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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TRICLOSAN IN DENTAL FORMULATIONS

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ABSTRACT: The present study is a simple rapid, sensitive, selective and reproducible RP-HPLC method has been developed for the estimation of Triclosan in dental formulations. In present work a simple, sensitive and specific method (RP-HPLC assay, stability indicating RP-HPLC) has been developed by using a phenomenex, BDS C18, column having 5 µm particle size and 150 mm × 4.6 mm in length and gradient mode, with mobile phase containing acetic acid buffer (pH 2.5, adjusted with formic acid) and acetonitrile in the ratio of 30:70. The flow rate was 1.2 mL/min and effluents were monitored by PDA detector at 281nm. Injection volume was 20 µL. The method is linear (1-50 µg/mL). The regression equation was y = 7311x-2307 (r^2 = 0.997). The method was validated for linearity, precision, accuracy, ruggedness, and forced degradation studies were performed. Estimation of triclosan was done in 3 dental formulations. Recoveries from formulations were between 98% and 102%. Limit of detection and limit of quantification was 0.101433 and 0.3333µg/mL, respectively. All the validation parameters are within the acceptance range.

INTRODUCTION: Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) **Fig. 1** is a strong broad-spectrum anti-microbial agent used in many consumer products, such as anti-bacterial soaps, toothpaste, cosmetics, clothing, kitchenware, and toys[1]. Triclosan is a relatively small molecule , with a molecular weight of 289.54 g/mol and a diameter of about 7.4 Å[2]. It is a white solid at standard temperature and pressure, with a boiling point in the range of 280-290 °C and a melting point in the range of 56-58 °C. Triclosan has a low partition coefficient (log *P*o/w= 4.7) [3]. The partition coefficient is a ratio of solubility between two liquids, typically octanol and water.

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Triclosan discharged into surface waters through wastewater effluents react photochemically to form polychlorinated dibenzo-p-dioxins [4]. From the literature review, it is known that various methods have been reported. A gas chromatography-tandem mass spectrometry (GC-MS/MS) [5] has been developed for the simultaneous analysis of different classes of preservatives like benzoates, bronidox, 2-phenoxyethanol, parabens, BHA, BHT and triclosan in cosmetic products.

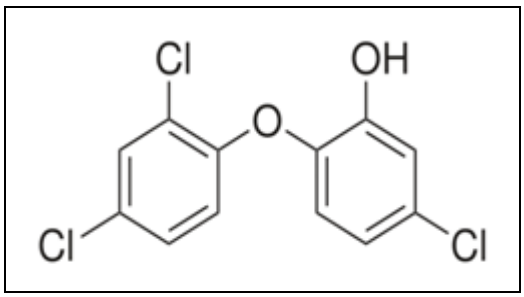

FIG 1: STRUCTURE OF TRICLOSAN

Liquid chromatography (HPLC)/GC-MS method for triclosan in personal health care products[6-9],

human milk [10], GC–MS analysis in Indian rivers [11], dentifrices [12] hydrogel patches [13], and Dioxin [14] photoproducts has been developed. Some combination analytical methods developed for triclocarban (TCC), triclosan (TCS) and Methyl-triclosan (MTCS) in environmental water, by using silicon dioxide/polystyrene composite micro-spheres solid-phase extraction and detection with HPLC-ESI-MS method [14].

From the literature review, it is known that various methods have been reported for the estimation of triclosan, and it was found that stability indicating methods for triclosan in dental formulations by RP-HPLC was not reported. Hence an attempt has been made to develop a simple and accurate method for the estimation of triclosan in dental formulations.

The objective of the study was to develop and validate new stability indicating RP-HPLC method for estimation of triclosan in dental formulations.

MATERIALS & METHODS:

Instrumentation: Chromatographic separation was performed on Shimadzu UFLC system consist of model LC20AD having autosampler and photo diode array (PDA) detector. LC solution software was applied for data collecting and processing.

Chromatographic Conditions: The method has been developed by using a phenomenex, BDS C18, the column having 5 µm particle size and 150 mm × 4.6 mm in length and gradient mode, with mobile phase containing acetic acid buffer (pH2.5, adjusted with O-phosphoric acid) and acetonitrile in the ratio of 30:70. The flow rate was 1.2mL/min, and effluents were monitored by PDA detector at 281 nm.

