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Study of Homosalate Stability in Chlorinated Water and Identification Chalogenated By-Products by Gas Chromatography-Mass Spectrometry.

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ABSTRACT

The increasing use of sun-creams containing organic UV-filters has led to increased concentration of these compounds in aquatic environment. Chlorinated water can convert these chemicals into chlorinated products whose toxic effects are of primary concern. The new compound may be more toxic than the starting primary compound. Many studies have shown that UV filters absorb UV light and decompose under solar irradiation, due to their unstable properties. This may lead to formation of certain by-products with harmful effects. Their decomposition products can cause allergic and toxic reactions to the human skin. This study follows the stability of most commonly used UV filters, homosalate, in conditions that include those existent in swimming pools. Stability of the homosalate in chlorinated water was studied in simulated swimming pool water samples. UV spectroscopy was used to follow the reaction of homosalate in presence of free chlorine. Water samples were filtered, acidified, and extracted by use of solid-phase extraction. Gas chromatography with mass spectrometry was used to identify the major transformation by-products. Under the experimental conditions, homosalate reacted with chlorine following zero order reaction. The chemical transformation of the homosalate in chlorinated water led to formation of chlorinated by-products that was identified as: monochloro-homosalate, dichloro-homosalate and two diastereoisomers monochloro-homosalate.

Keywords: homosalate, free chlorine, solid phase extraction (SPE), GC-MS, by-products

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INTRODUCTION

Sunlight on the skin surface is absorbed and may cause a variety of damages, such as burns and erythema. By using sunscreens, the skin burns are avoided, since UV filters absorb UV light instead of the skin and protect against the harmful effects of UV rays [1-4].

UV filters are organic compound with one or more aromatic rings, which are sometimes joined by double carbon-carbon bond of carbonyl group. It used for skin protection, light stability, durability and also to expand the shelf life of beauty cosmetics products such as creams, lipstick, skin lotion, hair spray, hair dyes, shampoos and etc. They can be found not only in cosmetics but also in many industrial products such as paints, plastics or textile materials, to prevent degradation of polymers and pigments [5].

Increased production and consumption of products containing organic UV filters cause their presence in the environment in significant concentration, especially in water. Humans can be exposed to UV filters through two pathways; directly through using cosmetic (dermal absorption) and directly through the food chain [6-11]. Recently, there is increasing public concern regarding secondary effects of personal care products. Therefore, UV filters belong to a new class of organic pollutants [12]. Secondary pollutants are created when primary pollutants (UV filters) react with other active substances, present in the environment [13]. The new compound may be more toxic than the starting primary compound. Many studies have shown that UV-filters absorb UV-light and decompose under solar irradiation, due to their unstable properties. This may lead to the formation of certain by-products with harmful effects. Their decomposition products can cause allergic and toxic reactions to the human skin. The most of the previous researches in this area were based on the examination of these substances in their native form, and a few authors investigated the by-products that may occur because of the UV filters increased concentration in a small area (the water in the pools) [14]. Sakkas et al. [15] studied the degradation kinetics of the UV filter 2-ethylhexyl 4-(dimethylamino) benzoate (EHPABA) in water and chlorinated swimming pool water, under natural and artificial sunlight irradiation. In the case of irradiation (60 h) of chlorinated swimming pool water, previously spiked with the UV filter, several mono- and dichlorinated forms of EHPABA have been identified. In a more recent study by Negreira et al. [16] the stability of three UV filters EHPABA, 2-ethylhexylsalicylate (ES) and 2-hydroxy-4-methoxybenzophenone (BP-3) were studied in chlorinated water samples at neutral pHs and in the presence of potassium bromide salt. For EHPABA only mono halogenated products were detected whereas for BP-3 mono and dichloro-halogenated products were identified. The extension of ES halogenation reactions was found to be negligible in the experimental conditions under study. Nakajima et al. [17] studied the reaction kinetics of EHPABA and 2-ethylhexyl-4- methoxycinnamate (EHMC) in chlorinated aqueous solutions and various mono- and di-chlorinated by-products have been identified by gas chromatography–mass spectrometry (GC-MS). It had been found that UV-filters react slowly with chlorine, which is the most commonly, used chemical oxidant for drinking water disinfection**.** Particularly, it is used for hygiene in swimming pools. Numerous transformation products may be formed, due to oxidation/substitution reactions [18, 19]. Therefore it was necessary to examine not only the presence of UV filters in the water, but also the presence and realistic estimation of by-products that may occur, with particular attention paid to the small water areas, such as swimming pools. Besides the potential risk for human health of the UV filter chlorinated by-products, swimming pool effluents can present an additional threat in the aquatic environment because they can have a negative effect in ecosystems after being discharged.

It was our purpose with this study to follow the stability of most commonly used UV filters, homosalate, in conditions that include those existent in swimming pools. As far as we know, information about stability of homosalate in presence chlorine is not available.

