KINETICS AND MECHANISMS OF DEGRADATION OF SELECTED ENVIRONMENTAL PHARMACEUTICALS BY AQUEOUS CHLORINATION

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ABSTRACT

The occurrence of pharmaceuticals as pollutant in the environment has been widely reported across the world. It has been a growing concern due to it negative impacts of these pharmaceuticals on the ecosystem and living organism. During conventional water treatment processes, untreated pharmaceuticals are often exposed to chemical oxidation reaction during disinfection process. Among various disinfection methods, chlorination is one of the most commonly used methods. The reactive species in the chlorination process is hypochlorous acid (HOCl) which is known to react with organic pollutants.

The main objectives of this study were to determine the second-order rate constants (k_{app}) for the reaction between selected pharmaceuticals with free available chlorine (FAC) and identification of the transformation by-products generated from the chlorination of selected pharmaceuticals. According to the identified transformation by-products, the mechanism of the transformation pathway of selected pharmaceuticals in chlorination was elaborated. The efficiency of chlorination in the removal of selected pharmaceuticals in different matrices was also evaluated. The selected pharmaceuticals for this study were acebutolol and sotalol (β-blockers), mefenamic acid (nonsteroidal anti-inflammatory drugs), sulfacetamide and sulfanilamide (antibiotics). These pharmaceuticals have been frequently detected in the aquatic environment.

In the kinetics study, k_{app} for the reaction between selected pharmaceuticals and FAC were determined at 25 ± 0.1 °C. The result indicated that the degradation of selected pharmaceuticals by free FAC was highly pH dependence at the selected pH range. At pH 6 to 8, it was found that k_{app} of Abt, Stl, Mfe, Sfa and Sfn was ranged from 0.03 to 0.19 $M^{-1}s^{-1}$, 0.93 to 0.65 $M^{-1}s^{-1}$, 16.4 to 4.35 $M^{-1}s^{-1}$, 4.50 to 4.50 $M^{-1}s^{-1}$ and 2.20

to 0.97 $M^{-1}s^{-1}$, respectively. The elimination of the selected pharmaceuticals in different water matrices (lake water, ground water and secondary effluent) showed that the efficiency of chlorination in the pharmaceuticals removal was retarded by high TOC and COD concentrations. Chlorination showed the highest efficiency in the removal of selected pharmaceuticals containing in ground water which has the lowest TOC and COD concentrations. However, in secondary effluent that containing higher concentrations of COD and TOC, the percentage removal of selected pharmaceuticals was relatively poor as compared with other water matrices.

Characterization of the transformation by-products formed during the chlorination of selected pharmaceuticals was carried out using liquid chromatography coupled to quadrupole time-of-flight high resolution mass-spectrometry. The transformation by-products were determined after 24 h of FAC exposure. The result indicated that chlorination of pharmaceuticals could produce various transformation by-products. Overall, 18 transformation by-products were identified for the selected pharmaceuticals. These transformation by-products were mainly formed through the hydroxylation, chlorination, oxidation and dealkylation reactions.

In conclusion, this research showed that pharmaceuticals could react with HOCl during disinfection process. The reactivity of the selected pharmaceuticals towards the reaction with HOCl is highly pH dependent at the common pH range of natural water. Chlorination might remove some of the pharmaceuticals however its efficiency was depending on the characteristic of water matrices. Chlorination of pharmaceuticals was also found to form various transformation by-products.

ABSTRAK

Pencemaran alam sekitar yang melibatkan farmaseutikal telah dilaporkan secara meluas di seluruh dunia. Ini menimbulkan kebimbangan yang semakin meningkat disebabkan oleh kesan negatifnya ke atas ekosistem dan organisma hidup. Semasa proses rawatan air konvensional, farmaseutikal yang tidak dirawat seringkali terdedah kepada tindak balas pengoksidaan kimia semasa proses pembasmian kuman. Antara berbagai kaedah pembasmian kuman, pengklorinan adalah salah satu kaedah yang biasa digunakan. Spesies reaktif dalam proses pengklorinan adalah asid hipoklorus (HOCl) yang juga boleh bertindak balas dengan bahan pencemar organik. Objektif utama kajian ini adalah untuk menentukan pemalar kadar kedua (k_{app}) bagi tindak balas antara farmaseutikal yang dipilih dengan klorin bebas dan mengenalpasti produk sampingan yang terhasil daripada proses pengklorinan farmaseutikal. Dalam kajian ini, mekanisma bagi transformasi farmaseutikal yang dipilih semasa pengklorinan telah dihuraikan berdasarkan kepada produk sampingan yang dikenalpasti. Kecekapan pengklorinan dalam penyingkiran farmaseutikal yang dipilih dalam berbagai sampel air (air tasik, air bawah tanah dan efluen sekunder) yang berbeza juga telah dikenalpasti. Farmaseutikal yang dipilih untuk kajian ini ialah acebutolol dan sotalol (penyekat beta), asid mefenamik (ubat antiradang bukan steroid), sulfacetamide dan sulfanilamide (antibiotik). Farmaseutikal ini sering kali dikesan dalam persekitaran akuatik.

Dalam kajian kinetik, k_{app} bagi tindak balas antara farmaseutikal yang dipilih dengan klorin telah ditentukan pada suhu 25 ± 0.1 ° C. Hasilnya menunjukkan bahawa kadar tindak balas antara farmaseutikal yang dipilih dengan klorin bebas adalah bergantung pada keadaan pH yang dipilih. Pada pH 6 hingga 8, didapati bahawa k_{app} untuk Abt, Stl, Mfe, Sfa dan Sfn adalah dalam julat 0.03 hingga 0.19 $M^{-1}s^{-1}$, 0.93

hingga 0.65 $M^{-1}s^{-1}$, 16.4 hingga 4.35 $M^{-1}s^{-1}$, 4.50 hingga 4.50 $M^{-1}s^{-1}$ dan 2.20 hingga 0.97 $M^{-1}s^{-1}$. Pengklorinan farmaseutikal yang dipilih dalam air tasik, air bawah tanah dan efluen sekunder menunjukkan bahawa kecekapan pengklorinan dalam penyingkiran farmaseutikal yang dipilih dipengaruhi oleh nilai TOC dan COD. Pengklorinan menunjukkan keberkesanan paling tinggi dalam penyingkiran farmaseutikal yang dipilih yang terkandung di dalam air bawah tanah. Walau bagaimanapun, dalam efluen sekunder yang mengandungi kepekatan COD dan TOC yang lebih tinggi, penyingkiran farmaseutikal yang dipilih adalah tidak berkesan.

Pencirian produk sampingan yang terbentuk semasa pengklorinan farmaseutikal yang dipilih telah dijalankan dengan menggunakan kromatografi cecair spektrometer jisim resolusi tinggi. Produk sampingan ini ditentukan selepas didedahkan kepada klorin bebas selama 24 jam. Secara keseluruhannya, 18 transformasi produk telah dikenalpasti. Transformasi produk ini dibentuk melalui hidroksilasi, pengklorinan, pengoksidaan dan tindakbalas pendealkilan.

Kesimpulannya, kajian ini menunjukkan bahawa farmaseutikal boleh bertindak balas dengan asid hipoklorus semasa proses pembasmian. Kecenderungan farmaseutikal yang dipilih untuk bertindak balas dengan asid hipoklorus sangat bergantung kepada pH. Kecekapan pengklorinan juga didapati bergantung kepada jenis sampel air. Pengklorinan farmaseutikal juga membentuk pelbagai produk sampingan termasuk produk yang mengandungi klorin.

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Abt	:	Acebutolol
Stl	:	Sotalol
Mfe	:	Mefenamic acid
Sfa	:	Sulfanilamide
Sfn	:	Sulfanilamide
FAC	:	Free available chlorine
phar	:	Pharmaceuticals
k_{app}	:	Second-order rate constant
k_{obs}	:	First-order rate constant
WWTPs	:	Wastewater Treatment Plants
EE2	:	17α-ethinyloestradiol
E2	:	Oestrogen oestradiol
$t_{1/2}$	:	Half-life
VTG	:	Vitellogenin
GST	:	Glutathione-s-transferase
CO_2	:	Carbon dioxide
O_2	:	Oxygen
COD	:	Chemical Oxygen Demand
BOD	:	Biological Oxygen Demand
TOC	:	Total Organic Content
TSS	:	Total Suspended Solids
H_2O	:	Water

μGAC	:	Micro-grain activated carbon
THMs	:	Trihalomethanes
HAAs	:	Haloacetic acids
DCAcAm	:	2,2-dichloroacetamide
HOCl	:	Hypochlorous acid
OCl^-	:	Hypochlorite ion
NSAIDs	:	Nonsteroidal anti-inflammatory drugs
B-blockers	:	Beta-blockers
HPLC	:	High Performance Liquid Chromatography
LC	:	Liquid Chromatography
QTOF-MS	:	Quadrupole time-of-flight-mass spectrometry
ESI	:	Electrospray ionization

CHAPTER 1

CHAPTER 1

INTRODUCTION

1.1 Pharmaceuticals in the Environment

Pharmaceuticals are chemicals manufactured as medical drugs to improve human and animal health (Jelic et al., 2011; Sim et al., 2011). However, pharmaceuticals have also been found to pollute the environment due to its frequently used (Dong et al., 2015; Andreu et al., 2016). Research on the pharmaceuticals in the environment was published as early as 1970's (Tabak and Bunch 1970; Hignite and Azarnoff, 1977). The most noticeable issue on the pharmaceuticals pollution came in 2007. Since then, the presence of a wide variety of pharmaceuticals compounds (e.g. analgesics, antidepressants, β-blocker, antibiotics, anti-inflammatory, etc.) in the water environment all over the world have been frequently reported (Huerta-Fontela, et al., 2011; Jurado et al., 2012; Soufan et al., 2012; Gatica and Cytryn, 2013; Barbosa et al., 2016).

The major issue with pharmaceuticals in the environment is the continuous rising of its level (Verlicchi et al., 2010) and due to this reason, it has been classified as one of the major emerging water pollutants (Ahrer et al., 2001; Cahill et al., 2004; Westerhoff, et al., 2005; Lacina et al., 2013; Sun, et al., 2014; Jung, et al., 2015). Table 1.1 shows the distribution of several main classes of pharmaceuticals detected in different types of water bodies around the world. These water samples are include surface water, municipal wastewater,

underground water and drinking water. Due to their physico-chemical properties such as high water solubility and often poor degradability, pharmaceuticals are often penetrated through the surface of soil and lastly enter the groundwater, lake water and surface water (Jorgensen and Halling-Sorensen, 2000; Roberts et al., 2006; Buth et al., 2007; Ganiyu et al., 2015). In addition, poor efficiency of wastewater treatment plants in the removal of pharmaceuticals also contributed to the pharmaceuticals in the environment through the discharging of effluent. As example, estrogenic hormones were often detected in the surface water and river water due to the presence of pharmaceuticals in the effluents of wastewater treatment plants (WWTPs). The results showed that the concentration of estrogen was 220 ng/L in the effluent of WWTPs and 26 ng/L in the corresponding receiving river water (Gabet-Giraud et al., 2014). Drinking water treatment facilities was also found to be unable to completely remove the pharmaceuticals residues as well (Boyd et al., 2003; Chamberlain and Adams, 2006; Chang et al., 2012; Lacey et al., 2012; Wang et al., 2015). Kleywegt et al., (2011) found that 17 up of 48 targeted pharmaceuticals were detected in the finished drinking water in Canada. Benotti et al., (2009) reported that 9 up of 17 targeted pharmaceuticals were appeared in finished drinking waters of United States. Vulliet et al., (2011) discovered 18 up of 51 targeted pharmaceuticals in finished drinking water in France and Huerta-Fontela et al., (2011) reported 5 up of 55 targeted pharmaceuticals in the finished drinking water of Spain.

Table 1.1: Concentration of different classes of pharmaceuticals detected in different water bodies.

Pharmaceuticals	Surface water (ng/L)	Municipal waste water (ng/L)	Under ground water (ng/L)	Drinking water (ng/L)	River water (ng/L)	Country
Antibiotics						
Erythromycin[a]	292					Serbia
Ofloxacin[a]		220				Serbia
Trimethoprim[a]	8.1	259				Serbia
Azithromycin[e]			68			Serbia
Oxytetracylin[g]	50					Germany
Sulfamethoxazole[g]	20					Germany
Lincomycin[h]	250					Italy
Clarithromycin[i]	20					Italy
Psychiatric						
Carbamazepine[f]					25.3	China
Diazepam[f]				1.9	24.3	China
Oxazepam[f]					3.3	China
Estazolam[f]					1.2	China
Temazepam[f]				0.2	1.3	China
Alprazolam[f]				2.4	2.5	China
Lipid regulators						
Bezafibrate[e]			4.2			Spain
Gemfibrozil[e]			15.5			Spain
Beta-blockers						
Sotalol[a]		91.3		0.4		Serbia
Propranolol[a]	10.4	78.5				Serbia
Metoprolol[b]				0.6		China
Atenolol[c]				3		Spain

Table 1.1 (*continued*)

Pharmaceuticals	Surface water (ng/L)	Municipal waste water (ng/L)	Under ground water (ng/L)	Drinking water (ng/L)	River water (ng/L)	Country
Anti-inflammatories						
Ketoprofen[a]	45	247				Serbia
Phenazone[a]	12.5	13.5	23.4			Serbia
Codeine[a]	7.3	1017				Serbia
Ibuprofen[d]				5		Spain
Salicylic acid			2.5			Serbia
Naproxen[e]			27.6			Serbia

[a]Petrović et al., 2014 [b]Sun et al., 2015 [c]Huerta-Fontela et al., 2011 [d]Gros et al., 2012 [e]Sui et al., 2015 [f]Wu et al., 2015 [g]Hirsch et al., 1999 [h]Castiglioni, 2004 [i]Calamari et al., 2003

Eventhough the concentration of pharmaceuticals in the environment are very low in most of the cases (in between ng/L to µg/L) (Boyd et al., 2003; Dodd and Huang, 2007; Kasprzyk-Hordern et al., 2008; Lin et al., 2011; Bulloch et al., 2015) but their potential negative effect is an important issue as certain pharmaceuticals such as carbamazepine, citalopram, diazepam, lorazepam and atenolol (Moreno-González et al., 2016) can accumulate in living organism (Prasse et al, 2015; Lin et al., 2010) and remain bioactive at very low concentration. In addition, the continuous released of pharmaceuticals to the environmental may lead to high long-term concentration and promote to unnoticed adverse effects to the aquatic and terrestrial organisms (Petrović et al., 2005; Deblonde et al., 2011; Bulloch et al., 2015).

Researchers have reported various negative impact of pharmaceuticals to living organism such as generating antibiotic-resistant bacteria, transgender and growth inhibition (Hedgespeth, et al., 2012; Mohanta and Goel, 2014; Cizmas et al., 2015). A study by Borgatta et al. (2015) showed that the reproduction of *Daphnia pulex* dropped off after exposing to endoxifen and 4-hydroxy-tamoxifen. The concentration of endoxifen and 4-hydroxy-tamoxifen that affect the reproduction of *Daphnia pulex* were about 51.8 μg/L and 23.8 μg/L, respectively. This result showed that pharmaceuticals can be harmful to living organisms even at a very low concentration. These findings indicated that cautions must be taken to prevent the negative impact of pharmaceuticals to the living organism even at a very low concentration. In addition, biological effect of synthetic oestrogen, 17α-ethinyloestradiol (EE2), to the fish was also frequently reported. EE2 has been frequently detected at low level (ng/L) in surface water and WWTP effluents (Kolpin et al., 2002). EE2 has lower solubility than natural oestrogen oestradiol (E2) and is more persistent in aquatic environment with the estimated half-life ($t_{1/2}$) between 1.5 to 17 days (Zuo et al., 2006; Jürgens et al., 2002). This pharmaceutical has been shown to induce feminization in male fish; induction of the female yolk precursor, vitellogenin, (VTG) in male fish; formation of a female reproductive duct in the testis and induction of intersex (Corcoran, et al., 2010). Another research on EE2 that carried out at Canada's Experimental Lake, have shown the delayed in sperm cell development in fish and the male fish started to produce eggs after exposing to the discharged municipal wastewaters containing 5 ppt of EE2 for a year. As a result, the fish populations were reduced drastically (Kidd et al., 2007). The other study by Metcalfe et al., (2001) also showed that the presence of estrogens

that discharged in sewage treatment plant effluents may be responsible for the feminization of fish as well. Also, the 1.5 μg/L of gemfibrozil (human lipid regulator) was found to decrease testosterone levels by 50% after 14 days of exposure. This study provides the strong evidence that this compound may be function as an endocrine disruptor in fish (Mimeault et al., 2005). Therefore, it can be concluded that the exposure of relevant concentration of a pharmaceutical to an environmentally can induce adverse biologically effects to aquatic organisms.

The other researches by Rosen et al., (1988), Huggett et al., (2002), and Owen et al., (2007) on the toxicity of propranolol showed that the growth of a (*Japanese rich fish*) *Japanese medaka* was significantly reduced after 14 days exposure at the concentration of 0.5 mg/L. Although, two weeks exposure of *Japanese medaka* to propanolol with the concentration of ≤ 0.5 mg/L did not result in the decreased of egg production, but it was observed that the male and female plasma steroid levels were significantly decreased and their plasma estradiol levels were increased significantly. Another study by Maszkowska et al., (2014) towards propranolol on algae showed that propranolol was one of the most toxic pharmaceuticals to green algae which EC_{50} value was 24 mg/L. EC_{50} is the concentration required to achieve 50% of maximum effect. Besides, it is also used to evaluate the suitability and the performance of the pharmaceuticals (Sebaugh, 2010).

In terms of cytotoxic and oxidative effect of pharmaceuticals, it was found that estradiol-17β can cause oxidative damage to the rainbow trout's liver

(Gagné et al., 2006). The antidepressant such as fluoxetine was found to slow down the development of frogs and fishes (Halford, 2008). Anticonvulsant such as carbamazepine was found to influence the emergence of insects which is an important food for certain fish (Halford, 2008). Guiloski et al. (2015) conducted a study to assess the effects of exposing male fish (*Hoplias malabaricus*), to prey species that contaminated by diclofenac and dexamethasone. Twice per week, the fish were fed with *Astyanax sp.* that has been given intraperitoneal injections of either diclofenac or dexamethasone. The results showed the decreased of glutathione-s-transferase (GST) activity in the liver of *Hoplias malabaricus* and at the same time the decreased levels of testosterone were observed (Guiloski et al. 2015; Cizmas et al., 2015). Moreover, the studies from Hoeger et al., (2005) demonstrated that diclofenac residues have the potential to adversely affect various tissues such as gills and kidneys in brown trout at concentrations close to those regularly found in surface waters.