Chemicals and Reagents: Triclosan drug was obtained from Vivimed Private Limited, India. Acetonitrile of HPLC grade was procured from Merck Chemical Laboratories, Bangalore, India. Milli-Q-water was used throughout the process. Acetic acid, formic acid is of analytical reagent grade of Merck Pharmaceuticals. All chemicals and reagents used were of HPLC grade only.

Method Development:

Selection of Mobile Phase: The drug triclosan was injected with different mobile phases at different ratios with different flow rates till a sharp peak, without any interference peaks containing spectrum was obtained. The mobile phase selected was an acetic acid buffer (pH 2.5), and acetonitrile in the ratio 30:70.

Preparation of Mobile Phase:

Solution A: Acetonitrile;

Solution B (Buffer): Buffer solution is prepared by dissolving 5 mL of acetic acid in 395 mL of Millipore water and sonicated to dissolve, then filtered through 0.45µ membrane filter;

Mobile Phase: 30 volumes of solution A and 70 volumes of solution B.

Preparation of Standard Stock Solution: 10mg of Triclosan working standard was transferred into 100 mL volumetric flask add about 40 mL of Methanol and sonicated to dissolve, and volume made with diluents (100 µg/mL Triclosan). The calibration curve was drawn with concentration range 1-50 µg/mL. The peak was eluted at R_t 5.1 minutes, chromatograms of blank and standard triclosan were shown in **Fig. 2** and **3.**

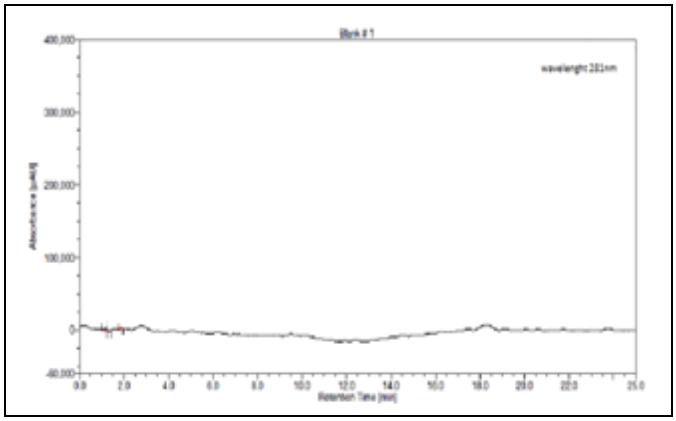

FIG. 2: CHROMATOGRAM OF BLANK

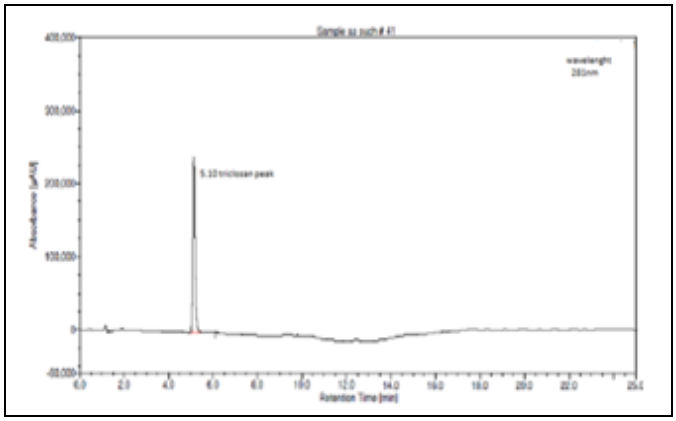

FIG. 3: CHROMATOGRAM OF STANDART TRICLOSA

Working Standard Solutions: Transfer aliquots of the above standard stock solution into a series of 10 mL volumetric flasks and dilute to volume with methanol to obtain a concentration range of 1-50 µg/mL.