Homosalate is weaker UVB absorber so they must be used in relatively high concentration. These chemicals are used to increase the effect of other UVB absorbers. Homosalate is a member of Salicylates. Homosalate (homomenthyl salicylate; max, 306 nm) [20].

Homosalate chemical structure (HMS; CAS No.118-56-9) is given Figure **1**. It is a viscous or light yellow, to slight tan liquid or oil. It has a boiling point of161-165 °C. Homosalate is mixture of isomers, where one isomer is much more present then the other. There are two homosalate isomers mixtures used: usually a mixture of 15% cis and 85% trans form, as well as the 40% cis and 60% trans form [21].

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HMS is likely converted to salic and is highly photostable agents used to reduce photodegradation of other active sunscreen ingredients [20].

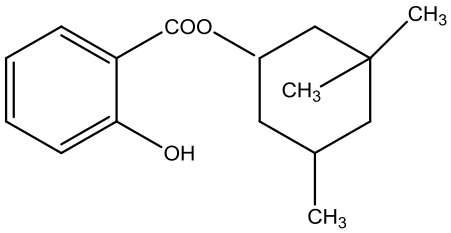

Figure 1: Chemical structure of Homosalate.

The kinetics of the reactions between the homosalate and chlorine in aqueous solution was studied UV/VIS spectrometry and the rate constants and half-lives, under specific experimental conditions, were determined. Solid phase extraction (SPE) coupled to gas–mass spectrometry (GC-MS) was used to identify the chlorination by-products. The experimental levels were selected according to expected values in swimming pool waters.

EXPERIMENTAL

Reagents, standards and materials

Standard Homosalate (HS) was purchased from Merck (Germany). Ethanol and methanol (HPLC grade) was acquired from Panreac Quimica (Espana), dichlormethane (HPLC grade) and ethyl acetate was from Sigma-Aldrich (Germany). A commercial sodium hypochlorite solution with chlorine content 2.5% was used in chlorination studies. This solution was stored at 4^0C and its exact concentration was determined by iodometric titration using standard procedures. Deionised water was used in all experiments. The pH of the solutions was adjusted to a pre-determined value with 1 M HCl. Pre-concentration of the water samples for GC-MS analysis was performed by Oasis HLB (500 mg) SPE cartridges were acquired from Waters (Ireland).

Study of the reaction kinetics of Homosalate (HS) in chlorinated water (spectrophotometric method)

For this study stock solution of HS (5 mg/ml) in ethanol were first prepared. The kinetics of the reactions were evaluated at room temperature (18 ± 2^0C) considering initial concentration of HS 500 ng/ml and free chlorine in the ranges of 0.2-0.6 µg/ml, respectively. The reactions were carried out in glass vessels containing 100 ml deionised water. Deionised water samples were spiked with a sodium hypochlorite solution to get the required initial concentration of free chlorine. Initial free chlorine content was determined using N, N-diethyl-p-phenylendiamine method with photometric detection. After that, samples were spiked with a solution of the HS in ethanol.

Experiment consists of two parts. In the first part, HS spectrum was recorder, without free chlorine addition, in order to determine absorption maximum. After that, HS solution spectrum with the largest concentration of free chlorine (0.6) was recorded, immediately after chlorine addition, after 30 minutes and after 24 hours. The aim was to determinate whether there is a change in HS spectral characteristics in the presence of free chlorine. The second part referred to determination order of chemical reaction of HS in the presence of free chlorine. Rank of chemical reaction has been determinate by monitoring the HS absorbance change in the presence of free chlorine in time, at the maximum absorption of HS.

Analytical procedure for identification of chlorinated by-products

The identification of the chlorine by-products was performed using reactions conditions similar to those used in the kinetics study. After 24 hours, the water sample (100 ml) were concentrated by SPE using Oasis HLB (500 mg) cartridges, previously conditioned with 5 ml of methanol followed by 5 ml of deionised water. The sample was adjusted at pH 3, passed through the SPE cartridge and the analyte were eluted with 5 mL of ethyl acetate. After this procedure the organic solvent was evaporated and 500 µL of dichlormethane

was added to the final residue for GC-MS analysis. The same procedure was carried out with a working solution of the HS without addition of free chlorine.

Spectrophotometric and GC-MS conditions

The reaction kinetic of HS in water containing chlorine was studied by UV-spectrophotometric in the ranges of 200-400 nm. HS and his by-products were identified by GC-MS using an Agilent 7890A gas chromatography connected to an ion-trap mass spectrometer Agilent 5973. Separations were carried out in a DB-5MS type capillary column (30 m x 0.25 m x 250 µm) purchased from JW Scientific. The temperature of the GC oven was programmed as follows: 100 C^0 (1 min.), rate at 15^0/min to 200^0C, rate at 15^0/min to 280^0C for 7 minutes. Helium (99.999 %) was used as carrier gas at a constant flow of 1 ml/min. The injector was maintained at 280^0C and injections (1µL) were made in the slitless mode with a purge time of 1 min.

RESULT AND DISCUSSION

Study of the reaction kinetics of HS in chlorinated water

At the beginning of the experiment the spectrum of the HS solution in ultra pure water was recorder in the wavelengths range 200-450 nm.

