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RESEARCH ARTICLE

TRICLOSAN, ANTIBACTERIAL AGENT: HARMFUL TO ENVIRONMENT AND HUMAN HEALTH

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ABSTRACT

Antibacterial agents are extremely common in everyday products, such as toothpastes, facial cleansers, hand soaps, body washes, cosmetics, and numerous other products. Aquatic ecosystems are continuously contaminated by manufactured household and personal care products (PCPs). Non-regulated, multi-purpose PCP contaminants enter aquatic systems through sewage/ wastewater treatment plants after consumption and use by humans and animals. One antibacterial agent under scrutiny at this time is triclosan (TCS). Triclosan, an antibacterial agent, receive increased attention worldwide since significant levels of contamination have been found in various environmental compartments and organisms. Industries are now avoiding the use of triclosan, since very minute quantities can pose a severe risk to marine life in aquatic ecosystems. The purpose of this research was to develop an efficient, eco-friendly, sensitive, rapid Gas chromatography-mass spectrometry (GC-MS) method to detect triclosan in toothpaste, soap, tiles cleaner, basin cleaner and detergent. The experimental conditions, such as column temperature, solvents, flow rate, analytes extraction methods, and experimental procedure, were all optimized to find the best experimental conditions for detecting triclosan in the samples. The ability to detect triclosan in personal care products, as well as in pool and river water samples, will hopefully encourage consumers to reduce or avoid the use of triclosan containing products. Using the optimized method developed, the average concentration of triclosan in the soaps and tiles cleaner were found 0.043% (w/w). The average concentrations in the toothpaste, basin cleaner and detergents samples were 0.021% (w/w), 0.020%(w/w) and 0.024% (w/w), 0.010% (w/w) respectively.

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INTRODUCTION

In modern life, one of the most important issues in the world is the exposure to man-made chemicals that cause interference of regular activities such as reproduction and development of different organisms in the environment (Stone, 1994 and Ashby *et al*, 1997). Some of them are hazardous and present potential or actual threat to human health, wildlife, aquatic organisms and environment (Ashby *et al*, 1997). Triclosan (TCS), 2,4,4-trichloro-2-hydroxydiphenylether, has been extensively used as an antibacterial agent in formulations of toothpaste, liquid hand-soap, facial cleansing cream, detergents, and other household products (Sabaliunas *et al* 2003; Fiss *et al*, 2007; Moldovan, 2006; Tsai *et al*, 2008). TCS released to the environment is acutely and chronically toxic to aquatic organisms (Bhardwaj *et al*, 2016 AlGhais 2020). However, TCS was removed in 2010 from the EU list of additives for use in plastic use food-contact materials (Commission decision 2010/169/EU).

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On September 9, 2016, the United States Food and Drug Administration (FDA) banned the incorporation of triclosan and 18 other antimicrobial chemicals from household soap products and the next year prevented companies from using triclosan in over-the-counter health care antiseptic products without premarket review. On the other hand, about 85% of the total volume of TCS is used in personal care products, compared to 5% for textiles and 10% for plastics and food contact materials (SCCS 2010). Newer analytical techniques have made it possible to identify these compounds at extremely low level of the order of sub-ng/g. These are frequently detected in different environmental compartments including surface waters, wastewaters, air, wildlife and fish, and had not been recognized previously at such low levels. These compounds are often referred to as "emerging contaminants" (ECs) because adequate information associated with their presence, occurrence, fate, transport and mechanisms are not available to assess their risk to human health and the ecosystem (Daughton *et al*, 1999). Emerging evidence from wildlife and laboratory studies indicates that some chemicals may interfere with the endocrine system. Compounds identified include pesticides, polychlorinated