Ecotoxicity of antibiotics have been frequently reported. For example, Huang et al. (2014) revealed the potentially adverse effects of sulfamonomethoxine on the microalgae (*C. vulgaris and I. galbana*). The 72-h EC_{50} values of sulfamonomethoxine for *C. vulgaris* and *I. galbana* were 5.9 and 9.7 mg/L respectively. These results also showed that sulfamonomethoxine affected the growth of the selected microalgae. Based on the environmental hazard assessment and classification of the European Commmunity, a chemical can be assumed as toxic to aquatic organisms when the EC_{50} of the chemical is between 1 to 10 mg/L (Carlsson et al., 2006; Torres et al., 2015). The growth inhibitions of freshwater green algae also have been reported by Eguchi et al.,

(2004). Erythromycin showed the growth inhibition of *S. capricornutum* at EC_{50} of 0.037 mg/L. Therefore, Erythromycin is expected to cause damage to green algae in the ecosystem if they were released into the environment even at low concentrations.

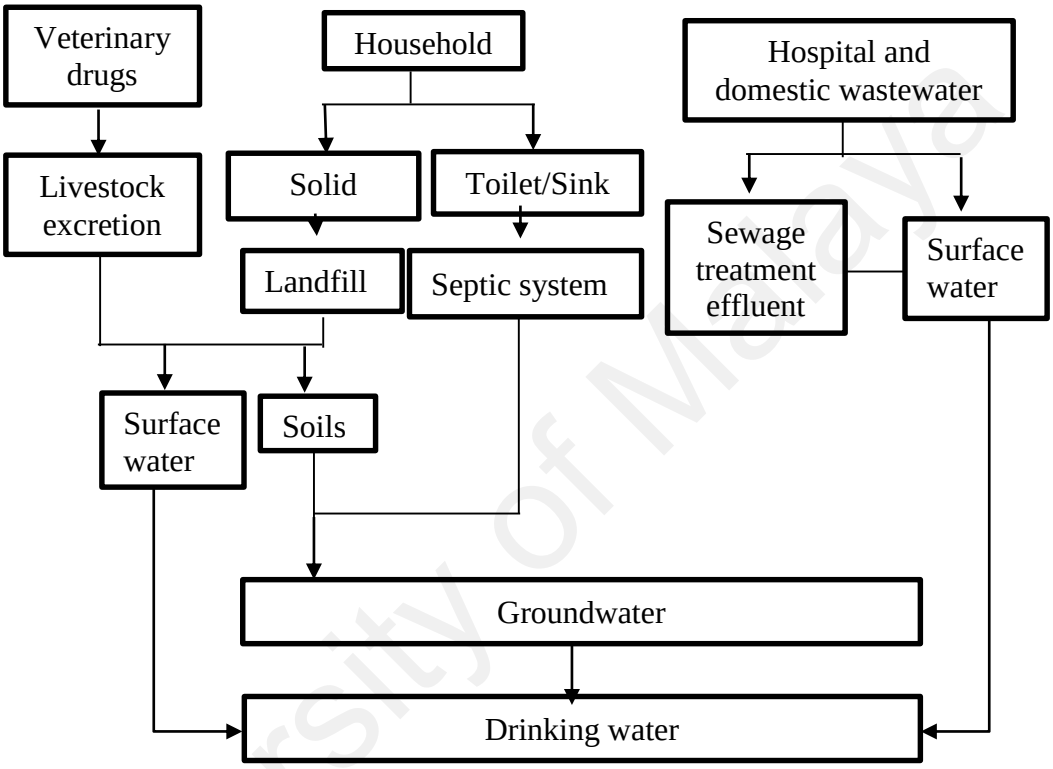


Figure 1.1: The possible sources of pharmaceuticals in the environment.

Figure 1.1 shows the main possible sources of pharmaceuticals in the environment. These sources are including veterinary drugs, household, hospital and domestic wastewater. Pharmaceuticals from household can enter the sewer system through several pathways such as excretion from human body through urine and fecal, or flushing of unused medications and washing-off medications via sinks or washrooms (Halling-Serensen et al., 1998; Heberer, 2002; Hilton and Thomas, 2003; Thomas and Hilton, 2004; Ellis, 2006; Kasprzyk-Hordern et

al., 2008; Musson and Townsend, 2009; Aukidy et al., 2012; Wang et al., 2015; Gonzalez-Marino et al., 2015). Nowadays, the most common practice for the disposal of household waste is landfilling. Landfills are the final depositories for various solid and semi-solid wastes which include household unwanted medications. Once discarded into landfills, pharmaceuticals may either decomposed by microorganisms, absorbed to the solid wastes and dissolved in landfill leachates (Musson and Townsend, 2009). The landfill leachate is a liquid that percolating through the waste within the landfill site. This liquid medium dissolved the nutrients, heavy metals, and organic contaminants including pharmaceuticals and capable in polluting the environments (Shehzad et al., 2015). In addition, the anaerobic condition in landfills are likely to slow down the biodegradation of organic compounds in leachate and allow the pharmaceuticals to reach the environment. Hence, the abundance and persistence of pharmaceuticals are detected in water sample such as groundwater due to the leachate contaminations (Kosjek et al., 2009).

Meanwhile, septic system can also be one of the important sources for pharmaceuticals in surface water and ground water. A septic system is a small-scale sewage treatment system commonly seen in rural areas with no connection to main sewage pipes. It allows water used domestically to be treated and recycled to substitute local groundwater supplies (Sui et al., 2015). Incomplete treatments in septic system often allow the pharmaceuticals to percolate through vadose zone of soils and enter the groundwater (Schaider et al., 2016). The leaky sewers are also proposed as one of the potential sources for the pharmaceuticals contamination in urban aquifers. The defected sewer pipelines may relate to the

long-time overuse without repair, as well as poor materials and negligence during the construction. Hence, a variety of pharmaceuticals may enter to soil zone through exfiltrating sewage with great potential to impact groundwater.

The used of veterinary drugs has also become a growing concern and has been identified as the pathway for environmental pharmaceuticals contamination (Kim et al., 2008; Bártíková, et al., 2016; Vassilis, et al., 2016). For example, antibiotics is one of the most commonly used pharmaceutical as veterinary drugs. The veterinary antibiotics such as tetracycline, sulfadiazine, sulfamethizole and sulfamethazine are often not completely metabolized and it can be excreated by the feedstock through urine and fecal. Kim et al., (2011) reported that approximately 50-100% of the antibiotics are excreted through urine and feces and then released to the environments by the feedstocks (Kim et al., 2011). Therefore, veterinary antibiotics are commonly transported from the farm area to the soils, ground water and surface water. The extensive and continuous release of pharmaceuticals from household, veterinary drugs and industrial and hospital wastewaters is a potential risk to non-target organisms due to the ubiquitous occurrence of pharmaceuticals in the environment (Wen et al., 2014).

1.2 Wastewater Treatment Plants (WWTPs) and Environmental Pharmaceuticals Pollution

Among various sources, the effluents of WWTPs have been classified as one of the most important sources of pharmaceuticals in the aquatic environment

(Daouk et al., 2016). Pollution occurred when treated wastewaters containing various chemicals including the pharmaceuticals are directly discharges into water bodies without undergo proper water treatment. Consequently, pharmaceuticals have been found to pollute the receiving surface waters with pharmaceuticals (Mompelat et al., 2009; Acero et al., 2013; Gonzàlez-Mariño et al., 2015). As reported by USEPA (2010) and Luo et al., (2014), there are no municipal sewage treatment plants that are engineered specifically for pharmaceuticals removal (USEPA, 2010; Luo et al., 2014). Therefore, the removal of pharmaceuticals from treatment plants varies based on the chemical properties of pharmaceuticals and the available individual sewage treatment facilities.

Table 1.2 shows the concentration of pharmaceuticals in the influent and effluents of WWTPs. These studies have investigated the removal efficiency of pharmaceuticals by various WWTPs. These WWTPs consisted of conventional preliminary treatment, primary treatment, secondary treatment, tertiary treatment and disinfection process. The result clearly indicated that the commonly found pharmaceuticals are not completely removed by conventional WWTPs.

Table 1.2: Concentrations of pharmaceuticals in the influents and effluents of wastewater treatment plants.

Pharmaceuticals	Influents (µg/L)	Effluents (µg/L)	Removal rate (%)	References
β-blockers				
Acebutolol	0.34	0.14	58.2	Deblonde et al., 2011
Atenolol	2.64	0.82	69	Gros et al., 2012
Propanolol	0.17	0.09	47	Gros et al., 2012
Nadolol	0.04	0.01	75	Gros et al., 2012
Sotalol	1.67	0.79	52.6	Deblonde et al., 2011
NSAIDs				
Mefenamic acid	17	5	70.5	Lacey et al., 2012
Diclofenac	1.48	0.17	88.6	Bo et al., 2015
Naproxen	0.39	0.16	59.7	Bo et al., 2015
Acetaminophen	16.72	0.34	98	Gros et al., 2012
Ketoprofen	1.13	0.56	50	Gros et al., 2012
Salicylic acid	4.85	0.11	98	Papageorgiou et al., 2016
Paracetamol	1.42	0.3	79	Papageorgiou et al., 2016
Antibiotics				
Sulfonamides	153	101	34	Hendricks and Pool, 2012
Ciprofloxacin	0.25	0.24	4	Gros et al., 2012
Trimethoprim	0.2	0.1	50	Gros et al., 2012
Metronidazole	0.15	0.12	20	Gros et al., 2012
Ofloxacin	0.3	0.19	37	Gros et al., 2012
Moxifloxacin	0.43	0.09	79	Papageorgiou et al., 2016
Ampicilin	1.24	0.15	88	Papageorgiou et al., 2016
Antihypertensives				
Losartan	0.37	0.08	78	Gros et al., 2012
Valsartan	3.7	0.57	85	Gros et al., 2012
Temazepam	10.6	2.3	78	Wu et al., 2015
Alprazolam	7.6	4.9	36	Wu et al., 2015
Estazolam	6.1	2.9	52	Wu et al., 2015
Fluoxetine	2.6	1.4	46	Wu et al., 2015
Oxazepam	9.2	6.8	26	Wu et al., 2015
Carbamazepine	9.2	0.07	99	Papageorgiou et al., 2016
Tranquilizer				
Azithromycin	0.2	0.17	15	Gros et al., 2012
Erythromycin	0.06	0.01	83	Gros et al., 2012
Clarithromycin	0.46	0.19	59	Gros et al., 2012

1.3 Conventional Wastewater Treatment Process (WWTP) and Pharmaceuticals removal

In nature, small amount of organic wastes undergo self-purification which dissolve oxygen is sufficient to oxidize small amount of wastes by aerobic microbial process and consequently they are converted to carbon dioxide (CO_2). Then, CO_2 are used in the photosynthesis process by aquatic plant under direct sunlight to produce oxygen (O_2). But self-purification is often forbidden due to the increase of the amount of degradable organic matters and producing anoxic conditions.

Wastewater is simply the part of the liquid waste produced by community or by the industry. Wastewaters can be divided into different classes according to its physical properties, chemical properties and biological properties. Chemical characteristics of wastewaters are often described by its organic and inorganic content. Many parameters have been used to measure the concentration of organic matter in wastewater. These parameters such as chemical oxygen demand (COD), biological oxygen demand (BOD) and total organic content (TOC). Table 1.3 showed the chemical characteristic of typical domestic wastewaters. The basic function of WWTPs is to speed up the natural processes by which water purifies itself (USEPA, 2004). It is designed to treat and improve wastewater quality before releasing it to the nature. WWTPs are required to reduce the organic content, suspended solids and pathogens in order to avoid water pollution (Marx et al., 2015). Regulation of the water quality of effluent WWTPs is mainly based on these parameters (Table 1.4) and seldom

focuses on the concentration of micropollutants such as pharmaceuticals. Exponential growth in human population has created a corresponding increase in the demand for the clean freshwater (Kolpin et al., 2002). The discharge of incompletely treated wastewater to surface water may contaminate the drinking water resources. Therefore, the performance of WWTP is regulated by the water quality standard for the effluent water quality.

Table 1.3: Chemical characteristics of typical domestic wastewater.

Constituents	Concentration (mg/L)
Total solids	350-1200
Dissolved solids	250-850
Suspended solids	100-350
Nitrogen	20-85
Phosphorus	6-20
Chloride	30-100
Alkalinity ($CaCO_3$)	50-200
Grease	50-150
BOD_5[2]	100-300

Source: UN Department of Technical Cooperation for Development (1985)

Table 1.4: Guidelines for domestic effluent quality.

Constituents	Concentration (mg/L)
Total Suspended solids	26
BOD_5[2]	22
Grease	16
pH	6.0-9.0

Source: USEPA, Effluent Guidelines and Standard (2014)

The wastewater treatment processes generally involve different stages, namely preliminary treatment and primary treatment, secondary treatment, tertiary treatment and disinfection process (Molinos-Senante et al., 2015). Figure 1.2 below showed the flow diagram for the process of wastewater treatment plants (WWTPs). The first stage of WWTPs is known as preliminary treatment. At preliminary treatment, the wastewater is passed through a screen to remove large pieces of debris such as sticks, stones, and plastic bags. Then, it enters a grit chamber, where the water flow is slowed just enough to allow coarse sand and gravel to settle out to the bottom. Then, the primary treatment is implemented to remove remaining fine particles.

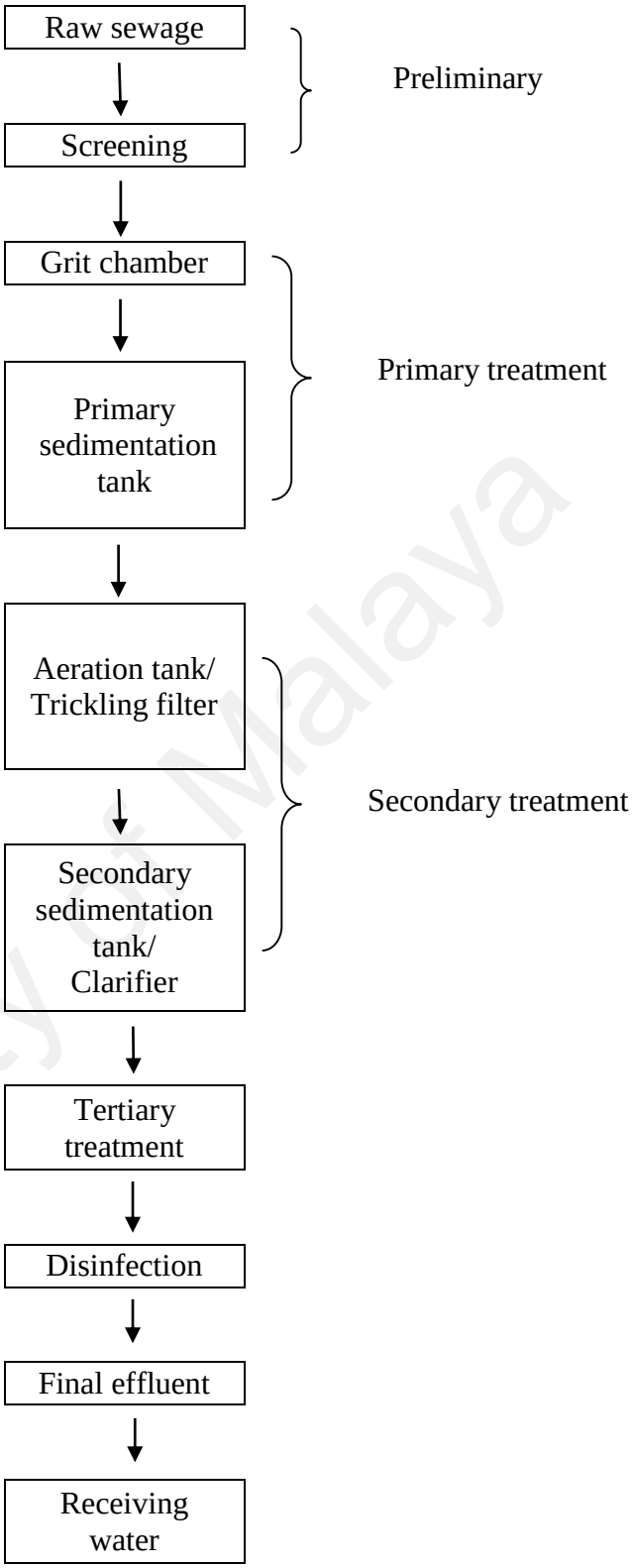


Figure 1.2: Flow diagram for wastewater treatment plants (WWTPs).

In primary treatment, coagulation is a process whereby destabilization of a given colloidal suspension or solution is taking place. The function of coagulation is to overcome the factors that promote the stability of a given system. It is achieved with the use of appropriate chemicals such as alum, ferric sulfate, ferric chloride, ferrous sulfate, and sodium aluminate. The process is used to enhance the degree of removal of total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and bacterial population in primary settling facilities, as well as to improve the performance of secondary treatment processes (Semerjian and Ayoub, 2003). Flocculation refers to the destabilization of fine particles to form large agglomerates which can be separated through gravity settling. Coagulation usually completes in a very short periods of time, whereas flocculation required longer time than coagulation. During this physical treatment process the organic compounds, including pharmaceuticals that are more likely to partition onto the suspended solid can exhibit higher removal efficiency (Westerhoff et al., 2005). However, most of the polar pharmaceuticals (e.g. aceclofenac, carbamazepine, diclofenac, trimethoprim, ibuprofen, clofibric acid, phenazone, etc.) (Koutsouba et al., 2003; Celiz et al., 2009) are not likely to associate with particles during the treatment process and consequently pharmaceuticals will be released into the secondary treatment process (Heberer and Stan, 1997; Benotti et al., 2009; Kuch and Ballschmiter, 2001; Carmona et al., 2014; Yang et al., 2014).

Secondary treatment used biological activity by microorganism to break down the organic matter to CO_2 and H_2O. The main objective for secondary treatment is to provide further treatment of the effluent from primary treatment

to remove the residual organics. In this process, mixtures of microorganisms or so called activated sludge are added to the primary treated effluent. The air is vigorously bubbled through pipes into the effluent. Then, the aerobic bacteria digest the organic material and break it down into CO_2 and H_2O. The activated sludge are returned to the aeration tank. Decomposition of pharmaceuticals through this biological treatment is relatively poor. Deng et al., (2016) reported that the removal efficiency of activated sludge process in the removal of sulfadiazine, sulfamethoxazole, roxithromycin, and diclofenac were 40%, 41%, 39% and 52%, respectively. The study by Guerra et al., (2014) showed that the removal of anti-inflammatory pharmaceuticals such as ibuprofen and naproxen was 74% and 63%, respectively.