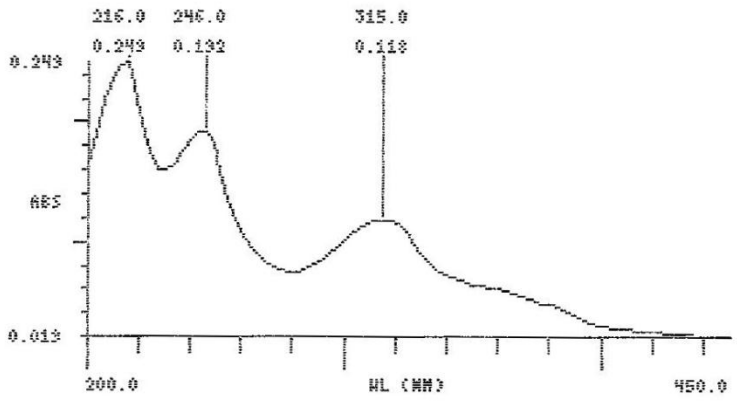

Figure 2: HS apsorbtion maximum in ultra pure water without free chlorine.

Recorded UV spectrum showed that, in ultra pure water, HS had two absorption maximums: 315 nm and 246 nm (figure 2.).

After that, HS solution spectrum was recorder in ultra pure water and free chlorine concentration of 0.6 µg/ml, after 30 minutes and 24 hours.

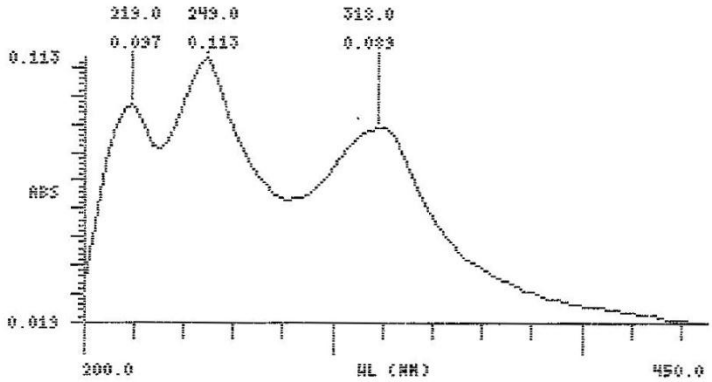

Figure 3: HS apsorbtion maximum in the presence of 0.6µg/ml free chlorine after 30 minutes

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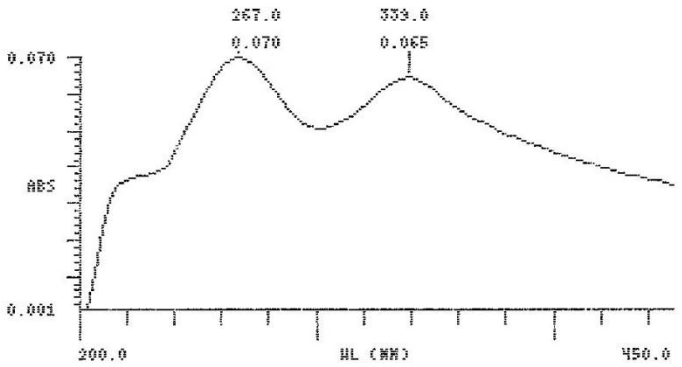
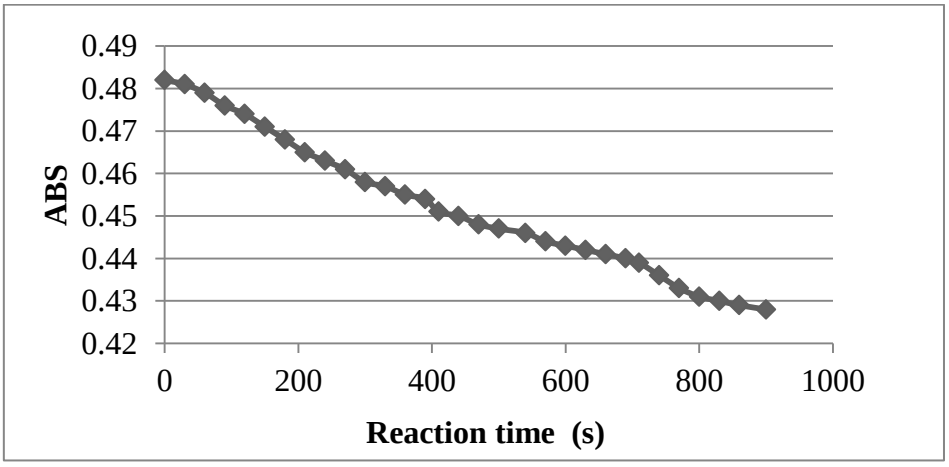


Figure 4: HS apsorbtion maximum in the presence of 0.6µg/ml free chlorine after 24 hours