biphenyls, dioxins, furans, alkyl phenols, and steroid hormones. These chemicals are routed to ecosystems through wastewater treatment plants. Several studies reported that many ECs present in municipal wastewater effluent can act as endocrine disruptors at concentrations capable of inducing fish feminization (Hahlbeck *et al*, 2004 and Harris *et al*, 2011). Personal care products (PCPs) and household products are a major class of ECs commonly used in human and animal applications. PCPs are many chemical compounds with a variety of chemical structures, conformations, functional groups, polarities and characteristics. Tons of these chemicals are produced annually worldwide (Christen *et al*, 2010). After consumption, PCPs are released into ecosystems via urine, feces or residues as either parent compounds or their metabolites. PCPs enter the environmental system through effluent discharge from wastewater/ sewage treatment plants, inappropriate disposal, shower drain, residues from drug manufacturing companies, nursing homes and hospital facilities. The widespread use of TCS, and the increasing public concern over health and environmental issues have stimulated our interest to investigate the content of TCS in PCPs. Therefore, the purpose of this research was to develop a sensitive, ecofriendly and rapid method to detect and quantify triclosan in personal care and household products using gas chromatography-mass spectrometry. The fact that triclosan is present and can be detected, in these products and, it is hoped to encourage consumers to reduce, or avoid the use of triclosan-containing products.

MATERIALS AND METHODS

Experimental Instrument: The instrument used in this research was Nexus GC-2030 gas chromatograph with GCMS- QP2020NX Mass spectrometer Shimadzu, hotplate Stirrer-Wise stir MSH-20D, analytical balance- Radwag PS2100R2, vertex-QLSMX2800- QLS.

Materials and Reagents: Triclosan was purchased from HiMedia, India. Methanol, acetonitrile, and acetic acid, were purchased from Eurolab, Germany. Any water used in standard or sample preparation was milliQ water. All reagents were of analytical grade.

Preparation of Standard Solutions and Solvents: A Standard stock solution of triclosan (10 ppm) was prepared by weighed accurately 10 mg of triclosan standard to 100 mL with methanol and dilute up to mark with methanol. Further diluted 1 mL of triclosan standard (10 ppm) to 100 ml with acetonitrile. From this stock solution, three different concentrations of triclosan solution were prepared with acetonitrile i.e. 0.02 ppm, 0.04 ppm, 0.06 ppm for linear concentration solution. Acetonitrile was used as blank. The standards were run in triplicate on the GC-MS to produce a standard curve.

Preparation of Samples: The samples of toothpaste (2 samples), soap (2 samples), tiles cleaner (2 samples), basin cleaner (2 samples) and detergent (4 samples) were purchased from local supermarkets of Ras Al Khaimah, UAE. Initially, weighed 5g of sample and mixed with 100 ml of methanol and kept it for stirring for 12 hr. in closed container (Note: at least ¼ th part should be dissolved in methanol if not dissolve then extend the stirring time as per type of sample). The solution was then centrifuged at 3500 rpm speed for 30min at room temperature. The clear supernatant was then collected and further diluted with acetonitrile (20 µL of solution to 100 ml). This was final sample solution used for analysis.

GC-MS Analysis: The instrument used in this research was Nexus GC-2030 with GCMS- QP2020NX Mass spectrometer Shimadzu. Inject the sample at injector temperature 250°C having ramp rate 90°C initial hold for 5 min and increase @ rate of 10 °C per min up to 140 °C and hold for 5 min again then increaser @ 10°C up to 280 °C and hold for 1 min. Mass detector parameter as interface temp 290°C and ion source tem is 230°C. Having linear velocity 35cm/sec with helium gas. The instrument parameters are outlined in Table 1 below.

Table 1. GC-MS optimized method parameters

Injector temperature	250°C
Interface temperature	290°C
Ion source temperature	230 °C
Linear velocity	35 cm/sec
Column	GC-RXI 5@sil-MS
Oven Ramp	90°C (5min Hold) Increase @10°C to 140°C (5min hold) and increase @10°C to 280°C (hold for 1 min)
Injection volume	1 µL
Mode	Split less

All samples were then run on the GC-MS with the instrument parameters set to the parameters listed in Table 1.