Extraction of Triclosan from dental formulations:

Method: The samples of Pepsodent sensitive (sample1), Senoquel-F (sample 2) and SHYOR mouth wash (sample 3) were purchased from the local supermarket. Initially, 20 or 50 mg of sample was placed in a 40 mL glass-centrifuge tube and mixed with 20 mL of ethanol. The mixture was immersed in an ultrasonic bath and extracted for 20min at room temperature. The solution was then centrifuged at 6000 rpm for 10minutes; the supernatant 50 µL was transferred to a vial and are analyzed.

RESULTS AND DISCUSSION:

Method Validation:

System Suitability: To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. System suitability tests are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard solutions. Results are shown in **Table 1.**

TABLE 1: SAMPLE SUITABILITY PARAMETER

Parameters	Values
The wavelength of the max absorbance (nm)	281
Retention time (min)	5.15±0.10
Tailing Factor	0.947
Theoretical Plate	13777.964
R.S.D of multiple injections (Amount, six replicates)	0.012
Limit of detection (LOD) (µg/mL)	0.101
Limit of quantification (LOQ) (µg/mL)	0.338

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of an analyte in samples within a given range. The study was performed by preparing standard solutions of the Triclosan at eight different concentrations, and analyzes were performed in triplicate. The calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in **Table 2**. The linearity curve of triclosan was represented in **Fig. 4.**

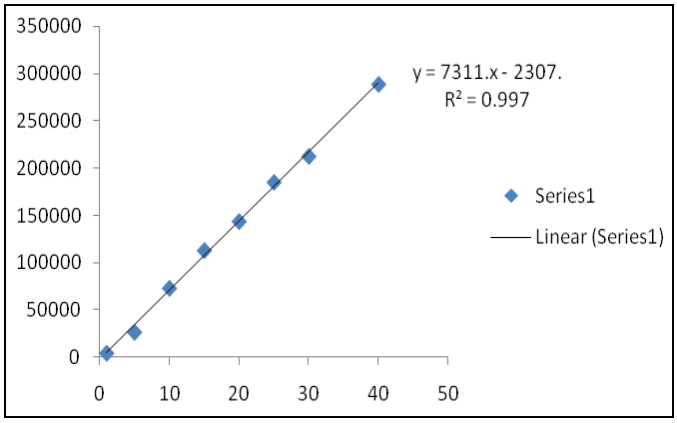

FIG. 4: CALIBRATION CURVE OF TRICLOSAN

TABLE 2: LINEARITY PARAMETERS

Parameters	Values
Linearity range (µg/mL)	1-50
Regression equation	Y=7311x-2307
Regression coefficient (R^2)	0.997
Slope	7311
Intercept	2307

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of the series of measurement.

Method Precision: In method precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results for a single batch. The method precision was performed on triclosan formulation. The % RSD of the assay value for six determinations shall not be more than 2.0%. Results are shown in **Table 3** and **4.**

TABLE 3: INTRADAY PRECISION

Concentration (µg/mL)	Inj 1 Peak area	Inj 2 Peak area	Inj 3 Peak area	Average	Std. Deviation	% RSD
5	27661	27281	27192	27196.3	73.1	0.2
20	145921	145856	145661	145821.7	135.30	0.09
50	288541	288321	288756	288539.3	217.5	0.07

TABLE 4: INTERDAY PRECISION

Concentration (µg/mL)	Inj 1 Peak area	Inj 2 Peak area	Inj 3 Peak area	Average	Std. Deviation	% RSD
5	15239	15809	15269	15439	320.7	1.69
20	117093	119752	117752	118199	1384.7	0.956
50	255632	259059	254895	256528	2222.1	0.86

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. Accuracy is performed in three different levels for Triclosan, and three different triclosan formulations were analyzed. A spiked known quantity of Triclosan at 50%, 100%, and 150% levels. Analyzed sample in duplicate for each level. From the results, % recovery was calculated. % Recovery at each spike level shall be not less than 98.0 and not more than 102.0. % RSD for the duplicate observations shall be not more than 2.0. Overall, % RSD for the % Recovery shall be not more than 2.0. Assay of three formulations was shown in **Table 5**. Peak results for Triclosan in Pepsodent sensitive, Senoquel-F, and SHYOR mouth wash were shown in **Table 6, 7,** and **8,** respectively**.**