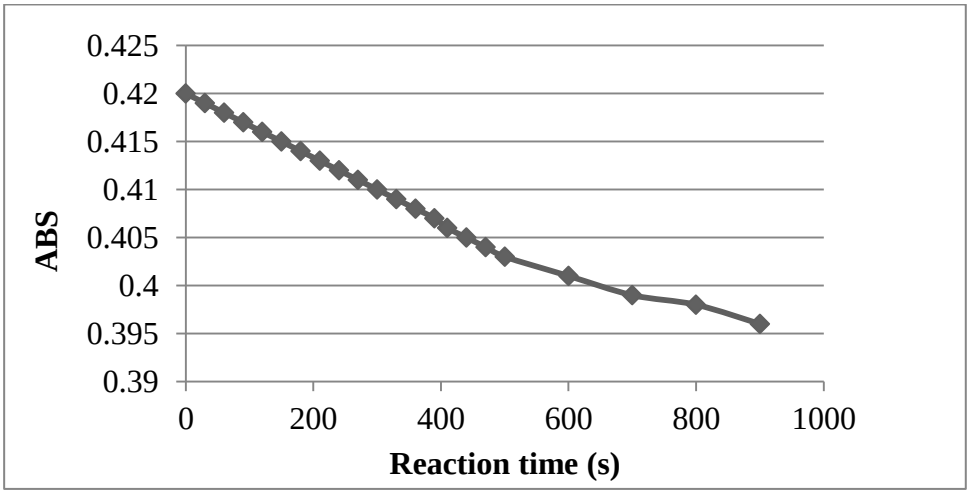
After 30 minutes, in the presence of 0.6 mg/ml of free chlorine, HS has maximum absorbance at 318 nm and 249 nm (Figure 3). 24 hours later, absorbance maximums for HS significantly changed. After 24 hours absorption peaks are at 339 nm and 267 nm (Figure 4).

Determination the rank of chemical reaction and half-time ($t_{1/2}$) for HS

In order to determine the half-life ($t_{1/2}$) for HS, first the rank of chemical was determined reactions. The rank of chemical reaction is determined by monitoring the change in absorbance (ABS) of HS in time, at the maximum absorption of HS (315 nm) in an aqueous solution and various concentrations of free chlorine.



Graph 1: 5000 ng/ml HS in presence of 0.2 µg/ml of free chlorine



Graph 2: 5000 ng/ml HS in presence of 0.6 µg/ml of free chlorine

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Graphs 1 and 2 shows the curves representing the change of HS absorbance (ABS) at different concentrations of free chlorine in time. From curves it is found that the reaction is of a zero rank compared to HS.

From the shape of curves obtained by monitoring the HS absorbance change in time at the tested concentrations of free chlorine (graph 1, 2), conclusion was made that reaction of HS was of a zero rank compared to investigated UV filter. This data tells us that the reaction between HS and free chlorine proceeded much more slowly. The appearance of curves indicates that the half-life time is relatively long. The obtained data show that HS is relative stable in the presence of free chlorine. These results were correlated with the results obtained at the beginning of the experiment (maximum absorption change in observed time).