Conventional tertiary treatment help to improves wastewater quality before it is discharged to the environment by removing the remaining nitrogen and phosphorus containing nutrients (Feng et al., 2013). Excessive released of nutrients can encourage the overgrowth of algae and weed which lead to the eutrophication process. The different treatment processes are required to remove nitrogen and phosphorus. Nitrogen is removed through the biological oxidation of nitrogen from ammonia to nitrate (nitrification), followed by denitrification, which is the reduction of nitrate to nitrogen gas. Meanwhile, phosphorus can be removed by chemical precipitation by using ferric chloride, alum or lime (Zhou et al., 2008; Zhibin et al., 2013). Due to the concern on the presence of various organic pollutants, various advanced tertiary treatments such as micro-grain activated carbon, combination of granular activated carbon adsorption with deep-bed filtration have been introduced (Voulvoulis et al., 2015; Altmann et al.,

2016). These methods were found to be effective in the removal of pharmaceuticals. For example, the study on the tertiary treatment of wastewater by micro-grain activated carbon (μGAC) was evaluated by Mailler et al., (2016). The results showed good pharmaceuticals removal up to more than 80-90% for atenolol, carbamazepine, ciprofloxacin, diclofenac, oxazepam, and sulfamethoxazole. Although advanced tertiary treatment was found to be effective in the removal of pharmaceuticals. However these methods are seldom been implemented in WWTPs due to higher cost. As a result, the untreated pharmaceuticals from secondary treatment are often exposed to the disinfection process.

1.3.1 Disinfection and Chlorination

Disinfections refers to the disruption of pathogens by using chemicals such as chlorine, bromine, ozone or by using other methods such as ultraviolet irradiation (Lee et al., 2015; Song et al., 2016; Uslu et al., 2016). It is the most important stages in the water treatments as it provide a protection on the transmission of waterborne disease. The effectiveness of disinfection depends on the quality of the water being treated (e.g., cloudiness, pH, ammonia content, etc.), the type of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Today, chlorination is one of the most popular disinfection method for treatment of wastewater all over the world (Abdullah et al., 2009; Gonzalez-Marino et al., 2015).

Chlorination was first discovered in Sweden in 1744. The application of chlorination in water treatment began in Great Britain and expanded to United States in 1908 (Water Quality and Health Council, 2016). Although less reactive than ozone, chlorination is frequently used in water treatments due to its cost effectiveness (Sharma, 2008; Deborde and von Gunten, 2008; Prasse et al., 2015; Negreira et al., 2016). Chlorination is a proven method to prevent the regrowth of pathogen in treated water due to its disinfectant's stability (Wu et al., 2009; Noutsopoulos et al., 2015). Chlorine disinfection is reliable and effective against a wide spectrum of microorganisms (Gonzalez-Marino et al., 2015). Instead of disinfection, chlorination can also remove the unpleasant odour from water (USEPA, 1999; Guo et al., 2016).

1.4 Chemistry of Chlorination during Water Treatment

Different chemical oxidants have been used for chlorination and these chemicals are chlorine gas (Cl_2), sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$) and chloramines. NaOCl and $Ca(OCl)_2$ are more expensive disinfectants than chlorine gas, but these chemicals is a preferred oxidants because of safety propose. Chlorination was first carried out for drinking water disinfection in 1908 using sodium hypochlorite (NaOCl) (The National Academic Press, 1980). NaOCl dissolves in water to form hypochlorous acid (HOCl) Equation 1:

$$NaOCl + H_2O \rightarrow HOCl + NaOH \quad (1)$$

In common wastewater chlorination, chlorine is introduced in the form of molecular chlorine gas (Cl_2) or as NaOCl at concentration of 10 mg/L for about 1 hour. When molecular chlorine is added into water, molecular chlorine rapidly hydrolyzes and forms HOCl (Equation 2). HOCl is a weak acid, therefore, it is dissociated to form hypochlorite ion (OCl^-) in aqueous solution as indicates by Equation 3.

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \quad (2)$$

$$HOCl \rightleftharpoons OCl^- + H^+ \quad (3)$$

Molecular chlorine, HOCl and OCl^- are the main reactive species that present in water and is known as free available chlorine (FAC) (Buth et al., 2007; Acero et al., 2013). During water treatment, FAC inactivate microbes, oxidize metals, and organic material. At acidic and neutral pH, HOCl is the predominant species in the water, while at alkaline pH the OCl^- dominates. HOCl is a stronger disinfectant than OCl^- and thus the chlorine disinfection is more efficient at acidic pH values. An advantage of disinfection by chlorination over the other disinfection methods (ozone and UV) is that some of the chlorine residue remains dissolved in water (National Academy of Sciences, 2007). The effectiveness of chlorination depends on the dose, the chlorine demand of the wastewater, and contact time. The suggested doses by USEPA (1999) are ranged from 5 to 20 ppm. Sulfur dioxide or sodium thiosulfate is frequently used to scavenge free chlorine before discharging the treated wastewater into the environment (Prasse et al., 2015).

1.5 Formation of Disinfection by-products (DBP) and Transformation by-products during Chlorination

Today, wastewater chlorination is widely practiced to reduce microbial contamination and potential disease risks to exposed populations. Disinfection by chlorination can be problematic in some circumstances because chlorine as oxidizing agent can react with naturally occurring organic compounds containing in the wastewater to produce disinfection by-products which is recognized as potent carcinogen at low levels of concentrations (Quintana et al., 2012). Organic matters from WWTPs effluent may content nitrogen, therefore the reaction between organic matters with chlorine produces nitrogenous and carcinonogenic halogenated disinfection by-products such as haloacetonitriles, halonitromethanes, trihalomethanes (THMs) and haloacetic acids (HAAs) (Nieuwenhuijsen et al., 2000; Kim et al., 2002; Ge et al., 2006; Barber et al., 2015). Several studies also reported that the chlorination of water producing variety of chlorinated organic compounds and some of them have been associated with adverse health effects (Triebskorn et al., 2004; Santos et al., 2012). For example, 2,2-dichloroacetamide (DCAcAm) which was formed during chlorination of drinking water was detected in chlorinated drinking water. DCAcAm can be easily accumulated and cause the acute metabolism damage in zebrafish (Yu et al., 2015).

Due to ineffectiveness of primary and secondary treatment, the tendency of the pharmaceuticals to be exposed to disinfection process is very high. Even

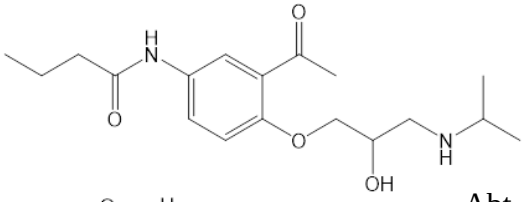
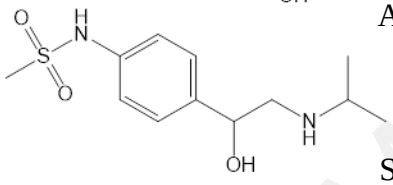
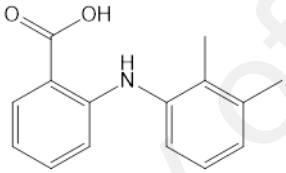
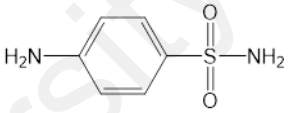
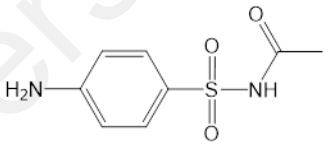
though pharmaceuticals may be degraded in chlorination through the reaction with HOCl, but their threat to aquatic environments may not be eliminated. Chlorination of pharmaceuticals usually results in chlorinated, oxidized, or fragmented by-products rather than complete mineralization (Postigo and Richardson, 2014). In some cases the toxic transformation by-products can be produced and may cause possible health risks (Zhou et al., 2016). Previous study by Buth et al., (2007) showed the transformation by products of cimetidine during aqueous chlorination formed unexpected by-products. Among four transformation by-products detected, two of them were predicted to be more toxic than cimetidine. These findings suggested that cimetidine can be degraded by free chlorine but chlorination does not eliminate the environmental threat, but it increases the threat to the environment. Therefore, the toxic by-products need to identify so that environment can be protected from these undesired by-products.

1.6 Background of Selected Pharmaceuticals

In this study, the fate of 5 pharmaceuticals were selected namely acebutolol (Abt), sotalol (Stl), mefenamic acid (Mfe), sulfacetamide (Sfa) and sulfanilamide (Sfn) were studied in chlorination. Ace and Stl are β-blockers, Mfe is a non-steroidal anti-inflammatory drugs (NSAIDs) and Sfn and Sfa are the sulfonamides antibiotics. These pharmaceuticals are frequently used and detected in the environment worldwide (Kasprzyk-Hordern, 2010). The chlorination of these selected pharmaceuticals were study since it has not been

reported elsewhere. Table 1.5 shows the chemical properties of selected pharmaceuticals.

Table 1.5: The chemical properties of selected pharmaceuticals.

Class	Structure		Chemical formula	pK_a
β-Blockers		Abt	$C_{18}H_{28}N_2O_4$	9.21[a]
		Stl	$C_{12}H_{20}N_2O_3S$	9.01[a]
NSAIDs		Mfe	$C_{15}H_{15}NO_2$	4.2[b]
Antibiotics		Sfn	$C_6H_8N_2O_2S$	10.6[c]; 2.27
		Sfa	$C_8H_{10}N_2O_3S$	5.27[a]; 1.76

[a] Babić et al., 2007 [b]Bones et al., 2006 [c]Ramnani et al., 1997

1.6.1 β-blockers

β-blockers are used to treat cardiovascular diseases such as hypertension, angina pectoris, and arrhythmia (Maurer et al., 2007; Sirès et al., 2010). Figure 1.3 shows the chemical structure of commonly used β-blockers such as atenolol, bisoprolol, metoprolol, and propranolol. β-blockers control heart rate and are

also used in the treatment of abnormal heart rhythms. As a result of their common usage to control chronic diseases, β-blockers are always detected in municipal wastewater treatment plant with the concentration ranging from μg/L to mg/L (Quintana et al., 2012; Petrović et al., 2014). Some of these β-blockers (such as atenolol) are even almost exclusively excreted unchanged through urine (Mehvar and Brocks, 2001).

The presence of β-blockers in the environment can lead to unexpected effects towards different organisms. Although β-blockers are safe for human and veterinary, but the negative impact of these drugs to the ecosystem has been frequently reported (Maszkowska et al., 2014). According to Santos et al., (2010), β-blockers showed deleterious effects on the different organisms such as fish, invertebrates and green algae. The selected β-blockers in this study were acebutolol (Abt) and sotalol (Stl) (Table 1.5).

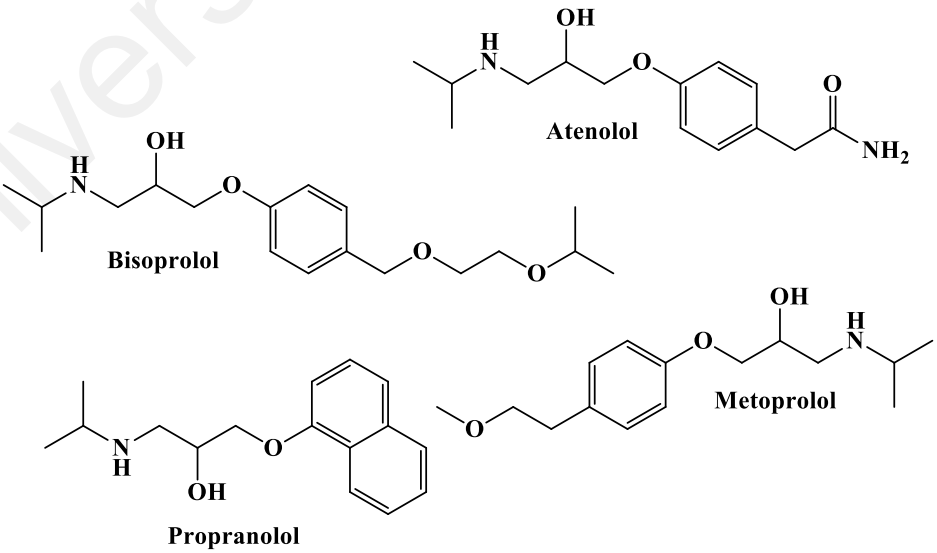


Figure 1.3: Chemical structure of β-blocker group.

1.6.2 Nonsteroidal anti-inflammatories (NSAIDs)

NSAIDs have been widely used as analgesics, antipyretics, anti-inflammatory agents, and for pain relief (Fent et al., 2006; Ziylan and Ince, 2011). They are the sixth most sold drugs worldwide (Parolini et al., 2009). The consumption in USA, United Kingdom, Japan, France, Italy and Spain has increased largely at a rate of 11.9% each year (Feng et al., 2013). It has been frequently reported that NSAIDs such as ibuprofen, naproxen, mafenamic acid and ketoprofen have been detected at trace level in the effluent of wastewater treatment plant, as well as in lakes, ground water, surface water and drinking water (Werner et al., 2005; Boleda et al., 2011; Chang et al., 2012; Khalaf et al., 2013). Figure 1.4 shows the chemical structure of common NSAIDs detected such as diclofenac, ibuprofen, naproxen, celecoxib, and indomethacin.

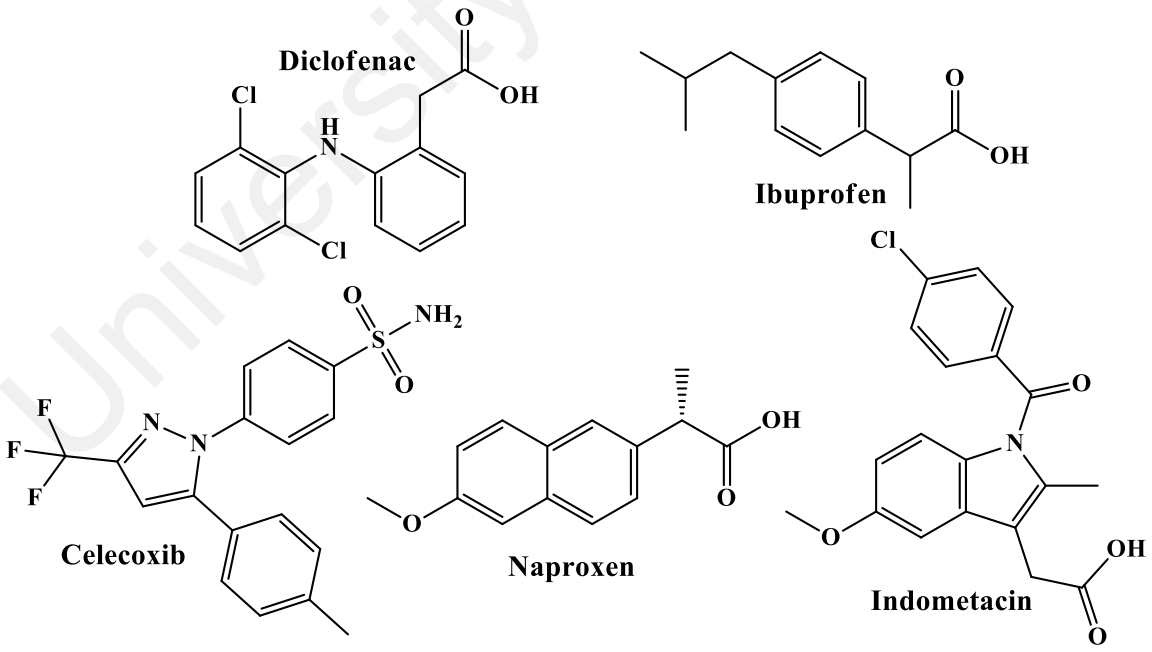


Figure 1.4: Chemical structure of NSAIDs group

The selected NSAID for this study was mefenamic acid (Mfe). Mfe is a third priority compound on the European Union list of priority pollutants and most toxic compound that frequently detected in river waters and wastewaters (Li M.H., 2013; Abdolmohammad-Zadeh et al., 2014). Mfe decreases the inflammation and uterine contractions by inhibiting the prostaglandin synthesis (Dhumal et al., 2014). 67% of injested Mfe is excreted unchanged through urine (Medsafe, 2015). Therefore, the exposure of Mfe to wastewater deserves investigation. Mfe is detected in effluent at the concentration of 10.7 to 1327 ng/L (Nebot et al., 2015). As reported by Collard et al., (2013) the exposure of Mfe zebra fish could affect sex hormone in balance and potentially reproduction damage and reduced the potential growth rate as investigated. In addition, due to the high lipophilicity of Mfe, it is expected to be persistent in the environment and consequently accumulated in living organism.

1.6.3 Antibiotics

In the late 1930s, natural and synthetic antibiotic to fight against infectious disease caused by bacteria and other microbes (Todar, 2008) were introduced and their usage has increased tremendously for human and animal production (Ricci and Cross, 1993; Lin et al., 1997; Zhang et al., 2012; González-Pleiter et al., 2013; Tongur and Yildirim, 2015). Antibiotics are considered as "pseudo-persistent" pollutants in the environment (Seifrtová et al., 2009; Yin et al., 2014). There are more than 10 different classes of antibiotic, namely β-lactams, macrolides, fluoroquinolones, aminoglycosides, sulfonamides and tetracycline (Li, 2014). For veterinary medicine, antibiotic are used to treat diseases or to

increase feed efficiency and improve growth rates (Kümmerer 2003; Chamberlain and Adams, 2006; Sarmah et al., 2006; Zhang et al., 2013; Brandt et al., 2015). Based on the previous research, a total of 110 tonnes of antibiotics such as sulfonamides are used as growth promoters in livestock production or as feed additives in fish farms production per year (Halling-Serensen et al., 1998). About 23000 tonnes of antibiotics are produced in the US each year, of which about 40% is used in agriculture sector (Sanderson et al., 2004).

After ingestion, both the metabolized and unmetabolized antibiotics are often excreted through urine and feces and it have already been frequently detected in wastewater treatment plants and surface water (Bu et al., 2013; Berendsen et al., 2015). Around 50% of the administered antibiotics by human are excreted unchanged (Chamberlain and Adams, 2006). These antibiotics generally undergo improper treatment by the municipal wastewater facilities and therefore they have found their way into streams and even present in the sources of drinking water (Rodriguez-Mozaz et al., 2015). Antibiotics also entered the aquatic environments at high concentrations through animal manure, overflow of treated aquaculture ponds, industrial and hospital effluents (Huang et al., 2014). Studies have reported that low concentration (μg/L to ng/L) of veterinary and human antibiotics have been detected in drinking water and ground water in US (Sui et al., 2015), northern China (Hu et al., 2010), and Switzerland (Morasch, 2013). Among various classes of antibiotics, sulfonamides are the first antimicrobials used in clinical practice (Gaffney et al., 2016). The sulfonamides were discovered by Domagk in 1930. Domagk found that an orange-red sulfonamide dye named protonsil rebrum was safe and effective in curing

streptococcal infection in mice (Fuchs and Elsner, 2003). Then, he managed to confirm the antibacterial property of these substances through various experiments. The selected sulfonamides for this study are sulfanilamide (Sfn) and sulfacetamide (Sfa) (Table 1.5). Sulfonamides are the most extensively studied antibiotics and they have been found at high concentrations (up to 1080 ng/L) in several studies (Bu et al., 2013; Lu et al., 2015; Sui et al., 2015). Due to their low cost and relatively high efficiency against many common bacterial infections, sulfonamides are one of the widely used antibacterial veterinary drugs. Sfa is commonly added to the cream to treat bacterial infections on the skin. It is also added to the eye drops to treat eye infections (MedlinePlus, 2015). Sfa also has been used to treat urinary tract infections. For Sfn, it is commonly used to treat various bacterial infections which include urinary tract infections, vaginal infections and strep throat (Walker, 2016).