RESULTS AND DISCUSSION

Analysis of Standards: Upon running the triclosan standards through the GC-MS, the produced chromatograms were evaluated. An extracted ion chromatogram (EIC) was used to identify the peaks belonging to triclosan. For triclosan, an EIC mass range (m/z) of 288.00000 was used. All of the EICs (20ppb, 40ppb, 60ppb) showed large definitive peaks within the specified mass ranges. Upon analyzing these peaks for mass spectrum data, the standards mass spectra contained a very prominent peak at the m/z of 288.00, which is slightly less than triclosan's molecular weight of 289.54 g/mol. In other studies, the observed mass peak for triclosan was an identical 287.95 (Gonzales- Marino *et al*, 2009). This value is expected as the MS was operating in negative mode, so a value of (M-1) is expected. All of the standards mass spectra also contained a very prominent peak at the m/z of 227.11 as well, which is about 1 mass unit less than BPA's molecular weight of 228.29 g/mol (Bisphenol A, 2015). Again, since the MS was operating in negative mode, a value of (M-1) is expected. The EICs and mass spectra for triclosan (Figure 1) is shown below.

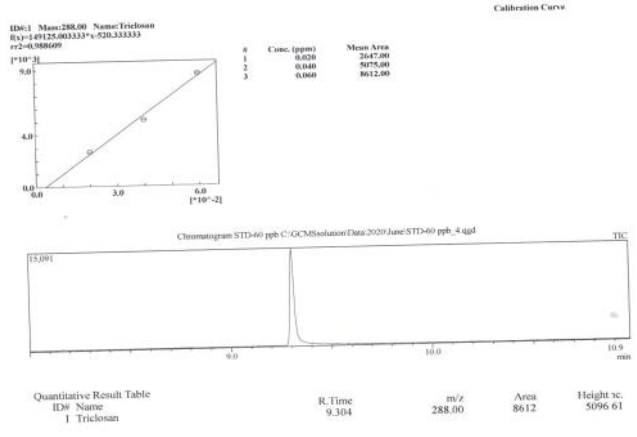

Figure 1. Coefficient correlation of Linearity curve of Triclosan standard

In order to determine the concentration of triclosan in the personal care and household products a standard curve had to be generated from the standards. Using the instrument's Mass software, the peak areas of the triclosan within their respective EICs were determined. The internal standard approach was used so that a triclosan peak area ratio was plotted against triclosan standard concentration to generate the standard curve (Figure 2). A coefficient of linearity (R2) value of 0.989 was obtained.

Figure 2. Triclosan Standard calibration curve

Analysis of Samples: The run time, along with the rest of the experimental parameters, was kept the same throughout the entire experiment due to the varying elution time of triclosan in the sample runs. Every sample was run in duplicate, just like the standards. EICs for each sample were analyzed in the same mass ranges as the standards to detect triclosan (m/z range of 288.00000). Prominent peaks for triclosan was found in almost every sample run. The Soap and tiles cleaner EICs and mass spectra are shown below (Figures 3). One of the four detergent runs did not show a triclosan peak. A possible reason for this could be due to temporary column clogging or could likely be due to extremely low triclosan levels, rendering the triclosan peak indistinguishable from the baseline signal noise peaks.

GCMS software was again used to determine the EIC peak areas of triclosan in each sample. The triclosan peak area ratio and the equation of the standard curve were used to determine the triclosan concentration. Working backwards through the dilution calculations, the original sample triclosan concentrations were determined. The table below shows the average of obtained original sample concentrations (Table 2). According to the research results, it was observed that all the samples contained triclosan but the products did not list the triclosan content and quantity on the leaflet. With obtained values of 425.25 ppm for the soap, and 419.75 ppm for the tiles cleaner, 210ppm for toothpaste, 201.5ppm for basin cleaner, 239.25ppm for detergents, respectively.

These recovery yields indicate that a triclosan is available in the products but not mentioned on leaflet of content list. The detection of triclosan in samples indicates its industrial scale use in everyday products and that needs to be reduced, restricted, or avoided. This also signals a need to successfully remove or destroy triclosan before it is released back into the environment. Similar results were reported by Cheng *et al*, 2011. Also, Mottaleb *et al*, 2015, reported the detection of emerging contaminants in fish.