Identification of chlorinated by-products

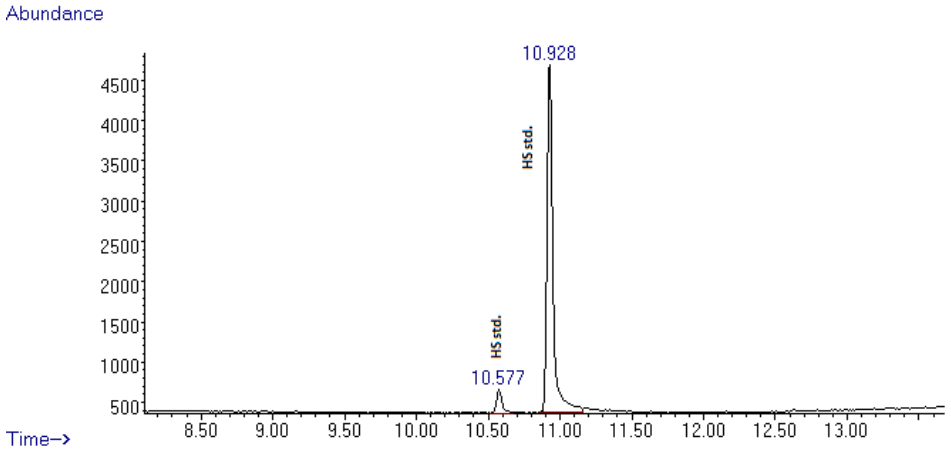

Figure 5: TIC chromatogram of standard homosalate

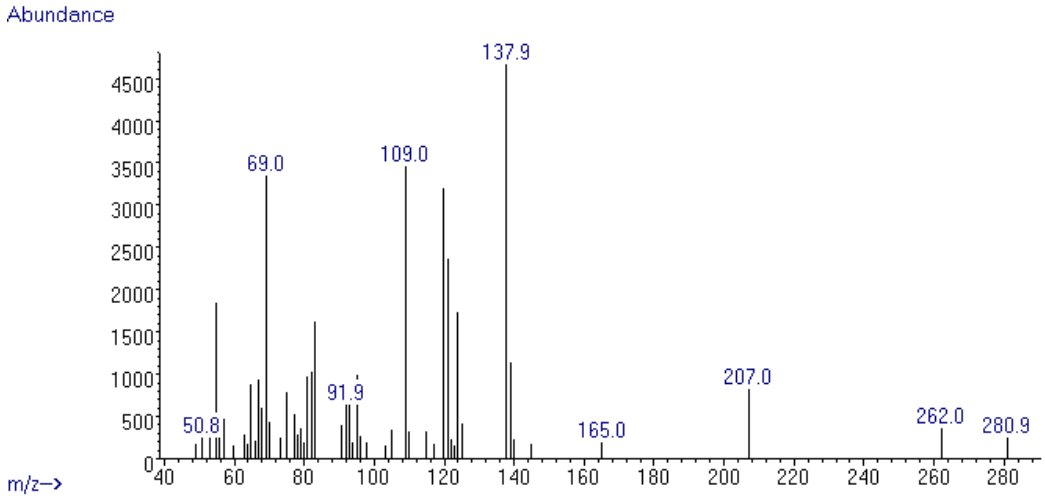

Figure 6: MS spectrum of standard homosalate

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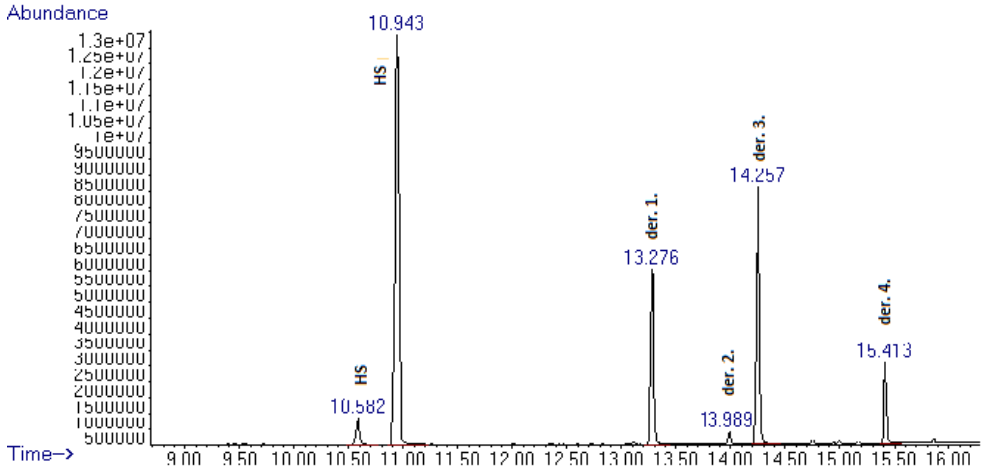

Figure 7: TIC chromatogram of homosalate by-products

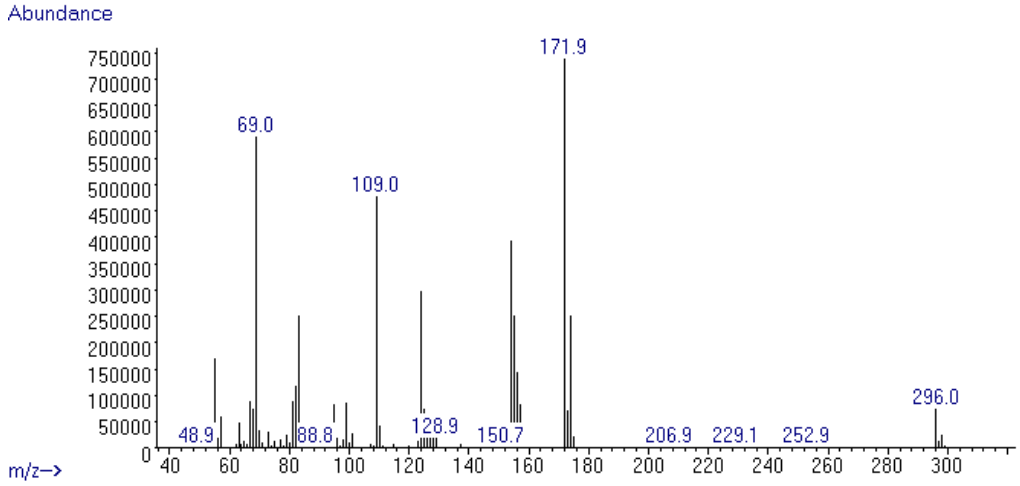

Figure 8: MS spectrum of homosalate by-product 1.