1.7 Objectives of study

The main objectives of this study were to study the fate of Abt, Stl, Mfe, Sfa and Sfn during chlorination process. The specific objectives are as below:

- to determine the second-order rate constants for the reaction between selected pharmaceuticals with FAC at environmental pH condition
- to identify the transformation by-products of the selected pharmaceuticals during chlorination
- to elaborate the mechanism of transformation of selected pharmaceuticals during chlorination
- to study the effect of different water matrices on the removal of selected pharmaceuticals during chlorination

These selected pharmaceuticals were frequently found in WWTPs. Therefore the tendency for these pharmaceuticals to be exposed to this disinfection process is high. Therefore, the study will provide a good understanding of the fate pharmaceuticals in the chlorination process.

CHAPTER 2

CHAPTER 2

Materials and Methods

2.1 Materials and Stock Solutions

Acebutolol hydrochloride, sotalol hydrochloride, mefenamic acid and sulfacetamide were purchased from Sigma (St. Louis, USA) and sulfanilamide was purchased from Riendemann Chmidt. Sulphuric acid, sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate were obtained from Merck (Darmstadt, Germany). Sodium hypochlorite solution with available chlorine 4.00-4.99%, potassium iodide (KI), sodium thiosulphate and formic acid were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile and methanol were obtained from RCI Lab Solution (Bangkok, Thailand). All chemicals were used without further purification.

The stock solutions with the concentration of 100 mg L^{-1} of acebutolol, sotalol, sulfacetamide and sulfanilamide were prepared by dissolving appropriate amount of selected pharmaceuticals in ultrapure deionized water (Elga, Buckinghamshire, UK) while mefenamic acid stock solution was prepared by dissolving appropriate amount of Mfe in 50% ultrapure deionized water and 50% of methanol. Stock solution of sodium hypochlorite was prepared freshly by diluting it with ultrapure deionized water.

2.2 Concentration of Free Available Chlorine (FAC)

The concentration of FAC stock solution was standardized using iodometric titration method as described by Adam and Gordon (1995). Briefly, 5 mL of concentrated sodium hypochlorite (NaOCl) was diluted to 100 mL using ultrapure deionized water. In a 500 mL conical flask, 10 mL of diluted NaOCl solution, 100 mL of ultrapure deionized water, 20 mL of concentrated KI and 50 mL of 10% H_2SO_4 were added. Then, the mixture of the solution was titrated with 0.1 M sodium thiosulphate ($Na_2S_2O_3$) until pale yellow solution was obtained. Then, the starch solution was added into the solution. The mixture of the solution was quickly titrated with $Na_2S_2O_3$ until the colour turned from blue to colourless. FAC concentrations were determined in triplicate.

2.3 Kinetics Experiments

All kinetic experiments were performed in 50 mL jacketed beaker at 25 ºC. For the determination of second-order rate constant (k_{app}), chlorination experiments were performed under pseudo first-order kinetics conditions where the concentration of HOCl was at least 20 times higher than the selected pharmaceuticals. The pH of the reaction mixtures was adjusted using phosphate buffer (50 mM) for 6 to 8 pH range. The chlorination was initiated by injecting an aliquot of sodium hypochlorite solution to the selected pharmaceuticals solution. The mixture was stirred with magnetic bar throughout the experiment and the temperature was kept at (25 ± 0.1) °C. 1 mL of the reaction mixture was withdrawn at define time interval and the reaction was quenched using either sodium thiosulfate (Abt, Stl, and Sfn) or ascorbic acid (Mfe and Sfa)

solution. Controls were prepared using similar method without the addition of HOCl. The reaction mixture was then subjected for HPLC analysis.

2.4 Chlorination of Selected Pharmaceuticals in Different Water Matrices

To stimulate the real water treatment conditions, experiments were performed using surface waters from lake water, ground water and also the secondary effluent collected around Kuala Lumpur.

For this experiment, each water sample was spiked with target compound at 1 μM concentration and transferred into 20 mL vials. Then, FAC doses ranging from 10 to 80 μM were added into the vials. The vials were shook vigorously after the addition of FAC solution for 24 h. The residual of target compound was measured by HPLC. All experiments were carried out at room temperature (25 - 30 °C).

2.5 Transformation by-products

For transformation by-products identification, chlorination was performed on 3.58 mM of Abt, 4.32 mM of Stl, 5.53 mM of Mfe, 3.87 mM of Sfn and 1.56 mM of Sfa solution at pH 6, 7 and 8. The molar ratios for HOCl to selected pharmaceuticals were kept at 0.5:1, 1:1 and 2:1. The samples were shook for 24 h. Then, 2 mL of the samples were filtered through 0.2 μm syringe filter and subjected for LC-QTOF-MS analysis without quenching. The control samples were prepared using similar method without the addition of HOCl. The controls were shook for 24 h as well.

2.6 Instrumental analysis

2.6.1 High Performance Liquid Chromatography (HPLC)

All HPLC analyses were performed using Shimadzu HPLC system consisted of a LC-20AT pump, a SPD-M20A diode array detector, a SIL-20AHT auto sampler, and a CTO- 10AS column oven and a CBM-20A communication bus module (Shimadzu, Japan). The reversed-phase Chromolith RP-18 monolithic column (100 mm×4.6 mm; Merck, Germany) or InertSustain C18 was used for separation. The flow rate was maintained at 0.25 mL min^{-1} for compound using InertSustain C18 while flow rate for Chromolith RP-18 was maintained at 1.0 mL min^{-1}. The solvent programs of HPLC are presented in Table 2.1.

Table 2.1: Applied HPLC solvent programs.

Compound	Solvent Program	Detection wavelength (nm)	Column
Abt	Isocratic with10A:90B for 15minutes (A=acetonitrile; B=0.1% formic acid)	240	Chromolith RP-18
Stl	Isocratic with 10A:10B:80C for 15minutes (A=acetonitrile; B=methanol; C=buffer pH8 (10mM))	237	InertSustain C18
Mef	Isocratic with 50A:50B for 9minutes (A=acetonitrile; B=0.1% formic acid)	209	Chromolith RP-18
Sfn	Isocratic with 5A:95B for 10minutes (A=acetonitrile; B=buffer pH8 (10mM))	257	InertSustain C18
Sfa	Isocratic with10A:90B for 15minutes (A=acetonitrile; B=0.1% formic acid)	254	InertSustain C18

2.6.2 Liquid Chromatography Mass Spetrometry /QTOF

The analysis of transformation by-products were performed using 6500 accurate mass quadrapole time-of-flight mass spectrometer bearing with electrospray ionization (ESI) source coupled to 1200 series rapid resolution LC system (LC-QTOFMS, Agilent Technologiest, Santa Clara, USA) as reported by previous study (Tay and

Madehi, 2015; Khalit and Tay, 2016). Briefly, 0.1% formic acid in water (A) and acetonitrile (B) were used as mobile phase at a flow rate of 0.25 mL min^{-1}. The gradient starts at 10% acetonitrile, follow by 10 min ramp to 100% acetonitrile and hold for 3 min. QTOF-MS system was operated in a high resolution mode at 4 GHz. Ions were generated using an electrospray ion source with Agilent Jet Stream Technology. The Agilent Jet Stream Technology uses superheated nitrogen sheath gas at the temperature of 300 °C and flow rate of 11 L min^{-1}. Analyses were performed in ESI positive ion mode using the following settings: nebulizer at the pressure of 35 psig, Vcap voltage of 3500 V, fragmentor voltage of 125 V, skimmer voltage of 65 V, nozzle voltage of 1000 V, and the collision energy was fixed at 15 V. A sprayer with a reference solution was used as continuous calibration in positive ion using the following reference masses: *m/z* 121.0509 and 922.0098. For MS/MS mode, the collision energy was adjusted at 20 V to obtain the optimal fragmentation. The recorded full-scan and MS/MS data was processed using Agilent MassHunter Workstation Software.

2.6.3 pH and Total Organic Carbon (TOC) analysis

The measurements of pH were carried out using Mettler Toledo FE20, which was calibrated with standard buffer solutions and the total organic carbon content was determined by using a Shimadzu TOC analyser programmed by TOC-Control V Software.

CHAPTER 3

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Kinetics reaction between selected pharmaceuticals with FAC

The reaction for the chlorination of organic and inorganic compounds in water is a second-order reaction: first order to the FAC and first-order to organic or inorganic compound as described by Equation 4 (Dodd and Huang, 2007; Deborde and von Gunten, 2008; Acero et al. 2010; Acero et al. 2013; Tawk et al., 2015):

$$-\frac{d[phar]t}{dt} = k_{app}[phar]t[FAC]t \quad (4)$$

where k_{app} is the second order rate constant, $[phar]_t$ and $[FAC]_t$ represent the concentration of selected pharmaceuticals and FAC at different reaction times, *t*. Consequently, in the presence of excess FAC, the rate of degradation of selected pharmaceuticals in water follows the pseudo-first-order reaction (Gallard et al., 2004) and Equation 4 can be simplified as Equation 5.

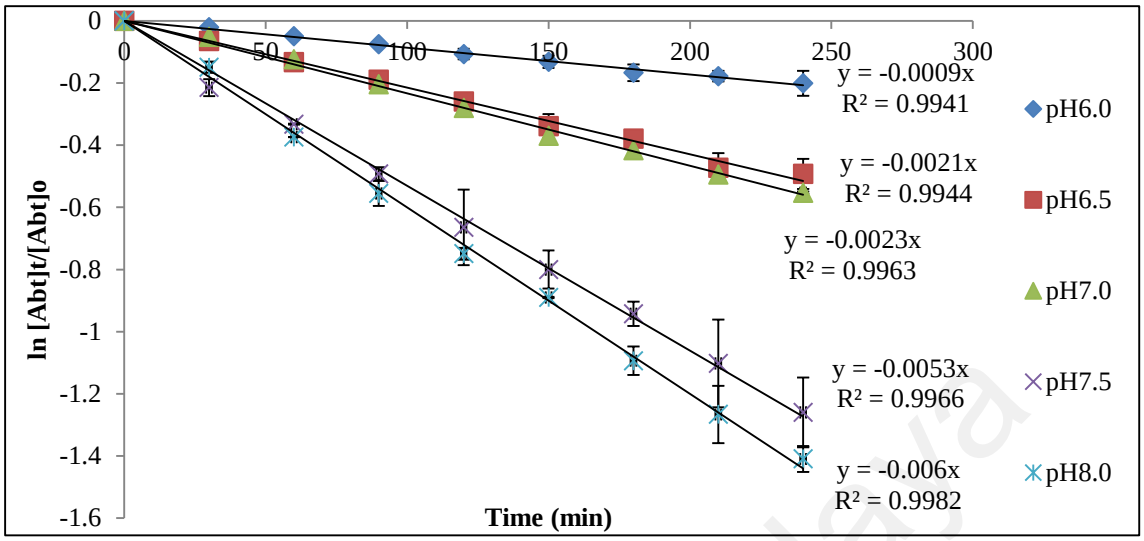
$$-\frac{d[phar]t}{dt} = k_{obs}\,[phar]t \quad (5)$$

$$\ln\left(\frac{[phar]t}{[phar]_o}\right) = -k_{obs}t \quad (6)$$

where k_{obs} (=k_{app}[FAC]) represents the pseudo-first-order rate constant. By integrating Equation 5, Equation 6 is obtained. In Equation 6, $[phar]_o$ represents the

initial concentration of the selected pharmaceuticals. k_{obs} can be determined experimentally by monitoring the concentration of selected pharmaceuticals versus time as indicated by Equation 6. Figure 3.1 shows the plot of $\ln([phar]_t/[phar]_o)$ as a function of reaction time for the selected pharmaceuticals. These results were obtained from the kinetics study involving the concentration of FAC which was more than 20 times of the concentration of selected pharmaceuticals. Under the selected pseudo-first-order condition, the initial concentration of FAC was found to be unchanged throughout the experiments. Figure 3.1 shows that the degradation of selected pharmaceuticals were well described by pseudo-first-order model with correlation coefficient (R^2) more than 0.95. The second-order rate constant (k_{app}) for reaction between FAC with selected pharmaceuticals was calculated by dividing the obtained k_{obs} with the initial concentration of FAC. k_{obs} values were obtained directly from the slope of the plot of $\ln([phar]_t/[phar]_o)$ versus reaction time.

(a) Abt



(b) Stl

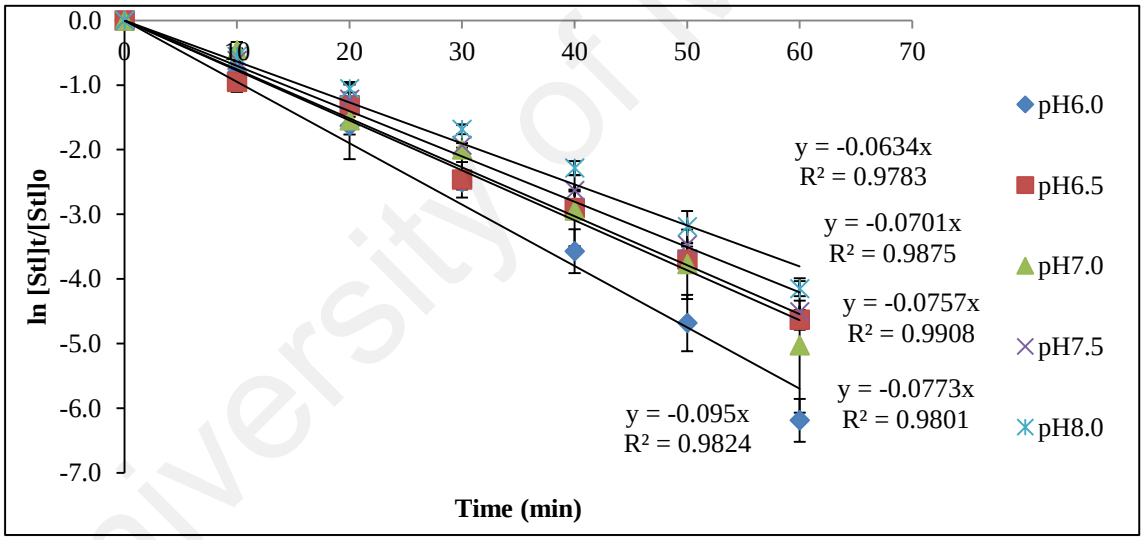
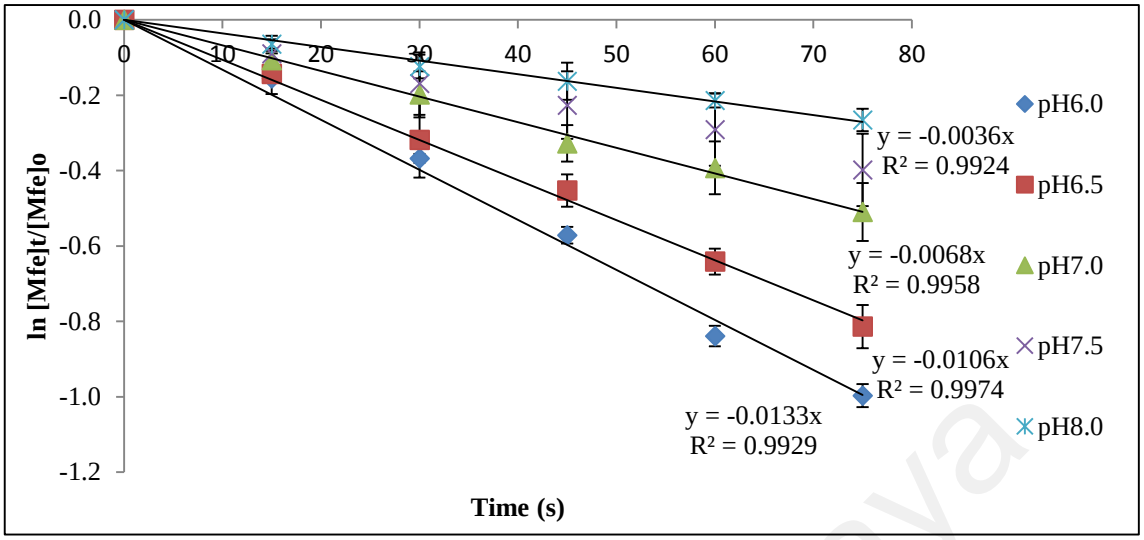


Figure 3.1: Pseudo-first-order kinetics plot for the degradation of selected pharmaceuticals via chlorination at pH 6 to 8. (Temperature = 25±0.1°C, [(a)$[Abt]_o$ = 2.68×10^{-5} M, $[HOCl]_o$ =5.37×10^{-4}M, (b)[Stl]o = 3.24×10^{-5}M, $[HOCl]_o$ =1.62×10^{-3}M, (c)$[Mfn]_o$ = 4.14×10^{-5} M, $[HOCl]_o$ =8.27×10^{-4}M, (d)$[Sfa]_o$ = 4.66×10^{-5} M, $[HOCl]_o$=9.33×10^{-4}M, (e)$[Sfn]_o$ = 5.80×10^{-4} M, $[HOCl]_o$=1.16×10^{-2}M]).