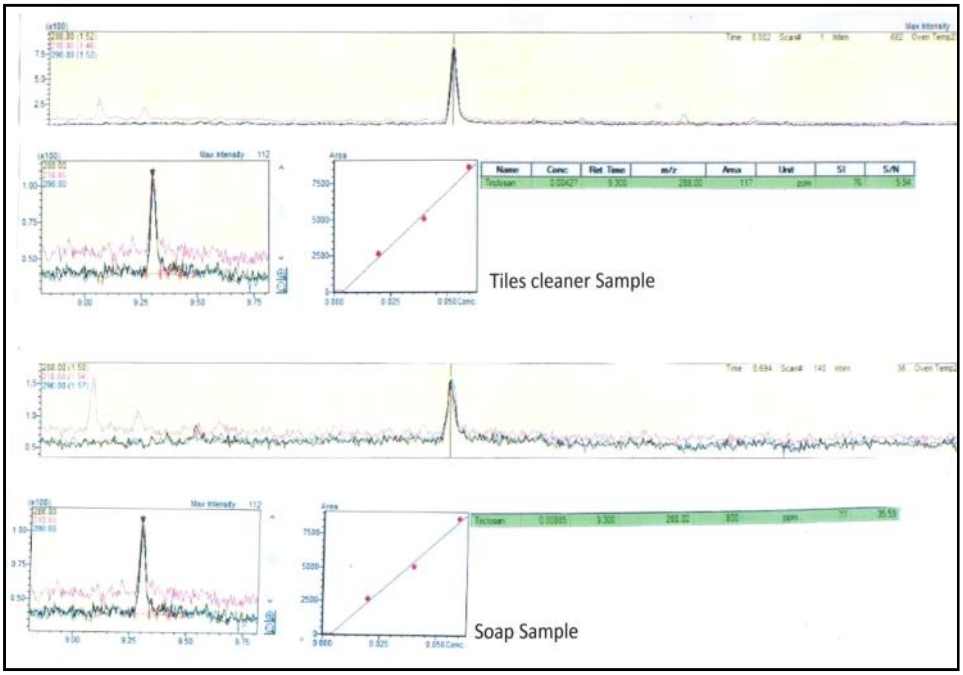

Figure 3. Mass spectra and EIC of Tiles cleaner and soap sample

Table 2. Triclosan concentration in different samples

Description	Sample	Concentration	Conc. ppm	Conc. percentage	Standard Limit
Soap	SPL-1	0.01145 0.00556	425.25	0.043	1) USA- Banned (google)
Toothpaste	SPL-2	0.00427 0.00413	210	0.021	2) Canada-NMT 0.03% (google)
Tiles Cleaner	SPL-3	0.00794 0.00885	419.75	0.042	
Basin Cleaner	SPL-4	0.00409 0.00397	201.5	0.020	
Detergent-1	SPL-5	0.00386 0	96.5	0.010	
Detergent-2	SPL-6	0.00441 0.00516	239.25	0.024	

Conclusion

Due to its properties and the widespread usage of triclosan, there is a need for monitoring and controlling the amounts present in personal care products, household products, cosmetics, sanitary products, wastewater effluents, river water, drinking water catchments areas, and drinking water. The environmental relevance of triclosan and its main metabolites in the environment calls for routine monitoring studies in order to control its presence in the environment and to assure water protection and food safety, with the utmost importance being placed on its control in marine and drinking water catchments areas.

Abbreviations

TCS: Triclosan **ppm:** parts per million **GCMS:** Gas Chromatography and Mass Spectrometry **PCP:** Personal care products **FDA:** Food and Drug Administration **EC:** Emerging contaminants **EIC:** Extracted ion Chromatogram **m/z:** mass to charge ratio **ppb:** parts per billion **conc:** concentration

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The relevant data and materials are available in the present study.

Competing interests: The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national). All institutional and national guidelines for the care and use of laboratory animals were followed

Funding: Not applicable.

Authors' contributions: VB supervised the entire project. Supervision of the laboratory work was performed by VB. VB analysed the data and wrote the manuscript. VB and PK did all experiment work. PK assist the experiment work.

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