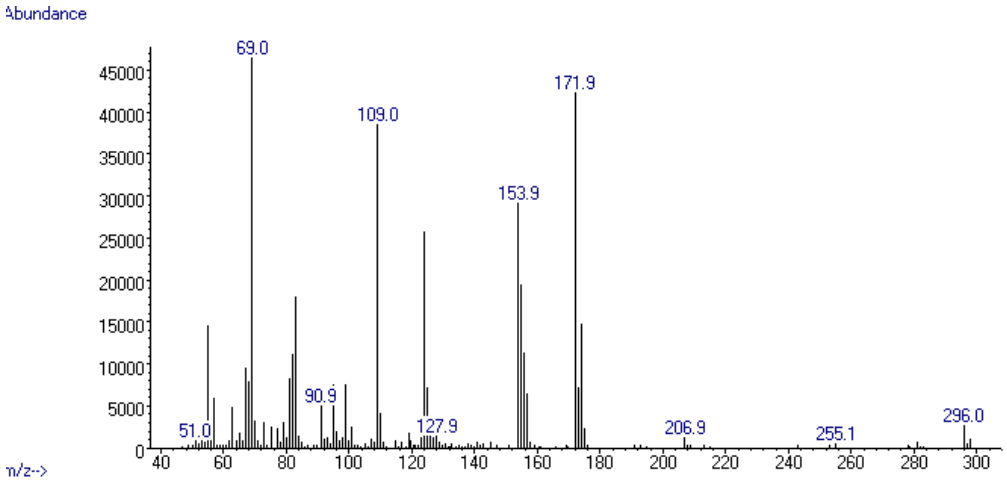

Figure 9: MS spectrum of homosalate by-product 2.

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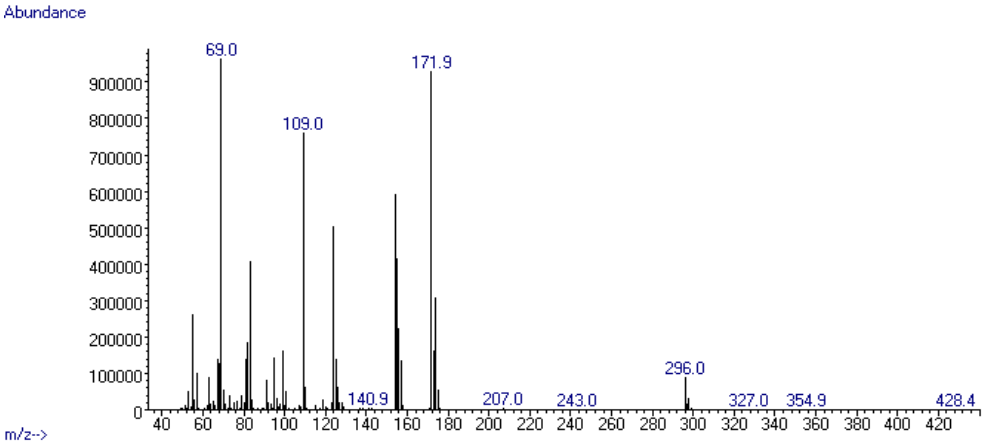
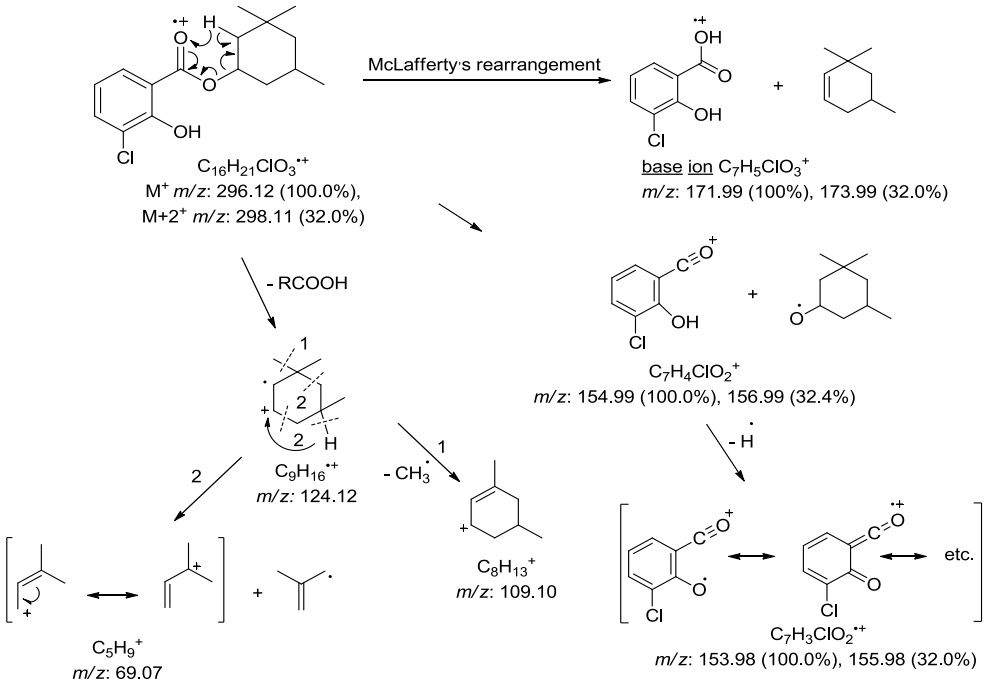


Figure 10: MS spectrum of homosalate by-product 3.