TABLE 5: ASSAY RESULTS OF TRICLOSAN

Formulations	Drug	Amount of drug labeled	Amount of drug estimated	% of label claim
Sample1	Triclosan	0.3%	0.28	93%
Sample 2	Triclosan	0.3%	0.27	90%
Sample3	Triclosan	0.3%	0.29	96.6%

TABLE 6: ACCURACY RESULTS OF PEPSODENT SENSITIVE

Target concentration	Weight of sample taken (mg)	Peak area	Total amount after spiking (mg)	Amount recovered (mg)	% recovery	% mean recovery	% RSD
80	0.6	128079	18	17.8	99.07	99.18	1.219
100	0.8	169721	24	23.5	98.04		
120	1	328195	45	45.2	100.45		

TABLE 7: ACCURACY RESULTS OF SENOQUEL-F

Target concentration	Weight of sample taken (mg)	Peak area	Total amount (mg)	Amount recovered (mg)	% recovery	% mean recovery	% RSD
80	0.6	133012	18	18.5	102	101.03	0.940
100	0.8	176619	24	24.5	101		
120	1	327056	45	45.05	100.1		

TABLE 8: ACCURACY RESULTS OF SHYOR MOUTH WASH

Target concentration	Weight of sample taken (mg)	Peak area	Total amount (mg)	Amount recovered (mg)	% recovery	% mean recovery	% RSD
80	0.6	129011	18	17.9	99.7	99.54	0.836
100	0.8	170619	24	23.6	98.5		
120	1	329056	45	45.3	100.1		

Procedure:

Accuracy at 80%, 100% and 120%: Accurately 0.6gm, 0.8gm, and 1gm of triclosan formulation was taken respectively for 80%, 100%, and 120% and extracted. From this 1mL was taken in each along with 15ppm standard was taken in 10 mL volumetric flask and sonicated it for 10 minutes. Filter this through 0.45µ nylon filter. Further volumes were made up to 10 mL with diluents and mixed well.

Robustness: Robustness was done by changing the column temperature (±5°C), flow rate (±10%), changing the wavelength (±5° nm), pH of buffer solution (±0.2 units) for15µg/mL concentration. All the system suitability parameters must be met as per the method. Robustness was analyzed by changing the parameters like flow rate, pH of buffer, and wavelength, and the results were recorded in **Table 9**.

TABLE 9: ROBUSTNESS

Parameter	Actual value	Changed value	R_t	Peak area	Changed value	R_t	Peak area
Flow rate	1.2 mL/min	1 mL/min	5.18	144842	1.3	4.18	150141
Buffer pH	2.5	2.4	5.10	171264	2.6	5.17	168825
Wavelength	281 nm	275 nm	5.13	144908	286	5.14	158742

Ruggedness: An aliquot (15µg/mL) was injected to study the ruggedness of Triclosan by two different analytical chemists (Analyst-1and Analyst -2) and the results were recorded in **Table 10** and are in the acceptable range for Triclosan. The results showed the % R.S.D. was less than 2% respectively.

TABLE 10: RUGGEDNESS CONCENTRATION PREPARED BY DIFFERENT ANALYST

Ruggedness	Analyst -1		Analyst -2	
No. of Injection	Retention time	Peak area	Retention time	Peak area
Injection-1	5.109	146782	4.921	147445
Injection-2	5.135	149892	5.112	141289
Injection-3	5.140	147888	5.141	142326
Injection-4	5.181	147347	5.128	142276
Injection-5	5.091	149895	5.151	144557
Injection-6	5.172	148654	4.968	147654
Average	5.138	148409.7	5.070	144257.8
Std Dev.	0.03	1304.9	0.099	2766.048
%RSD	0.67	0.879	1.959.	1.91

Forced Degradation Studies: The following are the stress conditions which were followed for forced degradation studies:

- Acid degradation
- Alkali degradation
- Peroxide degradation
- UV light degradation

Acid Stress Degradation: Test preparation was subjected to acid stress degradation by treating the sample with hydrochloric acid. The % degradation was evaluated by calculating the % assay and by comparing the assay results with the assay of unstressed sample.