(c) Mfe



(d) Sfa

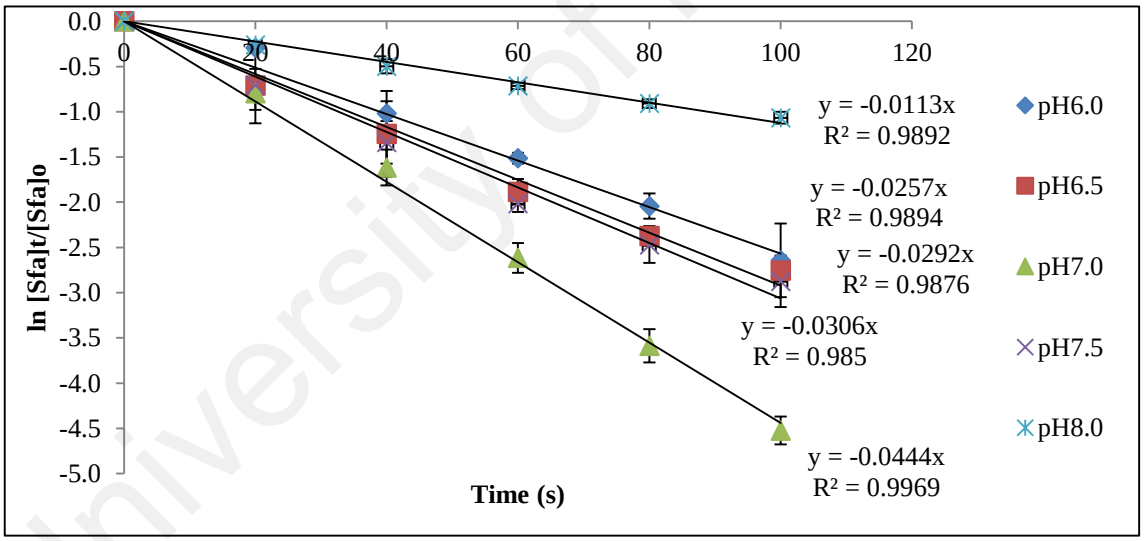


Figure 3.1*(continued)*

(e) Sfn

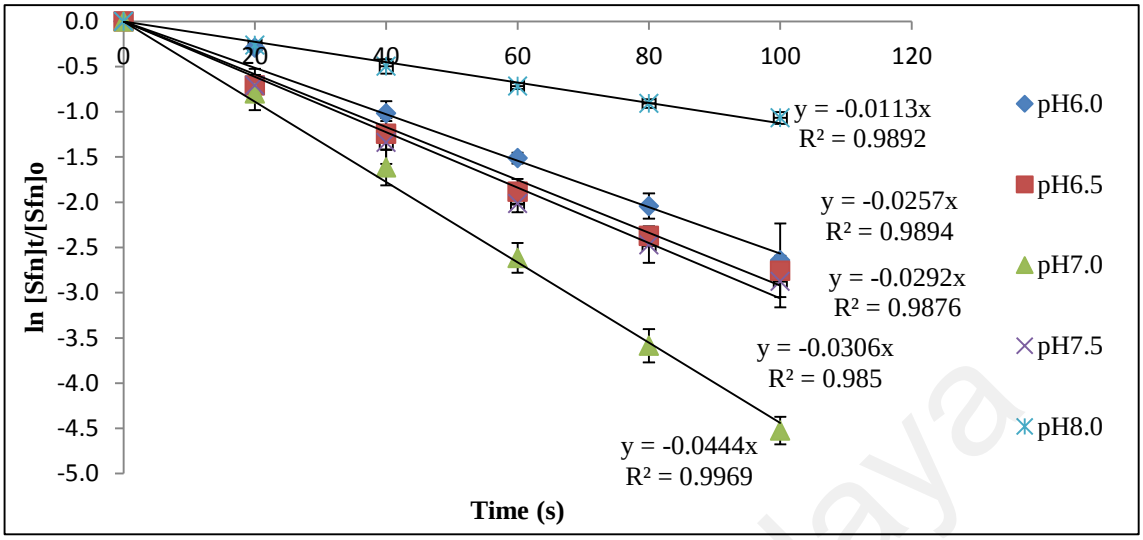


Figure 3.1*(continued)*

The effect of pH on k_{app} for the reaction between FAC and selected pharmaceuticals were investigated in detail since pH can significantly affect the speciation of FAC and selected pharmaceuticals. When NaOCl is dissolved in water, it forms HOCl and OCI^- (Deborde and von Gunten, 2008). The formation of OCl^- through the dissociation of HOCl which has the pK_a of 7.5 (at 25 °C) is a pH-dependent process (Ge et al. 2008). According to Chamberlain and Adams (2006), the mole fraction of HOCl and OCI^- representing by α_{HOCl} and α_{Cl^-} can be estimated using Henderson-Hasselbalch equation (Equation 7 and 8). Figure 3.2 showed the variation of mole fraction of HOCl and OCl^- which was calculated using Equation 7 and Equation 8. HOCl possessing a redox potential of 1.49 V is a stronger oxidant as compared to OCl^- with the redox potential of 0.84 V (Ge et al., 2008; Wenk et al., 2013). As shown by Figure 3.2, α_{HOCl} varies from 0.97 to 0.24 when the pH increases from pH 6 to 8. Consequently, pH of water is shown to influence the reactivity of FAC toward the degradation of organic pollutants by

varying the α_{HOCl}. In this study, the pH ranging from 6 to 8 was selected to study the influence of pH on the k_{app}. This selected pH range also represents the pH condition for most of the environmental water samples (Deborde and Gunten 2008).

$$\alpha_{HOCl} = \frac{1}{1+(10^{-7.5} \times 10^{pH})} \quad (7)$$

$$\alpha_{OCl^-} = 1 - \alpha_{HOCl} \quad (8)$$

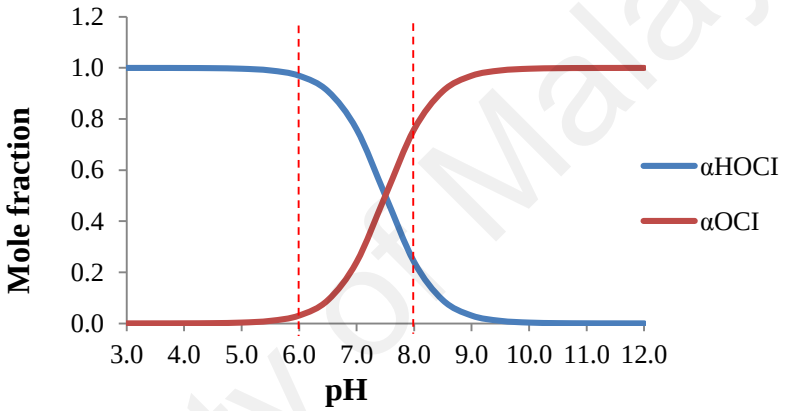


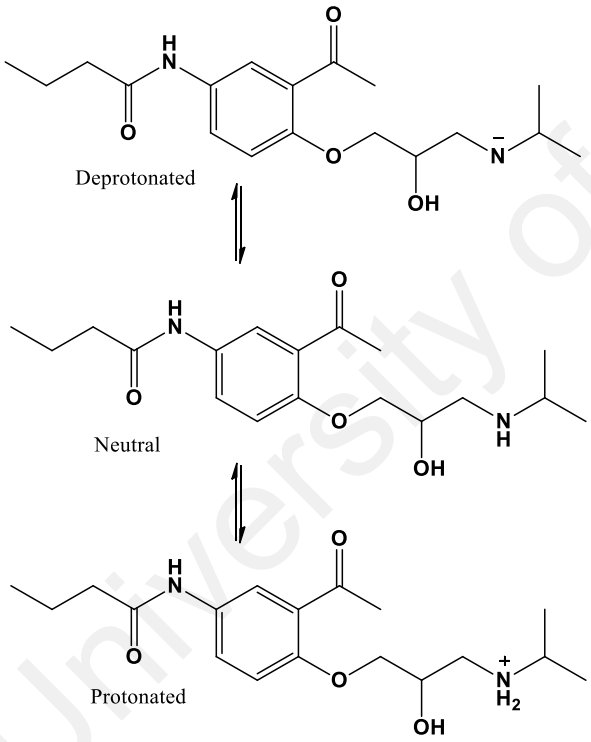
Figure 3.2: Relationship between pH with mole fraction of HOCl and OCl^-.

3.1.1 Abt and Stl

β-blockers are well known to be the basic drugs. The pK_a of Abt and Stl is 9.21 and 9.01, respectively (Babic et al. 2007). In water, Abt and Stl with secondary amine functional group can appear in neutral, protonated, and deprotonated forms (Figure 3.3). As HOCl, the ionization of Abt is also influenced by pH of the solution (Kibbey et al. 2007).

As shown by Figure 3.4, the determined k_{app} of Abt was found to increase with increasing pH from 6 to 8. This result indicated that the k_{app} was clearly pH dependent as shown in most of the chlorination reaction during water treatment (Gallard and Gunten 2002). HOCl is the predominant species during chlorination when pH is in the range of 3 to 7.5 (Zhang et al. 2013). However, when the pH increases from 7.5 to 8, the molar fraction of OCl^- increases from 0.5 to 0.76 and the molar fraction of HOCl decreases from 0.5 to 0.24.

(a) Abt



(b) Stl

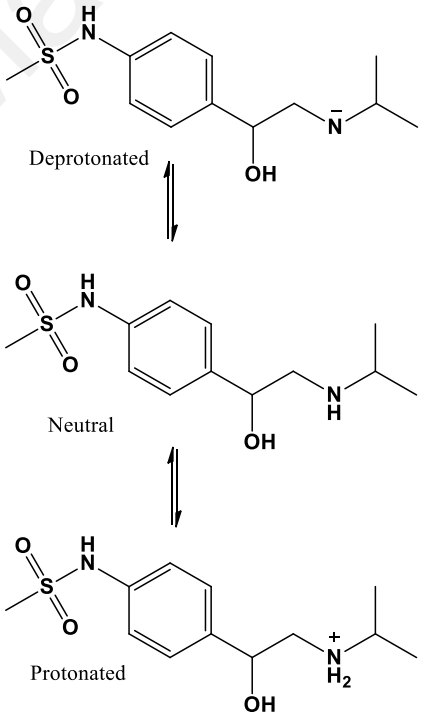


Figure 3.3: The neutral, protonated and deprotonated form of (a) Abt and (b) Stl in water.

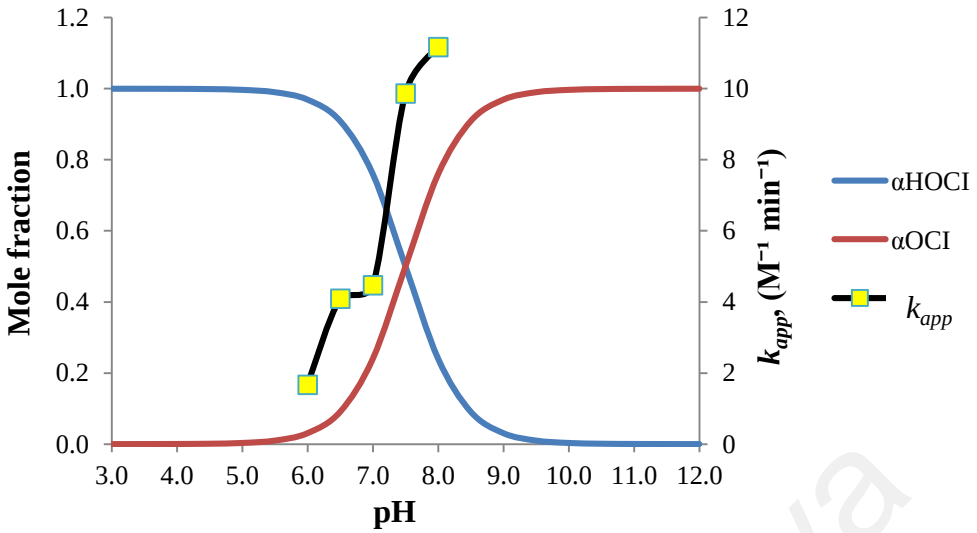


Figure 3.4: The variation of k_{app} for Abt with pH.

In most cases, the reaction between organic compounds with OCl^- can be considered as insignificant due to its low reactivity (Pinkston and Sedlak, 2004; Deborde and Gunten 2008; Xiang, et al., 2016). Therefore, the obtained result indicated that the degradation of Abt was not retarded by the increase of inactive OCl^- concentration. In this study, the selected pH conditions for chlorination were lower than the pK_a of Abt. Consequently, Abt can only appear as neutral or protonated form during the selected chlorination condition. When the pH increased from 6 to 8, the molar fraction of neutral Abt was found to increase, (Figure 3.5). In this study, the ratio of $\frac{[Abt]}{[Abt]^+}$ was also estimated using Henderson-Hasselbalch equation. The result indicated that, the increased of k_{app} with increasing pH showing that the neutral form of Abt is more reactive toward chlorination as compared to its protonated form. As reported by Abia et al (1998), secondary amine species reacted favorably with HOCl under basic condition to form chloramine as compared to acidic condition. Therefore, the increased of k_{app} with pH can be attributed to the increase of the concentration of Abt.

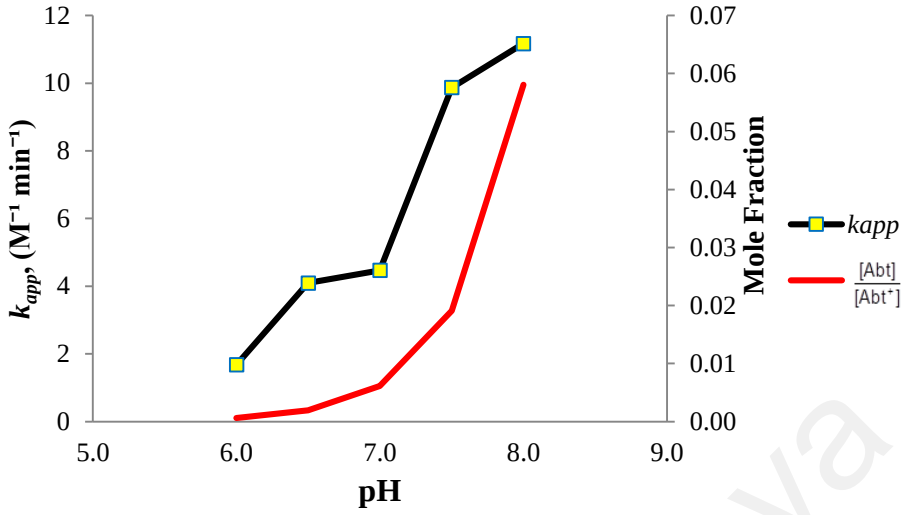


Figure 3.5: The variation of k_{app} and $\frac{[Abt]}{[Abt^+]}$ with pH.

According to Figure 3.6, the k_{app} of the reaction between Stl and FAC was found to decrease with increasing pH from 6 to 8. The rate of reaction between Stl with FAC decreased sharply with decreasing mole fraction of HOCl. This result showed that the degradation of Stl was dominated by reactions with HOCl. Similar observation was also obtained in the chlorination of sulfamethoxazole as explain by Dodd and Huang (2004) it was due to the decreases of α_{HOCl}.

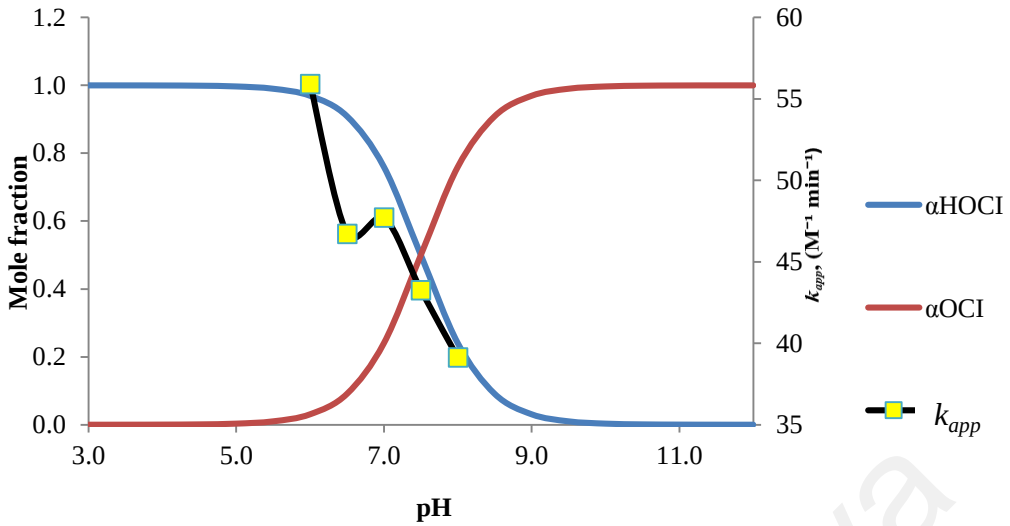
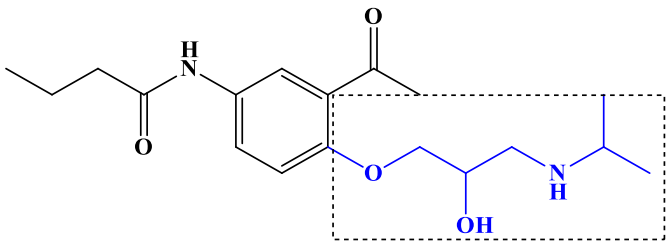


Figure 3.6: The variation of k_{app} for Stl with pH.

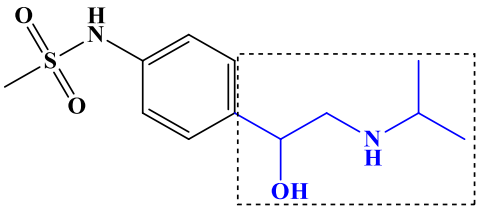
In general, Abt exhibited an increases in k_{app} with increasing pH. However, Stl exhibited the decreases in k_{app} with increasing pH. The variation of k_{app} with different pH for both Abt and Stl are different although they are from the same class of pharmaceutical. Based on the structure of Abt, 3-(isopropylamino)propane-1,2-diol (Figure 3.7a) substituent is attached to the aromatic ring via ether bond and consequently the lone pair electron of oxygen atom is delocalized and activate the aromatic ring of Abt. As for Stl, the substituent of *N*-(2-hydroxyethyl)propan-2-aminium group (Figure 3.7b) is attached directly to aromatic ring via C-C bond and therefore the aromatic ring of Stl is less reactive as compared to Abt. The tendency for aromatic ring of Stl to attack the positively polarized (HOCl) is less as compared to Abt. Therefore, it is proposed that the degradation of Stl is largely depending on the concentration of reactive HOCl due to its inactive nature. On the other hand, the degradation of more reactive Abt is less likely to be influenced by the concentration of HOCl.

(a)



3-(isopropylamino)propane-1,2-diol

(b)



N-(2-hydroxyethyl)propan-2-aminium

Figure 3.7: The structure of (a) Abt and (b) Stl.

3.1.2 Mfe

According to Figure 3.8, the k_{app} of Mfe was found to decrease from pH 6 to 8. Mfe was observed to give the highest reactivity at pH 6. At this point, HOCl is the dominant species in the chlorination process. The pK_a value for Mfe is 4.20 (Ni et al, 2002). Therefore, Mfe can appear as neutral (Mfe) or anion (Mfe^-) forms in solution under different pH conditions (Figure 3.9).