Scheme 1: Suggested structure and fragmentation path of by-products 1, 2 and 3, based on mass spectrogram

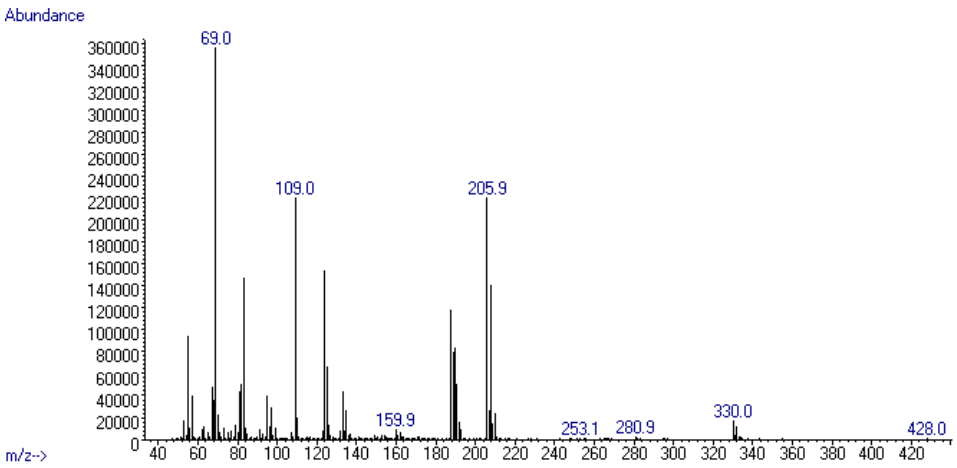


Figure 11: MS spectrum of homosalate by-product 4.

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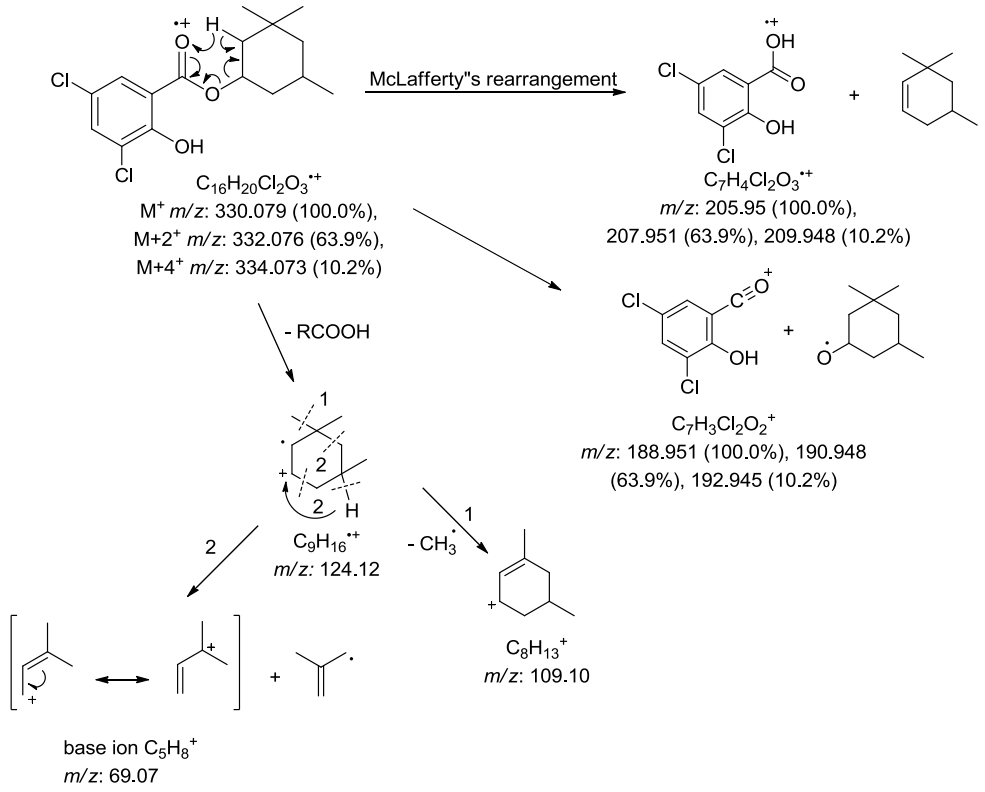

Scheme 2: Suggested structure and fragmentation path of by-product 4 based on mass spectrogram