Test Preparation: 50 mg of paste is weighed and extracted with 20 mL of ethanol. This is sonicated, centrifuged and the supernatant liquid is collected.8ml of the extract is transferred flask and added 1 mL 0.1N Hydrochloric acid, and allowed to stand for 4 h and neutralized with 1mL of 0.1N sodium hydroxide and filtered through syringe filters and injected. Acid degradation peak was shown in **Fig. 5**.

Alkali Stress Degradation: Test preparation was subjected to alkali stress degradation by treating the sample with sodium hydroxide.

Test Preparation: 50 mg of paste is weighed and extracted with 20 mL of ethanol. This is sonicated, centrifuged and the supernatant liquid is collected. 8 mL 0f extract is transferred flask and added 1 mL 0.1N sodium hydroxide, and allowed to stand for 4 h and neutralized with 1mL of 0.1 N hydrochloric acids and filtered through syringe filters and injected. Degradation peak was shown in **Fig. 6.**

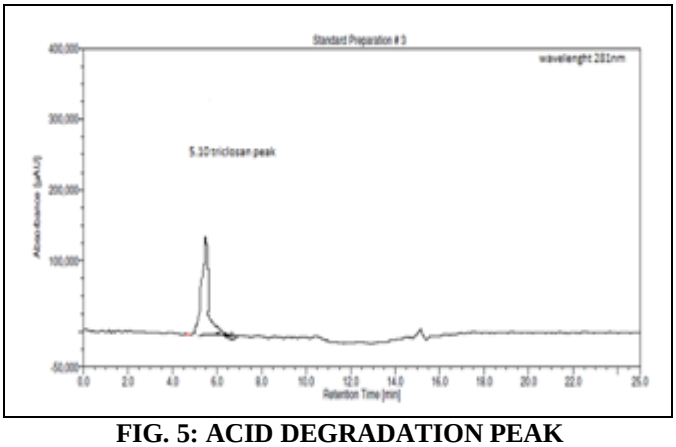

FIG. 5: ACID DEGRADATION PEAK

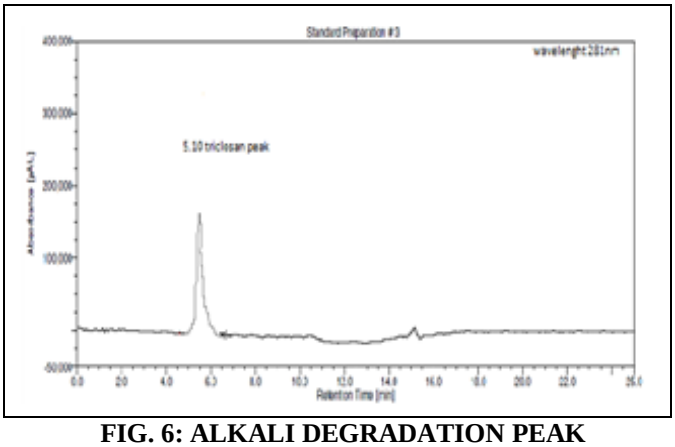

FIG. 6: ALKALI DEGRADATION PEAK

Peroxide Stress Degradation: Test preparation was subjected to peroxide stress degradation by treating the sample with peroxide.

Test Preparation: 50 mg of paste is weighed and extracted with 20 mL of ethanol. This is sonicated, centrifuged and the supernatant liquid is collected.

8 mL of extract is transferred flask and add 1 mL of 3% v/v Hydrogen peroxide, allowed to stand for 4 h and filtered through syringe filters and injected**.** Degradation peak was shown in **Fig. 7**.

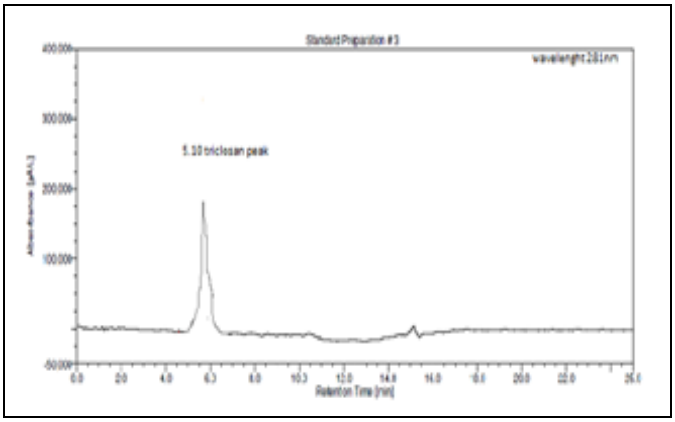

FIG. 7: PEROXIDE DEGRADATION PEAK

UV Degradation: Test preparation was subjected to UV light for a 4 h time.

