutions, system suitability tests were conducted to determine its efficacy [11]. Results shown in table 1

Wavelength of maximum absorbance (nm)	280
Retention time	4.6 ±0.2
Run time	10min
Injection volume	10µl
Injections	6
RSD% of multiple injection	0.13

Table 1

Linearity:
The linearity of the technique was evaluated throughout a variety of calibration points using the generated calibration point solution in order to determine the connection between the acquired response and analyte concentration. The devised technique was used to analyse the solution in triplicate for each concentration, with a blank analysis in between, ranging in concentration from 240, 270, 300, 330, and 360 ppm. The study was completed, and a linear graph with a regression coefficient value was used to depict the response against concentration calibration curve. [9]

Parameters	**Values**
Linearity range (µg/mL)	230-370
Regression equation	Y=3517x-3E+06
Regression coefficient(r^2)	0.9995

Table 2

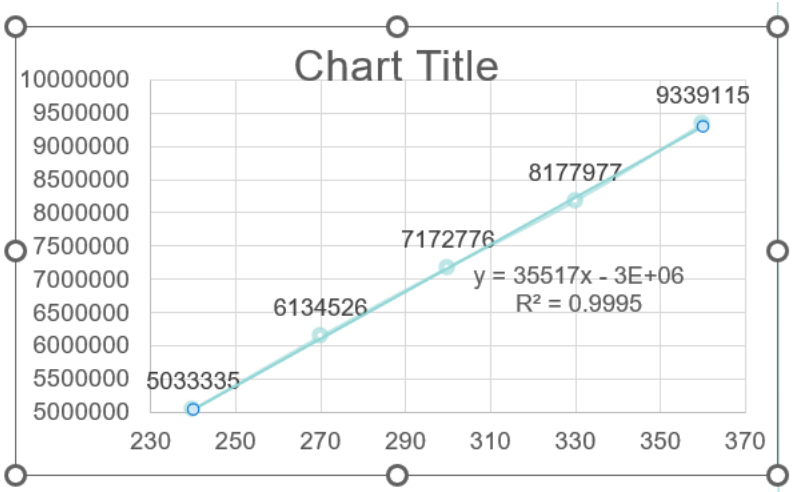


Figure 5. Chart

Accuracy:
To evaluate the accuracy of the developed procedure, a blank toothpaste sample was spiked with three different concentrations of the *Triclosan* reference standard, which are 80%, 100%, and 120% of the target concentration of 240 ppm, 300 ppm, and 360 ppm. The conclusion was analysed in three separate versions, and the percentage recoveries and relative standard deviation of the replies were...

%CONC	**AUC**	**%RECOVERY**
80	5184280	97.80

100	7290527	100.86
120	9207971	103.29

Table 3

Method precision:

A homogeneous sample from a single batch should be examined 12 times for method precision. This shows if a procedure produces reliable results for a particular batch. On the formulation of triclosan, the technique precision was applied. For 12 determinations, the assay value's percentage RSD cannot exceed 2.0%. [10]

No.	AUC1	AUC2	Mean	SD	RSD%
Injection-1	7006403	7090968	7048686	42282.5	0.599864
Injection-2	7076861	7054451	7065656	11205	0.158584
Injection-3	7071503	7071556	7071530	26.5	0.000375
Injection-4	7059945	7071403	7065674	5729	0
Injection-5	7078685	7064849	7071767	6918	0.097826
Injection-6	7060764	7070645	7065705	4940.5	0.069922

Table 4

Intermediate precision:

A *Triclosan* toothpaste solution with 300 ppm was injected 12 times, separated by 10 minutes. to determine intraday precision, the peak area and relative standard deviation were measured. On a different day, the intermediate precision was evaluated. the peak area was noted, and values of the relative standard deviation (RSD) were computed.

No.	AUC1	AUC2	Mean	SD	RSD%
Injection-1	7076423	7086117	7081270	4847	0.068448
Injection-2	6890762	7143977	7017370	126607.5	1.804202
Injection-3	7076336	7083302	7079819	3483	0.049196
Injection-4	7077509	7067233	7072371	5138	0.072649
Injection-5	7079914	7072388	7076151	3763	0.053179
Injection-6	7083048	7068791	7075920	7128.5	0.100743

Table 5

Method Precision vs Intermediate Precision:

Method Precision	%	Intermediate Precision	%
Sample 1	98.039	Sample 1	98.068
Sample 2	98.014	Sample 2	98.027
Sample 3	98.031	Sample 3	98.080
Sample 4	98.080	Sample 4	98.023
Sample 5	98.034	Sample 5	98.026
Sample 6	98.080	Sample 6	98.092
Mean	98.046		98.052
Sd	0.02501		0.028223
%RSD	0.02550		0.028784
%Relative difference		0.00327	

Table 6

Limit of detection and limit of quantitation:

The HPLC method of *Triclosan* detection serves as the basis for the limit of detection. The statistical equation is used to compute the LOD and LOQ values, and the LOQ value is multiplied by 3x. [12]

Equation: $LOD=\frac{3.3\Omega}{S}$ $LOQ=\frac{10\Omega}{S}$

LOD	3
Auc1	181824
Auc2	180236
Auc3	183071
Mean	181710.3
SD	1160.171
RSD%	0.638473

Table 7

LOQ	9
Auc1	597174
Auc2	599287
Auc3	590948
Auc4	594193
Auc5	596684
Auc6	601191
Mean	596579.5
SD	3325.909
RSD%	0.557496

Table 8

Where, Ω =standard deviation of the y-intercept of the calibration curve and
S = slope of the calibration line

Robustness:

Robustness was achieved by varying the wavelength (±5° nm) and flow rate (±10%). The method's requirements for system appropriateness must be fulfilled. Robustness was examined by varying factors such as wavelength, pH of the buffer, and flow rate. The outcomes were noted.[12]

Flow change table:

RT	**0.72ml/min**	**RT**	**0.88ml/min**	**RT**	**0.8ml/min**
5.12	7078435	4.012	7129533	4.6	7094021
4.98	7169492	4.002	7112170	4.68	7097537
5.11	6944404	4.00	7093761	4.6	7078547
Mean	7064110		7111821		7090035
SD	92448.36		14605.94		8249.088
RSD%	1.308705		0.205376		0.116348

Table 9

Change in Wavelength table

Wavelength	**AUC1**	**AUC2**	**AUC3**	**Mean**	**SD**	**RSD%**
278	7603411	7657246	7586313	7615657	30225.15	0.396882

280	7094021	7097537	7078547	7090035	8249.088	0.116348
282	7878579	7855530	7902218	7878776	19060.8	0.241926

Table 10

3. CONCLUSION

In conclusion, we have enhanced the High Performances Liquid Chromatography (HPLC) method by reducing the analysis time and creating a more efficient technique. HPLC is a commonly used analytical technique for developing new methods and separation techniques in mixtures of components. the primary component, which consists of a stationary phase C-18 column and a mobile phase that usually consists of acetonitrile and orthophosphoric acid. The sample was injected using a 25microliter syringe, and the mobile phase moved the sample to a detector that measures retention time, peak height, peak breadth, and area under the curve. and these factors are taken into account when calculating the method's repeatability, linearity, LOD, and LOQ.

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4. REFERENCES

1. Bhargava, H. N., & Leonard, P. A. (1996). Triclosan: applications and safety. American Journal of Infection Control, 24(3), 209-218.
2. Jones, R. D., Jampani, H. B., Newman, J. L., Lee, A. S., & Triclosan: (2000). A review of effectiveness and safety in health care settings. American Journal of Infection Control, 28(2), 184-196.
3. Schweizer, H. P. (2001). Triclosan: a widely used biocide and its link to antibiotics. FEMS Microbiology Letters, 202(1), 1-7.
4. Tata-raza-ko, T., Di Vaio, A., Vasudevan, N., & Papenbrock, J. (2004). Effects of the biocide *Triclosan*on different aquatic organisms. Marine Environmental Research, 58(4), 411-417.
5. Waller, C. P., & Kokane, M. S. (2009). Effects of *Triclosan*on microbial nitrogen removal from wastewater in an activated sludge system. Bioresource Technology, 100(3), 1236-1241.
6. Fernandes, P., Miranda, A. F., Komesu, A., Gimenes, M. L., & Higarashi, M. M. (2008). Effect of *Triclosan*(5-chloro-2-[2, 4-dichloro-phenoxy]-phenol) on growth and metabolism of the toxic cyanobacterium Microcystis aeruginosa. Brazilian Journal of Biology, 68(4), 795-802.
7. Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2013). Fundamentals of Analytical Chemistry. Cengage Learning. (This textbook provides comprehensive coverage of analytical chemistry principles, including chapters on chromatography techniques such as HPLC.)

8. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). Introduction to Modern Liquid Chromatography (3rd ed.). Wiley. (This book offers in-depth insights into liquid chromatography techniques, including HPLC theory, instrumentation, and method development strategies.)
9. Ahuja, S., & Dong, M. (2005). Handbook of Pharmaceutical Analysis by HPLC. Elsevier. (This handbook focuses specifically on pharmaceutical analysis using HPLC, covering method development, validation, troubleshooting, and applications.)
10. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). (This guideline provides detailed recommendations for the validation of analytical methods, including those involving HPLC.)
11. United States Pharmacopeia (USP) General Chapter <621> Chromatography. (This chapter of the USP provides general information on chromatography techniques, including HPLC, and outlines requirements for method validation.)
12. European Pharmacopoeia (Ph. Eur.) General Chapter 2.2.46. Chromatographic Separation Techniques. (This chapter of the European Pharmacopoeia provides guidance on chromatographic separation techniques, including HPLC.)