Before identification of HS by-products, gas chromatogram of HS was recorded and retention time as well (Figure 5). From the gas chromatograms it can be seen that there are two peaks for homosalate (HS). First peak is at 10.577 minutes, and the second is at 10.928 minutes. Specifically, homosalate is a mixture of isomers, where one isomer is much more present then the other. There are two homosalate isomers mixtures used: usually a mixture of 15% cis and 85% trans form, as well as the 40% cis and 60% trans form [20]. This explains the emergence of two peaks at different retention times on the gas chromatograms. By comparing the HS mass spectrograms (Figure 6) with the mass spectrograms from electronic databases (PMW Tox3.1; WileY7Nist05.L), HS standard identification was performed. The difference in structure cannot be determined on basis of mass spectrums. It is supposed that there are two diastereoisomers (two chiral centres in the cyclohexene part). Molecular ion (M+) m/z 262.16 corresponds to a molecular weight of homosalate. The base ion at mass spectrograms was m/z 137.9, which appeared by McLafferty's rearrangement in a homosalate molecule. Other ions in the mass spectrogram, on which the structures of homosalate were confirmed, were: m/z 121.03, m/z 120.02, m/z 109.10 and m/z 69.07.

After this, gas chromatograms of sample containing the HS by-products were recorded. At the gas chromatogram six peaks are visible (Figure 7). At the retention time 10.582 minutes and 10.943 minutes two peaks are present, whose retention time corresponding HS standard. Spectrograms of mass corresponding to the mass spectrograms of HS standard (Figure 6) also confirmed that it was about parent HS. Due to the action of free chlorine on the HS molecule, one part remained in its original form, while one part reacts with free chlorine.

At the time of retention of 13,276 minutes, there is a peak of the first by-product of HS. Ion M+2 m/z 296.12/298.11 was determinate according to the mass spectrogram (Figure 8). The ratio of signal intensity in M+ ion is 3:1, which indicates that it is monochlorine by-product. According to the mass spectrogram, fragmentation path was suggested, which in the first step included McLafferty's rearrangement in by-product 1. molecule and appearance of the base ion m/z 171.99/173.99.

It is followed by ions m/z 154.99/156.99, m/z 153.98/155.98, m/z 109.10 and m/z 69.07. According to fragmentation of by-product 1. it was concluded that it was about molecule of monochlorine homosalate (Scheme 1). Position of chlorine could not be experimentally confirmed. The assumption is that the chlorine

may be in a position *ortho* or *para* in regard to the OH group (*ortho* and *para* direction) in the homosalate molecule.

At retention times 13.989 minutes and 14.257 minutes, there are two peaks, recognized as by-product 2. and by-product 3. According to analysis of mass spectrograms of by-product 2. and by-product 3. (Figures 9 and 10), it was concluded that their mass spectrograms very similar mass spectrogram of by-product 1. M+2 ion is m/z 296.12/298.11 on both by-products. The difference between the mass spectrogram of by-product 1. and by-products 2. and 3. is in the base ion. Basic ion in the by-product 1. is m/z 171.9, while in by-product 2. and 3. it is m/z 69. In this case it is about diastereoisomer of monochlorine homosalate. Given the small amount of by-product 2. the assumption is that it arises from the less represented isomers in the initial HS standard.

A by-product 4. is present on the gas chromatogram retention time of 15.413 minutes (Figure 2). According to mass spectrogram of by-product 4. analysis, ions M+ m/z 330.079, M+2 m/z 332.076 and M+4 m/z 334.073 were identified (Figure 11). The ratio of signal intensity in the molecular ion was 9:6:1. These data indicate presence of two chlorine atoms in the molecule of by-product 4. According to mass spectrogram, the structure and fragmentation path of by-product is suggested 4 times (Scheme 2). In the first step, there is a McLafferty's rearrangement, whereat fragment m/z 205.95/207.951/209.948 occurs. After that, the following fragments are m/z 188.951/190.948/192.945, m/z 109 and base ion m/z 69.07.

Based on these data, we have come to the conclusion that the by-product 4. is dichlorine-homosalate. The exact position of the chlorine atom could not be determined. The assumption is that one chlorine atom is situated in the ortho and the other in the *para* position relative to the OH group.

CONCLUSION

In this paper, stability of the homosalate in swimming pool water was investigated. Results showed that HS had relative stability in the tested concentrations of free chlorine. After an extended period (24 hours), a change of its spectroscopic characteristics occurred due to the reaction with chlorine. According to the recorded spectra, it was found that in the presence of free chlorine, after 24 hours HS converted into the products that have different absorption maxima of the absorption maxima HS, which was recorded without the presence of chlorine.

Based on the gas chromatogram and mass spectra comparing of the HS standard and samples of HS with free chlorine, it was found that the reaction of HS with free chlorine gives the following derivatives: monochloro-homosalate, dichloro-homosalate and two diastereoisomers monochloro-homosalate. The position of the chlorine in the molecule of HS derivatives could not been confirmed experimentally.

From the point of view of the water quality of swimming pools where people use UV filters this work is quite relevant because the UV filters and their degradation products accumulate in the bathing water raising health concerns. Environmental risk can also arise after discharge of the swimming pool effluents into aquatic systems. Future work should investigate the presence of these chlorinated by-products in swimming pool water and waste water, and address potential risks for human health due to dermal contact and evaluate possible environmental toxic effects.

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