Test Preparation: 50 mg of paste is weighed and extracted with 20mL of ethanol. This is sonicated, centrifuged and the supernatant liquid is collected. 10mL of the extract is transferred flask and subjected to UV light for 4 h, and filtered through syringe filters and injected. Degradation peak was shown in **Fig. 8**.

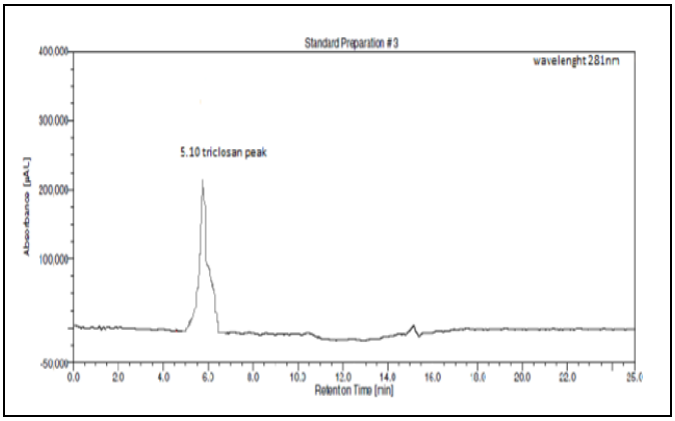

FIG. 8: UV LIGHT DEGRADATION PEAK

Based on the forced degradation studies, the proposed analytical method can be considered as stability-indicating the method and can be used for release and stability studies for effective evaluations. Degradation results for sample 1, sample 2, and sample 3 are shown in **Table 11, 12,** and **13,** respectively. Drug (triclosan) is stable to acid, alkali, peroxide, and UV light degradation within the range in the ICH guidelines.

TABLE 11: DEGRADATION OF PEPSODENT SENSITIVE

Parameter	Peak area of unstressed	Peak area of the stressed sample	% sample degraded
Acid	113292	97730	13.8
Base	113292	96945	14.5
Peroxide	113292	92977	17.9
UV light	113292	86933	23.27

TABLE 12: DEGRADATION OF SENOQUEL-F

Parameter	Peak area of the unstressed sample	Peak area of a stressed sample	% degraded
Acid	113292	79593	29.75
Base	113292	88160	22.19
Peroxide	113292	86248	23.81
UV light	113292	100794	11.04

TABLE 13: DEGRADATION OF SHYOR MOUTH WASH

Parameter	Peak area of the unstressed sample	Peak area of the stressed sample	% degraded
Acid	113292	83640	26.2
Base	113292	89012	21.5
Peroxide	113292	92040	18.8
UV light	113292	85454	27.1

CONCLUSION: The method involves a simple and precise method for the determination of triclosan in the dental formulation. The method was validated according to ICH guidelines. The triclosan was eluted at 5.1 minutes **Fig. 3**, with linearity in the range of 1-50 µg/mL and correlation coefficient (r^2) of 0.997. The limit of detection (LOD) was calculated and found to be 0.101µg/mL and the limit of quantification (LOQ) was found to be 0.338 µg/mL. Intraday precision values of %RSD were found to be in the range of 0.07-0.2% and interday precision values were 0.86-1.69% for triclosan. In degradation study, it showed no additional peaks in the chromatograms **Fig. 5, 6, 7** and **8** indicating that triclosan is stable in acidic, alkali, peroxide and photodegradation. Hence the proposed method is more precise, accurate and robust. So. this method can be applied for the estimation of triclosan in quality control studies for routine analysis.

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CONFLICT OF INTEREST: Nil

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