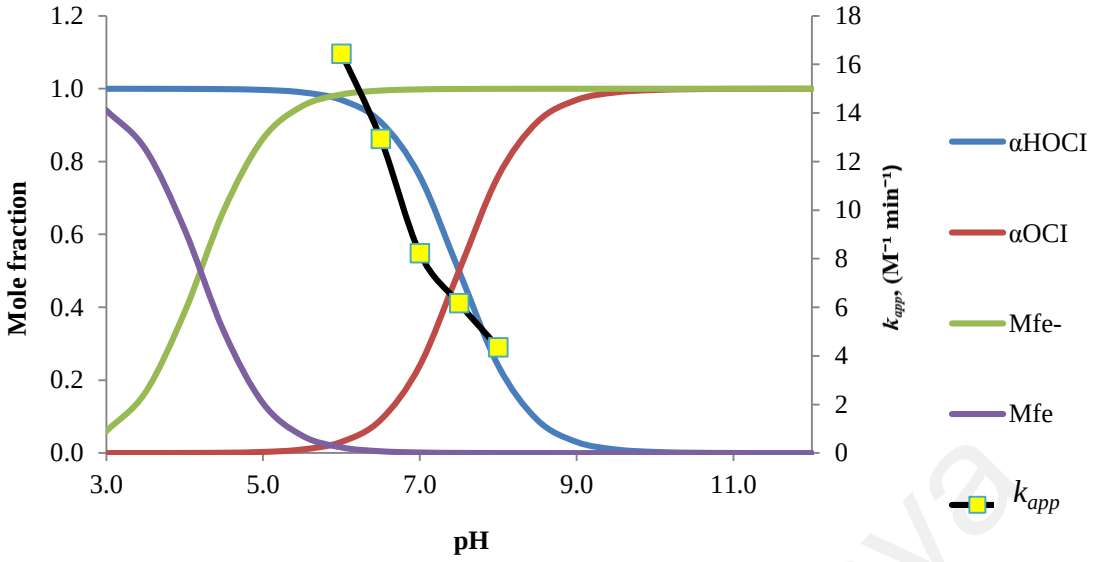


Figure 3.8: The variation of k_{app} for Mfe with pH.

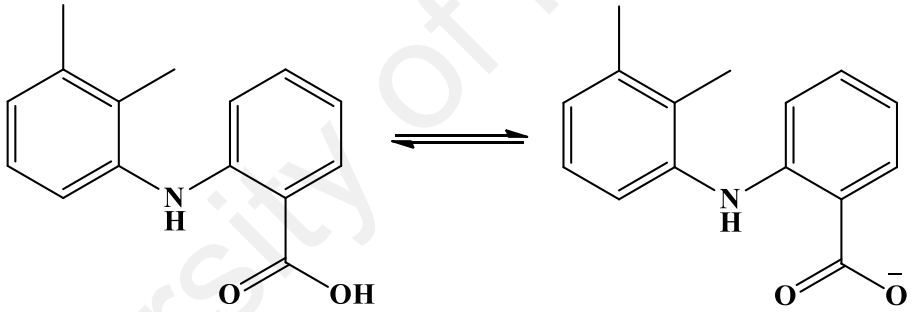


Figure 3.9: The neutral and deprotonated form of Mfe.

In this study, the molar fraction of Mfe and Mfe^- was also estimated using the Henderson-Hesselbalch equation as shown in Equation 9. Under the selected pH conditions, Mfe appeared dominantly as Mfe^- (Figure 3.8). Therefore, the effect of the degree of deprotonation of Mfe on the degradation of Mfe is negligible under the selected conditions (Deborde, and von Gunten, 2008; Acero et al., 2013). Consequently,

it can be concluded that the decrease of k_{app} with increasing pH of solution is mainly attributed to the decrease of the available HOCl in the solution. This results is also in agreement with the data from the chlorination of diclofenac which is one of the NSAIDs (Soufan et al., 2012).

$$\mathrm{pH} = \mathrm{pK_a} - \log_{10}\frac{[\mathrm{Mfe}]}{[\mathrm{Mfe^-}]} \quad (9)$$

3.1.3 Sfa and Sfn

The pK_a of Sfn are 2.27 and 10.6 while for Sfa the pK_a are 1.76 and 5.27. As other selected compounds, Sfn and Sfa can appear in protonated (Sfa^+, Sfn^+), neutral (Sfa, Sfn) and deprotonated (Sfa^-, Sfn^-) forms in water (Figure 3.10). In this study, the molar fraction of Sfa^+, Sfn^+, Sfa, Sfn, Sfa^-, and Sfn^- were calculated by using Equation 10, 11 and 12 (Chamberlain and Adams, 2006):

$$\alpha_{S^+} = \frac{1}{1+\left(\frac{K_1}{[H^+]}\right)+\left(\frac{K_1K_2}{[H^+]^2}\right)} \quad (10)$$

$$\alpha_{S^0} = \frac{1}{1+\left(\frac{[H^+]}{K_1}\right)+\left(\frac{K_2}{[H^+]}\right)} \quad (11)$$

$$\alpha_{S^-} = \frac{1}{1+\left(\frac{[H^+]}{K_2}\right)+\left(\frac{[H^+]^2}{K_1K_2}\right)} \quad (12)$$

where α_{S^+} refer to the molar fraction for protonated sulfonamides, α_{S^o} represents molar fraction for neutral sulfonamides, α_{S^-} is the molar fraction for deprotonated sulfonamides, $[H]^+$ is the concentration of hydroxonium ion, K_1 and K_2 are the acid dissociation of sulfonamides. Based on the Figure 3.11, the k_{app} of Sfa was found to increase from pH 6.0 to 6.5. Then, the k_{app} was found to decrease from pH 6.5 to pH 8.0. As shown in Figure 3.11, under the selected pH range, the dominant species of Sfa are Sfa^o and Sfa^-. When pH increases from 6 to 8, α_{Sfa^o} is decreased from 0.16 to 0.002 and α_{Sfa^-} is increased from 0.84 to 0.99. As reported by Dodd and Huang (2004), Sfa^- is more reactive than Sfa^o towards the reaction with HOCl during chlorination. Therefore, the increased of k_{app} (pH 6 to 6.5) can be attributed to the increases of more reactive deprotonated Sfa. On the other hand, the decreased of k_{app} (pH 7 to 8) can be attributed to the decreases of available HOCl (Deborde and von Gunten, 2008, Acero et al., 2013).

(a)

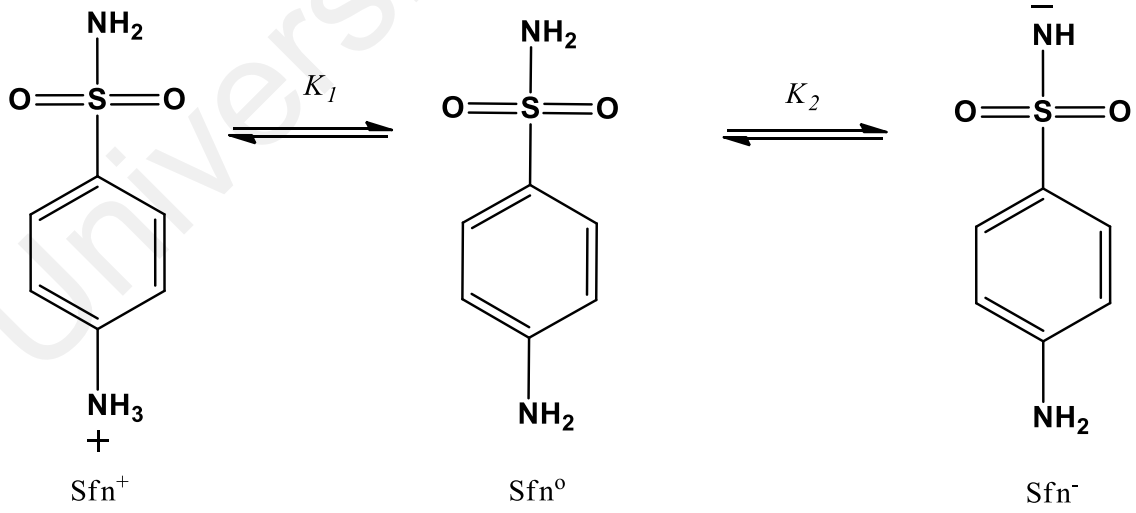


Figure 3.10: The protonated, neutral and deprotonated form of (a) Sfn and (b) Sfa (Chamberlain and Adams, 2006).

(b)

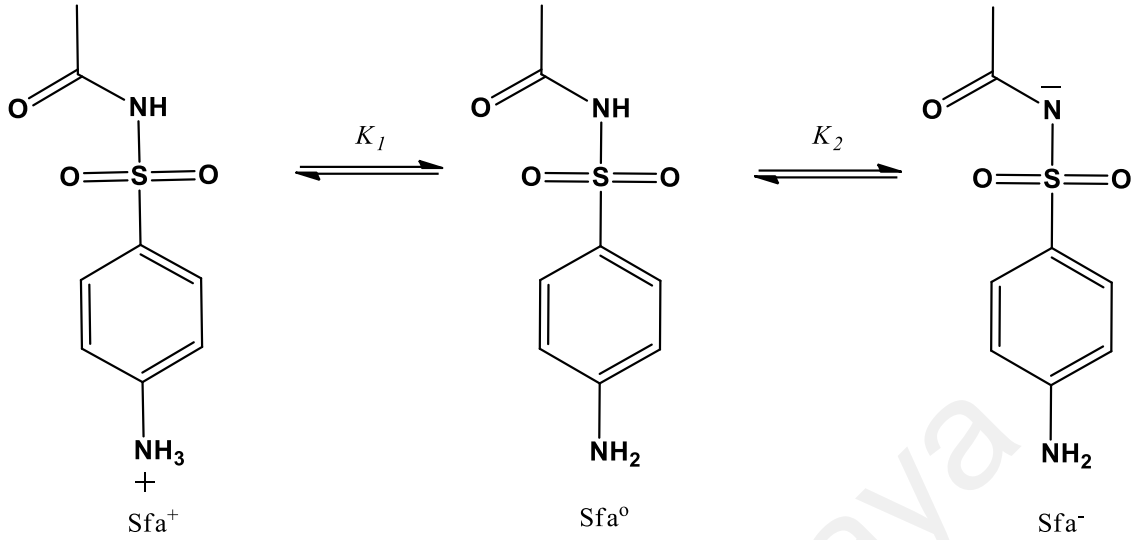


Figure 3.10 *(continued)*

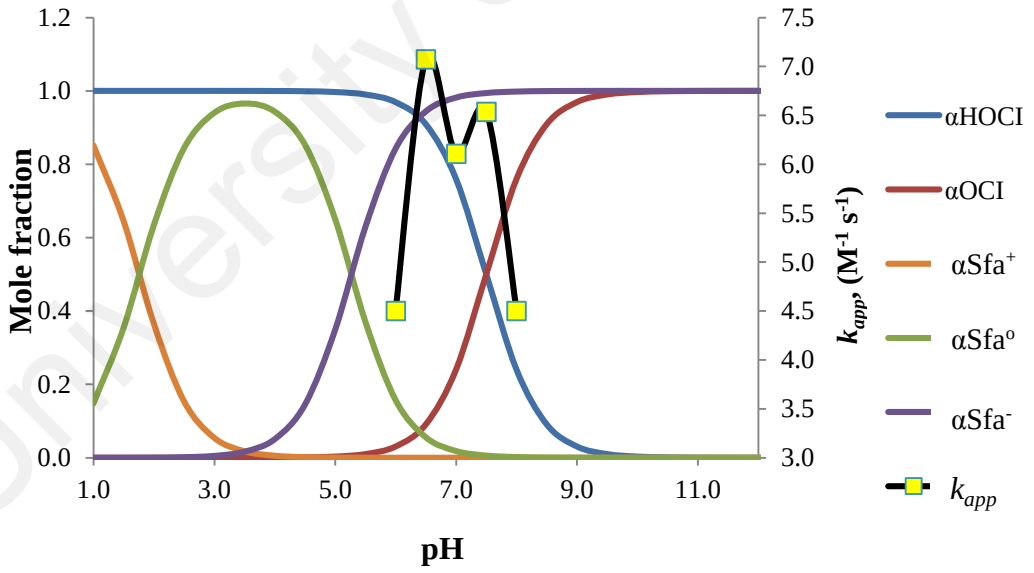


Figure 3.11: The variation of k_{app} for Sfa with pH.

Figure 3.12 presents the variation of k_{app} with pH for Sfn. As Sfn, the increased of k_{app} from pH 6 to 7 can be attributed to the increasing of neutral Sfn. Then, after pH 7 the k_{app} drop off sharply with decreasing α_{HOCl}. This result suggesting that k_{app} of Sfn are dominated by the reaction involving HOCl.

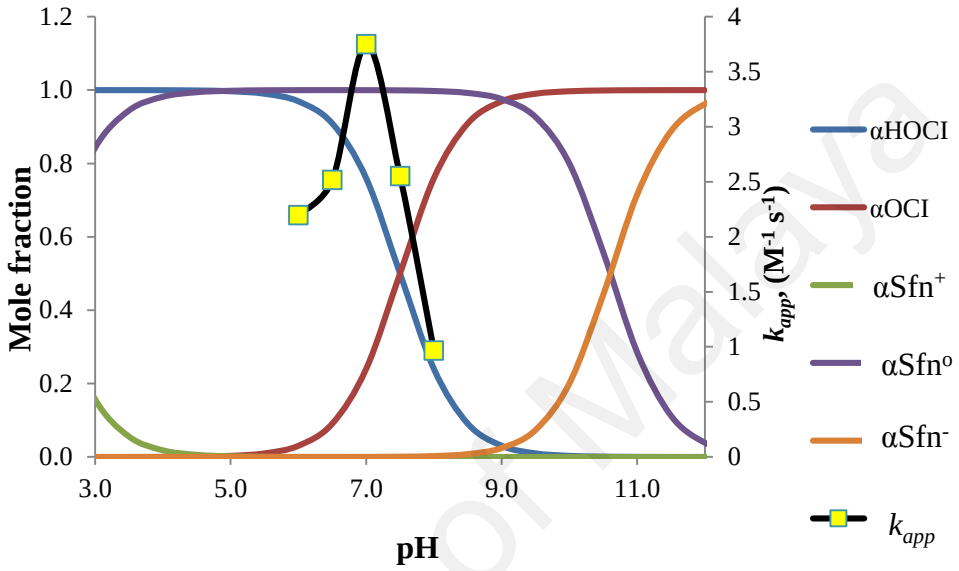


Figure 3.12: The variation of k_{app} for Sfn with pH.

The k_{app} values of Sfa was in the range (4.5-7.07 $M^{-1}s^{-1}$) while k_{app} values for Sfn was in the range (0.97-3.75 $M^{-1}s^{-1}$). Therefore, Sfa presents a higher k_{app} as compared to Sfn. The higher value of k_{app} for Sfa can be attributed to the resonance effect of the lone pair electron of nitrogen. The delocalization of lone pair electron of nitrogen into the carbonyl group forms the enolate species (*I*) with the negative charge. The presence of negative charge could increase the reactivity of Sfa via the nucleophilic attack on HOCl and consequently increase the rate of Sfa decomposition during chlorination (Figure 3.13).

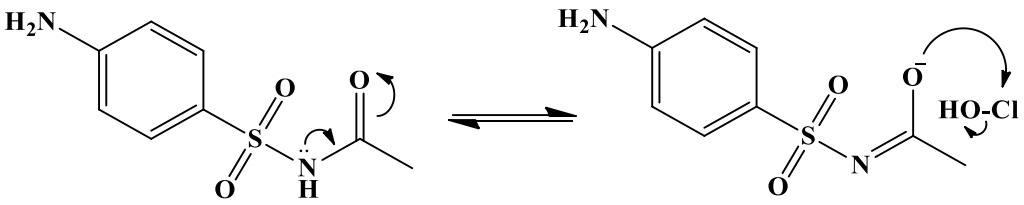


Figure 3.13: Delocalization of lone pair for Sfa.

3.2 Comparison of kinetics between selected pharmaceuticals at pH 7

From all the k_{app} values that are determined through this study, it is concluded that the removal of pharmaceuticals through chlorination was pH dependence. The results indicated that the pH could influence the rate of decomposition of selected pharmaceuticals through the dissociation of HOCl species and ionization of pharmaceuticals. Table 3.1 showed the summary of k_{app} value of the selected pharmaceuticals at pH 7. pH 7 is the nearest pH value of natural water. From the results, Mfe showed the highest k_{app} value which is 8.22 $M^{-1}s^{-1}$, while Abt showed the lowest k_{app} value which is 0.07 $M^{-1}s^{-1}$. Due to the low reactivity of β-blockers in the chlorination process, most of β-blockers could escape from the water treatment system and emerge in the environment. It can be concluded that the reactivity of selected pharmaceuticals towards FAC at pH 7 as follow:

Abt < Stl < Sfn < Sfa < Mfe

Table 3.1: Comparison of k_{app} value between selected pharmaceuticals at pH7.

Pharmaceuticals	k_{obs} x 10^{-3} (s^{-1})	k_{app} ($M^{-1}s^{-1}$)
Mfe	6.8 ± 1.2	8.22 ± 1.40
Sfa	5.7 ± 0.6	6.11 ± 0.59
Sfn	44 ± 11	3.75 ± 0.96
Stl	1 ± 14	0.80 ± 0.88
Abt	0.04 ± 0.10	0.07 ± 0.03

3.3 Half-life

3.3.1 Half-life of selected pharmaceuticals

From the kinetics study the half-life ($t_{1/2}$) for selected pharmaceuticals was calculated using Equation 13. $t_{1/2}$ represents the time for the concentration of pharmaceuticals to reduce by half of its initial concentration during chlorination process.

$$t_{1/2} = \frac{\ln 2}{k_{obs}} = \frac{\ln 2}{k_{app}[Cl]_o} \quad (13)$$

Table 3.2: The variation of $t_{1/2}$ for selected pharmaceuticals.

pH	Half-lifes, $t_{1/2}$ (min)				
	Abt	**Stl**	**Mfe**	**Sfa**	**Sfn**
6.0	768.0	7.7	0.8	2.8	0.5
6.5	315.0	9.2	1.1	1.8	0.4
7.0	289.0	9.0	1.7	2.0	0.3
7.5	131.0	9.9	2.3	1.9	0.4
8.0	116.0	10.9	3.2	2.8	1.0

According to Table 3.2, Sfn showed the highest reactivity towards FAC with the $t_{1/2}$ of 0.3 -1.0 min at pH ranging from 6 to 8. Abt showed the lowest reactivity towards FAC with the $t_{1/2}$ ranging from 116 to 768 min at the selected pH range. The order of the reactivity of the selected pharmaceuticals based on $t_{1/2}$ is as follow:

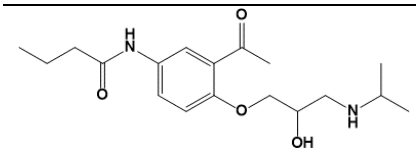
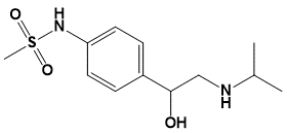
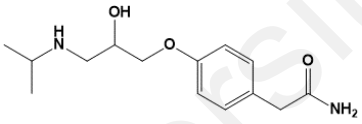
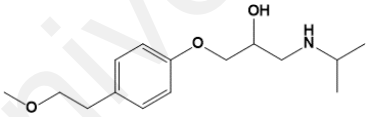
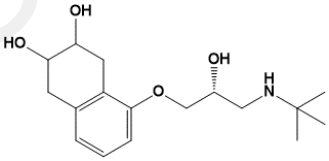
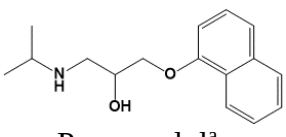
Abt < Stl <Sfa < Mfe < Sfn

Table 3.3 shows the $t_{½}$ of different β-blockers during chlorination at pH 7. Based on the k_{app} values published by Pinkston and Sedlak (2004), the $t_{½}$ of different β-blockers was calculated using the same initial concentration of FAC as this study. Abt was found to be more reactive towards FAC as compared with atenolol and metoprolol. However, Abt was less reactive towards FAC as compared with propranolol, Stl and nadolol. Therefore, it can be concluded that the reactivity of β-blockers towards FAC can be presented as follow:

Atenolol = Metoprolol < Ace < Nadolol < Stl < Propranolol

Propranolol shows the shortest $t_{½}$ followed by Stl. High reactive of propranolol might due to the presence of naphthalene moiety which can attack the positively polarized HOCl. Stl shows different aliphatic group (*N*-(2-hydroxyethyl)propan-2-aminium) as compared with other β-blockers. High reactivity of Stl suggesting the *N*-(2-hydroxyethyl)propan-2-aminium group as reaction site during chlorination.

Table 3.3: $t_{1/2}$ of different β-blockers in the chlorination process.

β-blocker	pH	Temperature (°C)	k_{app} (M^{-1} min^{-1})	$t_{½}$ ($\times 10^2$ min)
 Abt	7	25 ± 0.1	4.47	2.89
 Stl	7	25 ± 0.1	47.7	0.09
 Atenolol[a]	7	23 ± 2	1.02	12.7
 Metoprolol[a]	7	23 ± 2	1.02	12.7
 Nadolol[a]	7	23 ± 2	10.8	1.2
 Propranolol[a]	7	23 ± 2	450	0.03

[a]Pinkston and Sedlak (2004)

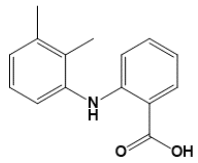
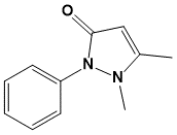
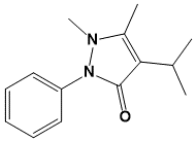
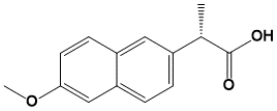
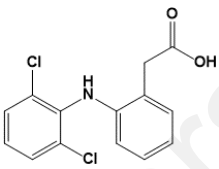
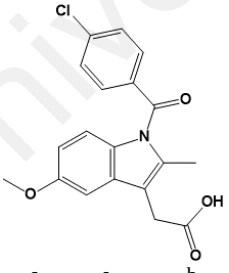
Table 3.4 shows the $t_{1/2}$ of different NSAIDs during chlorination at pH 7. Based on the published results by Radil et al. (2012) and Quintana et al. (2010), Mfe was found to be more reactive towards the reaction with FAC as compared to phenazone, propyphenazone, naproxen, and diclofenac but less reactive than indomethacine. The order of reactivity of NSAIDs towards FAC can written as follow:

Indomethacine < Mfe < Diclofenac < Naproxen < Propyphenazone < Phenazone

Table 3.5 showed the $t_{1/2}$ of sulfonamides. The most reactive sulfonamides towards FAC was Sulfathiazole while the less reactive was Sfa. Sulfonamide antibiotics sharing the same structure of 4-aminobenzenesulfonamide moiety with different substituents attached to sulfonamide moiety. Comparison of $t_{1/2}$ showed that the reactivity of sulfonamide antibiotics was largely influence by the substituents groups. Sfa and Sfn with the smallest substituents groups were the least reactive sulfonamides in chlorination process. Therefore, it can be concluded that the reactivity of sulfonamides towards FAC as follow:

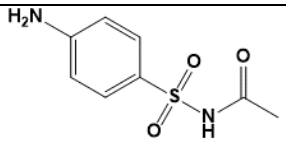
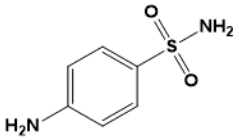
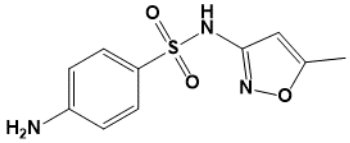
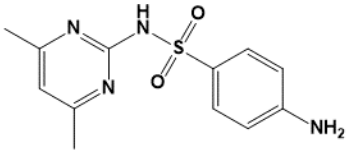
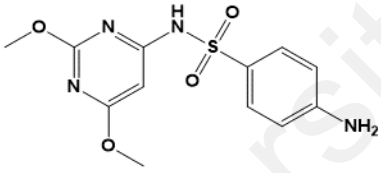
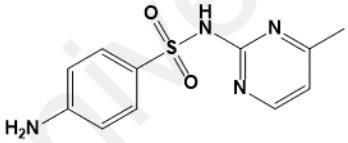
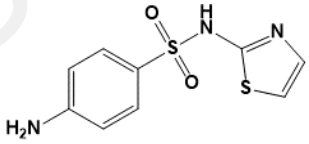
Sfa < Sfn < Sulfamerazine < Sulfamethoxazole < Sulfamethazine < Sulfathiazole < Sulfadimethoxine

Table 3.4: $t_{1/2}$ of different NSAIDs in the chlorination process.

NSAIDs	pH	Temperature (°C)	k_{app} ($M^{-1}s^{-1}$)	$t_{½}$ (min)
 Mfe	7	25 ± 0.1	8.22	1.7
 Phenazone[a]	7	20 ± 2	0.02	698
 Propyphenazone[a]	7	20 ± 2	0.03	466
 Naproxen[b]	7	-	0.29	48.2
 Diclofenac[b]	7	-	0.68	20.5
 Indomethacine[b]	7	-	10.58	1.32

[a]Rodil et al., 2012 [b]Quintana et al., 2012

Table 3.5: $t_{1/2}$ of different sulfonamides in the chlorination process.

Sulfonamides	pH	Temperature (°C)	k_{app} ($M^{-1}s^{-1}$)	$t_{½}$ (min)
 Sfa	7	25 ± 0.1	6.11	2
 Sfn	7	25 ± 0.1	3.75	0.3
 Sulfamethoxazole[a]	7	25	650	0.019
 Sulfamethazine[a]	7	25	790	0.016
 Sulfadimethoxine[a]	7	25	5630	0.002
 Sulfamerazine[a]	7	25	420	0.029
 Sulfathiazole[a]	7	25	1890	0.007

[a]Sharma 2008

3.4 Oxidation of pharmaceuticals during chlorination of natural waters and secondary effluent

In a following step, the removal of selected pharmaceuticals in different water matrices (secondary effluent, ground water and lake water) were investigated in order to evaluate the efficiency of the chlorination process in the removal of pharmaceuticals in real water samples. Table 3.6 shows the chemical characteristics of the selected water samples such as pH, TOC (total organic carbon), and COD (chemical oxygen demand).

Table 3.6: Quality parameters of the selected water matrices.

Type of water samples	pH	TOC (mg/L)	COD (mg/L)
Secondary effluent	7.0	13.4 ± 0.8	12.0 ± 0.5
Ground water	7.1	4.8 ± 0.1	9.5 ± 0.1
Lake water	6.9	6.4 ± 0.2	11.0 ±0.1

Figure 3.14 shows the percentage removal of pharmaceuticals during chlorination process in secondary effluent, ground water, and lake water. Different concentrations of FAC ranging from 10 to 80 μM were used for the chlorination process. It was clearly observed the positive influence of the increasing FAC dosage on the pharmaceuticals removal for the selected water samples. In ground water and lake water, 60 μM of FAC which is equivalent to 4.1 ppm was sufficient to remove more than 90% of the selected pharmaceuticals. Therefore, the suggested chlorine dosage by USEPA (1999) which is ranged from 5 – 20 ppm is expected to be sufficient for the

removal of pharmaceuticals in ground water and lake water. The effectiveness of chlorination in the removal of pharmaceuticals was relative poor in the secondary effluent. For secondary effluent, chlorination exhibited the lowest efficiency in the removal of Mfe (11%) and Sfa (2%). By using the dosage of 80 μM, the percentage removal of Abt, Stl and Sfn in secondary effluent was 81, 96 and 70% respectively. From Table 3.6, the TOC and COD values of secondary effluent were 13.4 and 12.0 mg/L, which is relatively higher than groundwater and lake water. High COD and TOC values indicated the presence of higher amount of organic matter in the secondary effluent. This result showed that the pharmaceuticals compete with the organic matter for the reaction with FAC. Therefore, in the presence of organic matter, the available HOCl for the oxidation of pharmaceuticals was largely reduced (Acero et al., 2010; González-Mariño et al., 2015; Negreira et al., 2015). Contrary, chlorination showed higher efficiency in the removal of selected pharmaceuticals in the ground water and lake water which has lower TOC and COD value. In general, it can be concluded that the efficiency of chlorination in the removal of pharmaceuticals are affected by other factors such as TOC and COD of the water samples.

Among the selected water matrices, chlorination achieved the highest efficiency in the removal of pharmaceuticals in ground water. Basically, the trend of the removal of some selected pharmaceuticals in cleaner water matrices can be predicted using the calculated $t_{1/2}$ when the FAC dosage is low. For example at the dosage of 10 μM of FAC, the percentage removal of Stl with $t_{1/2}$ of 9.0 min was 12%. On the other hand, the percentage of removal for Sfn with the shortest $t_{1/2}$ of 0.3 min was 98%.

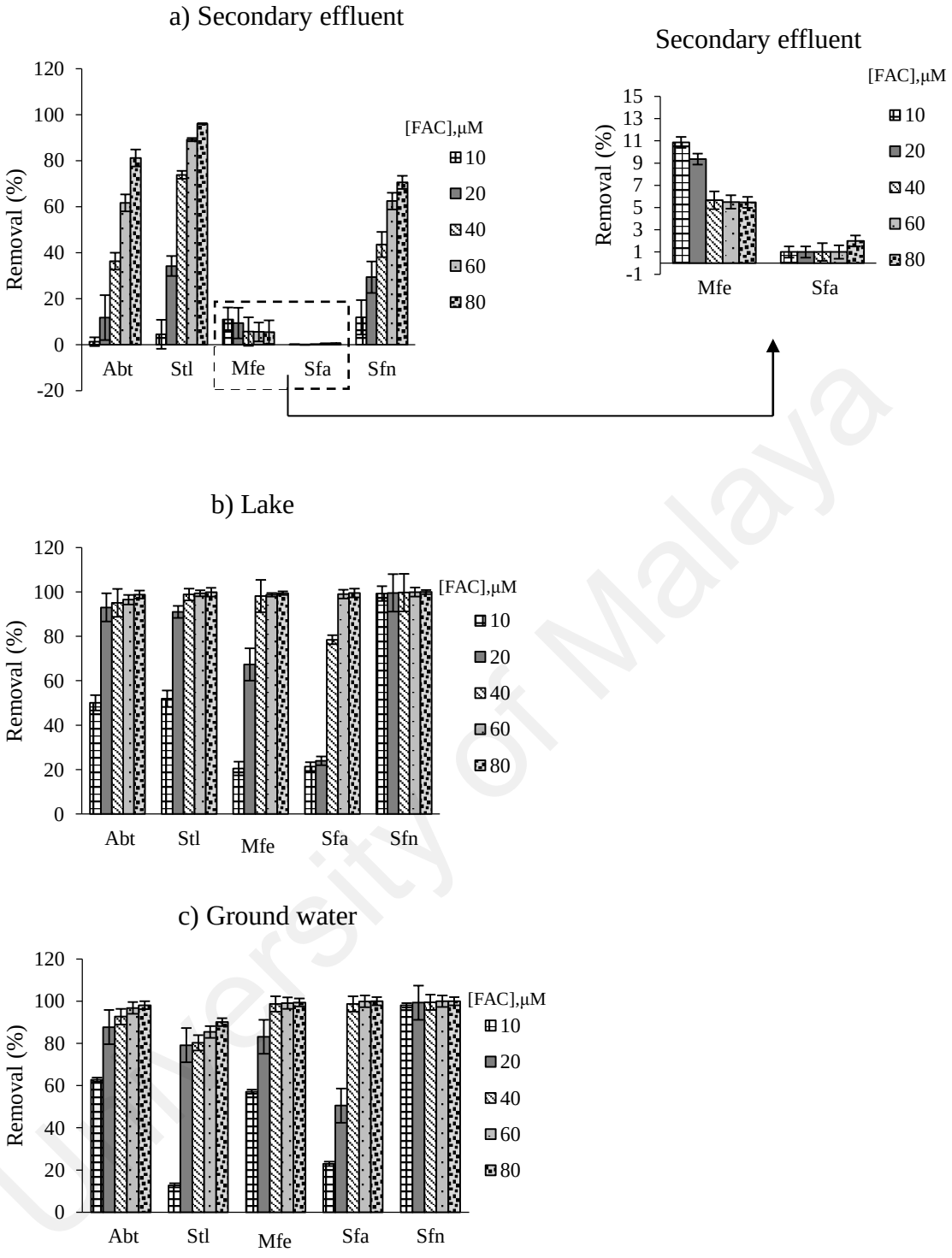


Figure 3.14: Influence of the initial chlorine dose on the selected pharmaceuticals oxidation on (a) secondary effluent (b) lake water (c) ground water.

[pharmaceuticals] = 1µM

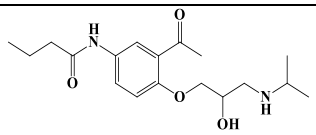
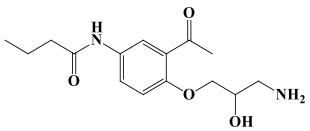
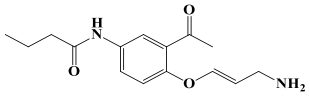
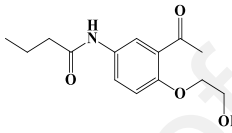
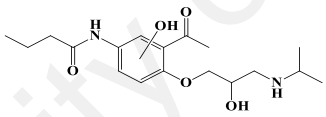
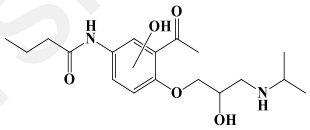
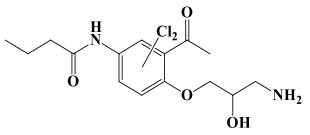
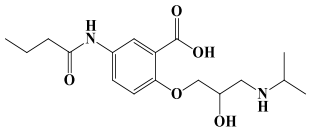
3.5 Identification of Chlorination by-products for Selected Pharmaceuticals

In order to obtain the transformation by-products of selected pharmaceuticals, chlorination was performed by allowing the selected pharmaceuticals to be exposed to FAC for 24 h. Under such conditions, the transformation by-products which are resistant against chlorination can be determined. In this study, structure elucidation of the transformation by-products was performed using LC-QTOFMS without quenching. This instrument produces MS and MS/MS with high mass accuracy for the quasi molecular ion $[M+H]^+$ and the fragment ions. Analysis was performed by comparing mass spectrometric data of the initial pharmaceuticals solution as control sample with the data collected from the chlorinated pharmaceuticals solution. The control samples were prepared as the selected pharmaceutical samples without FAC.

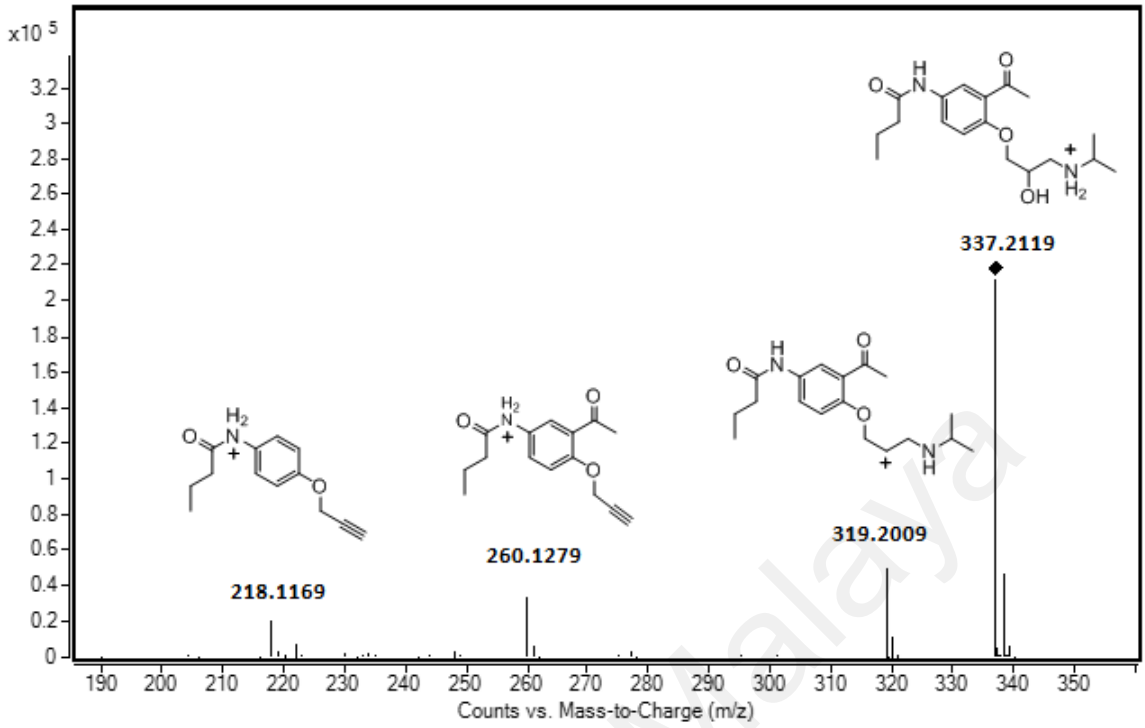
3.5.1 Transformation by-products of Abt

Table 3.7 shows the structure of Abt and the proposed structure of its transformation by-products. Seven transformation by-products of Abt were identified. The MS/MS spectrum of Abt is presented in Figure 3.15a. For Abt, a fragment ion at *m/z* 319.2009 and 260.1279 was formed through the loss of a H_2O molecule and propan-2-amine group from the $[M+H]^+$ ion, respectively (Tay and Madehi 2014). Further fragmentation of the fragment ion at *m/z* 260.1279 through the loss of acetaldehyde group formed the fragment ion at *m/z* 218.1169.

Table 3.7: Proposed transformation by-products of Abt during chlorination.

Quasi-molecular ion $[M+H]^+$	Chemical formulae $[M+H]^+$	Proposed structure (label-retention time)	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Reaction pH		
						6	7	8
337.2119	$C_{18}H_{29}N_2O_4^+$	 **Abt (6.91 min)**	336.2052	336.2049	-0.75	√	√	√
295.1643	$C_{15}H_{23}N_2O_4^+$	 **Abt-294 (4.68 min)**	294.1581	294.1580	-0.43	√	√	√
277.1555	$C_{15}H_{21}N_2O_3^+$	 **Abt-276 (3.79 min)**	276.1470	276.1474	1.54	√	√	√
266.1358	$C_{14}H_{20}NO_4^+$	 **Abt-265 (2.49 min)**	265.1302	265.1314	4.65	x	x	√
353.2094	$C_{18}H_{29}N_2O_5^+$	 **Abt-352a (3.11 min)**	352.1994	352.1998	1.06	√	x	x
353.2047	$C_{18}H_{29}N_2O_5^+$	 **Abt-352b (3.12 min)**	352.2000	352.1998	-0.59	√	x	x
363.0873	$C_{15}H_{21}CI_2N_2O_4^+$	 **Abt-362 (8.76 min)**	362.0800	362.0800	-0.01	√	√	x
339.1888	$C_{17}H_{27}N_2O_5^+$	 **Abt-338 (6.35 min)**	338.1830	338.1842	3.34	√	x	x

(a) Abt



(b) Abt-294

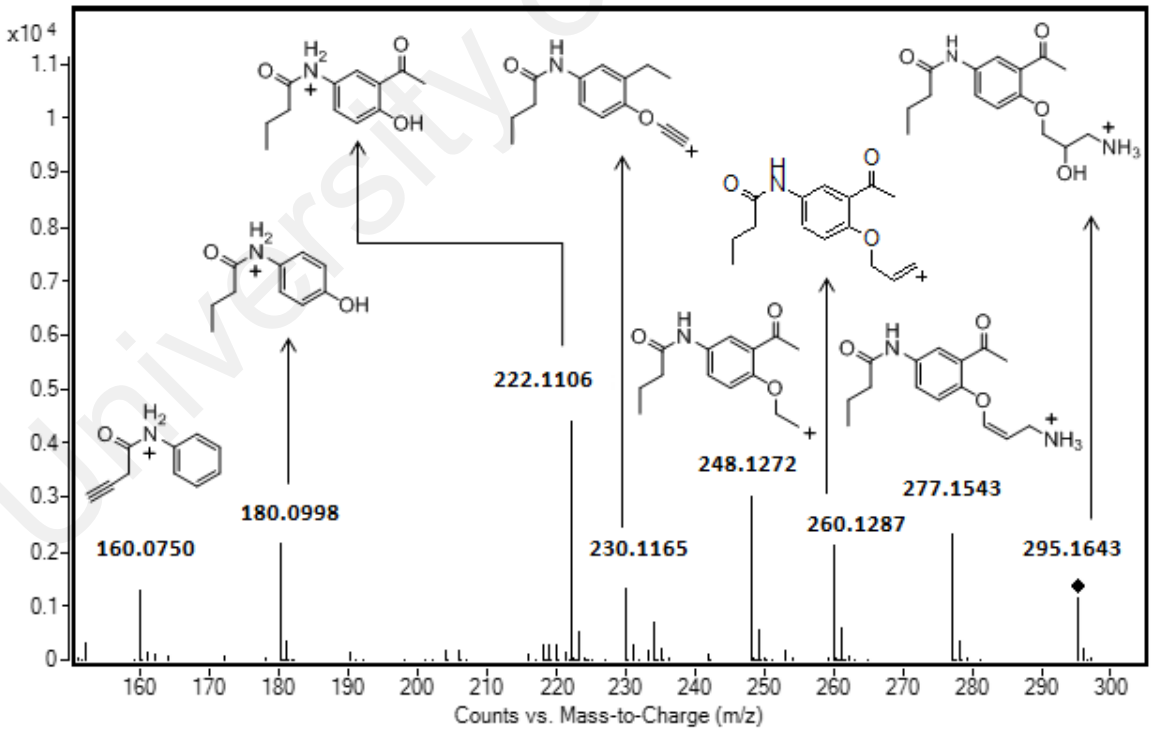
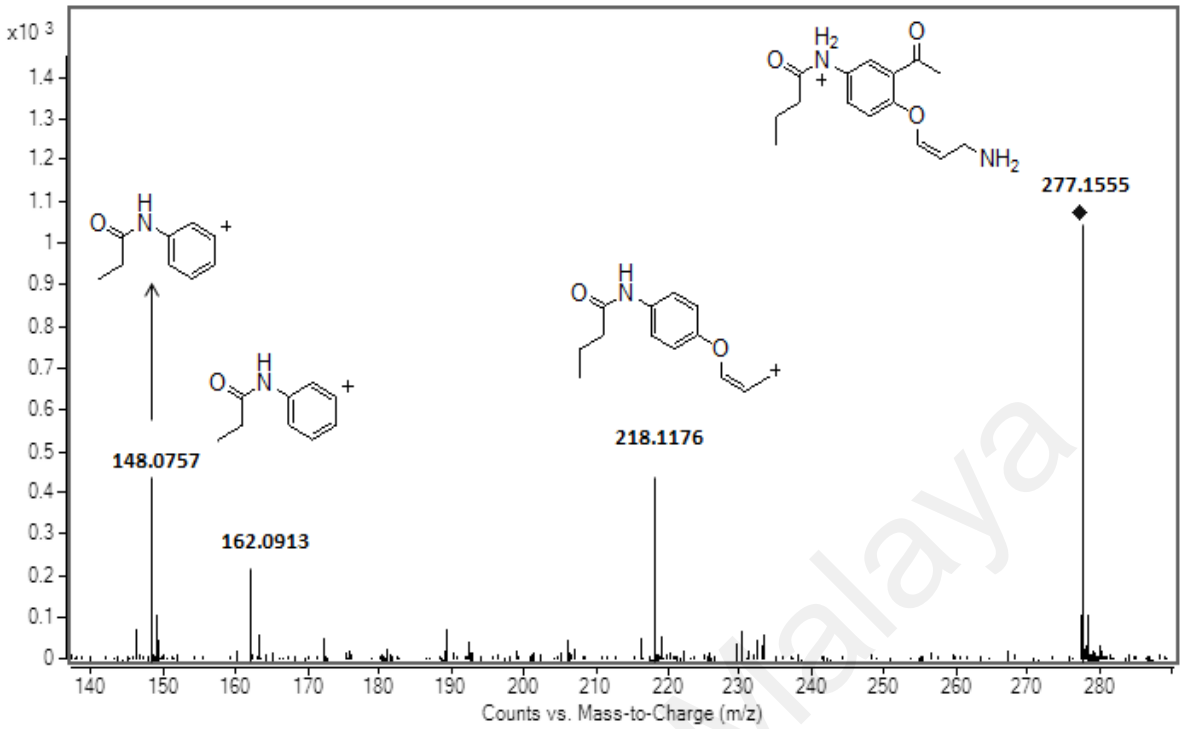


Figure 3.15: MS/MS spectrum of Abt and its by-products.

(c) Abt-276



(d) Abt-265

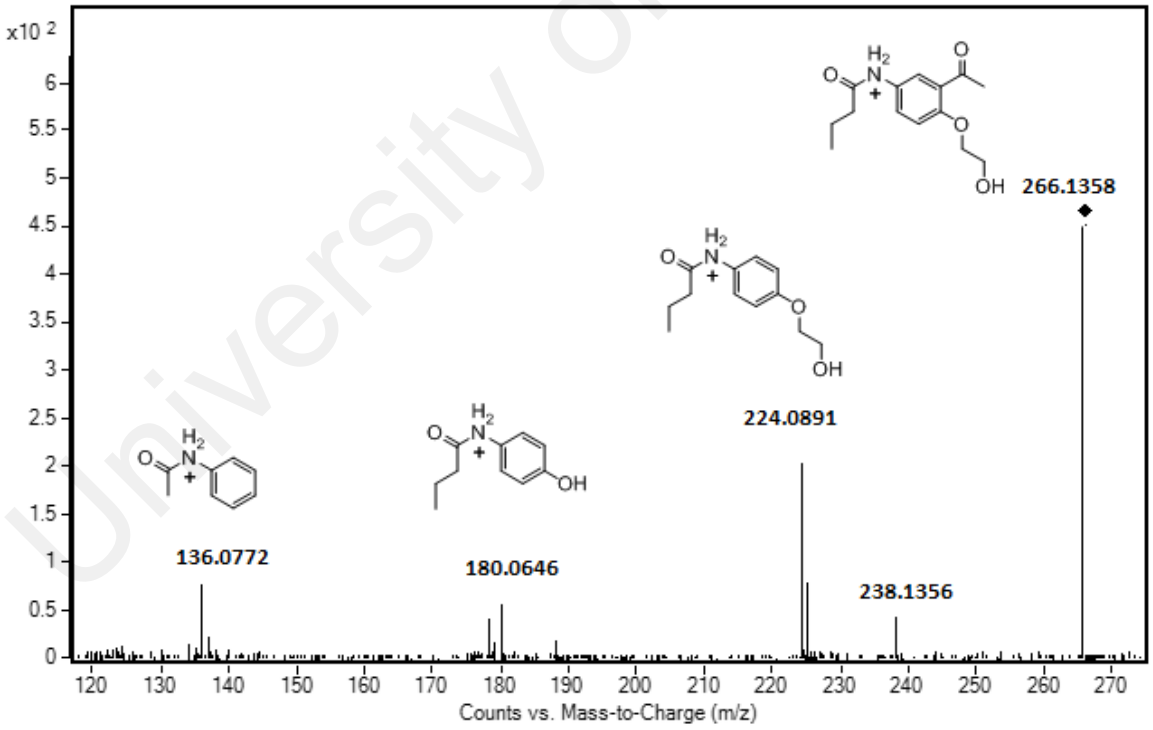
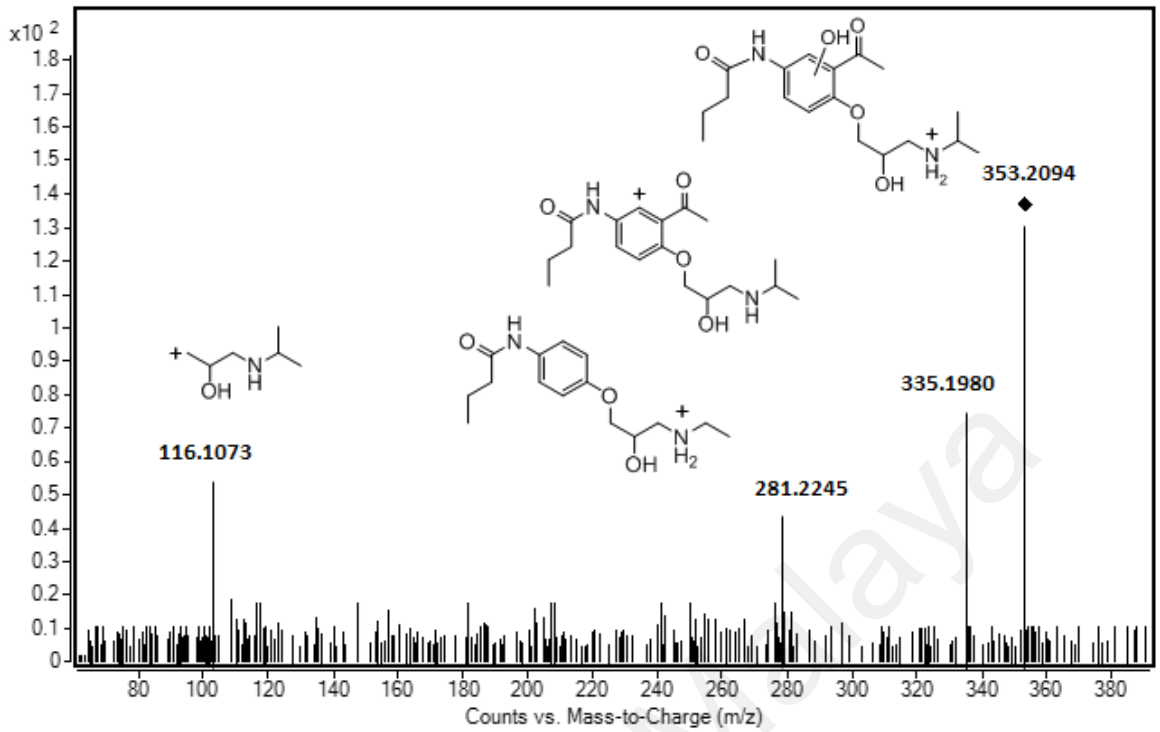


Figure 3.15 *(continued)*

(e) Abt-352a



(f) Abt-352b

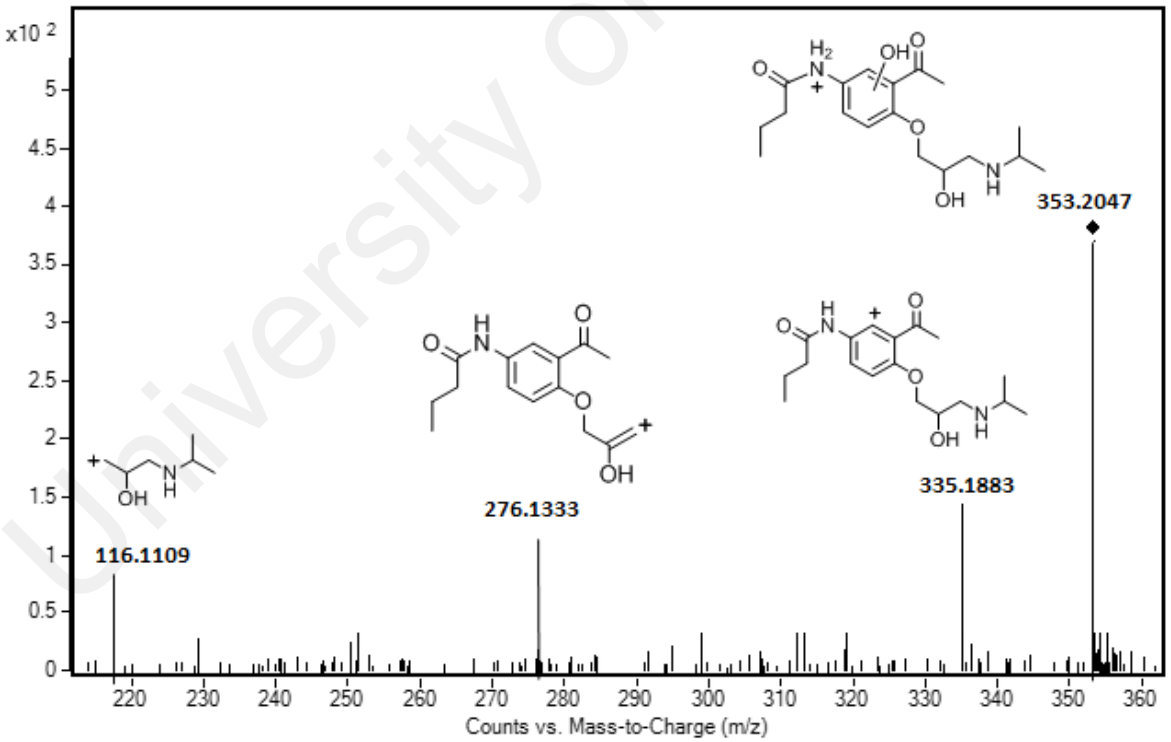
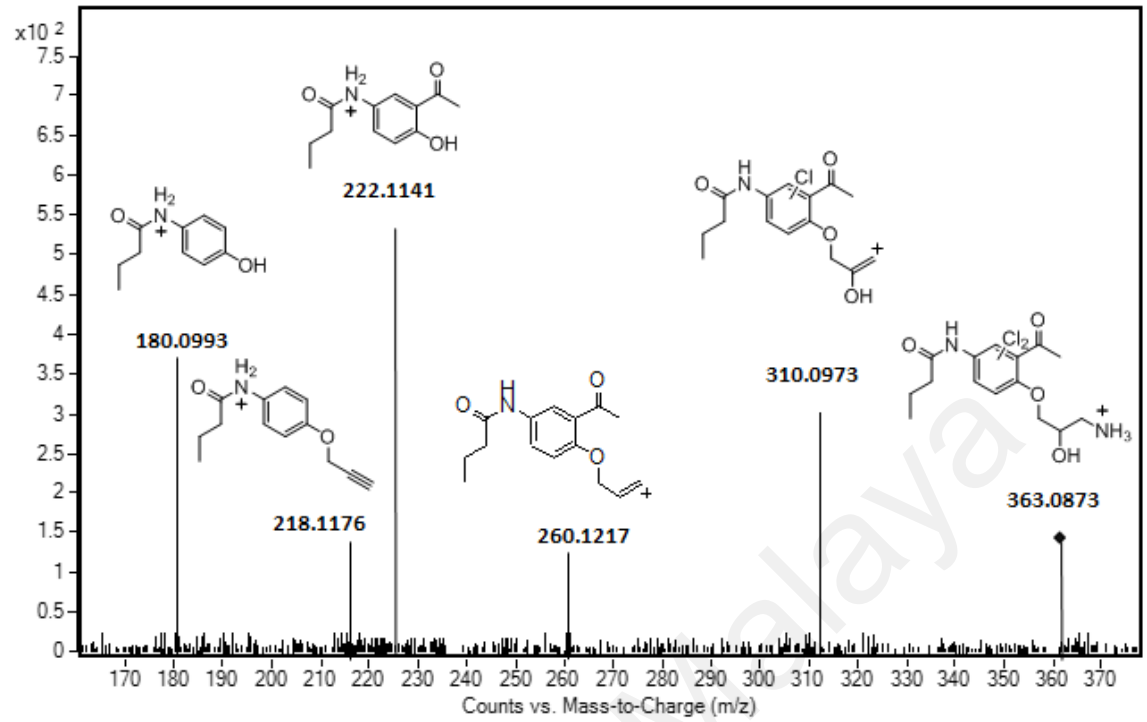


Figure 3.15 *(continued)*

(g) Abt-362



(h) Abt-338

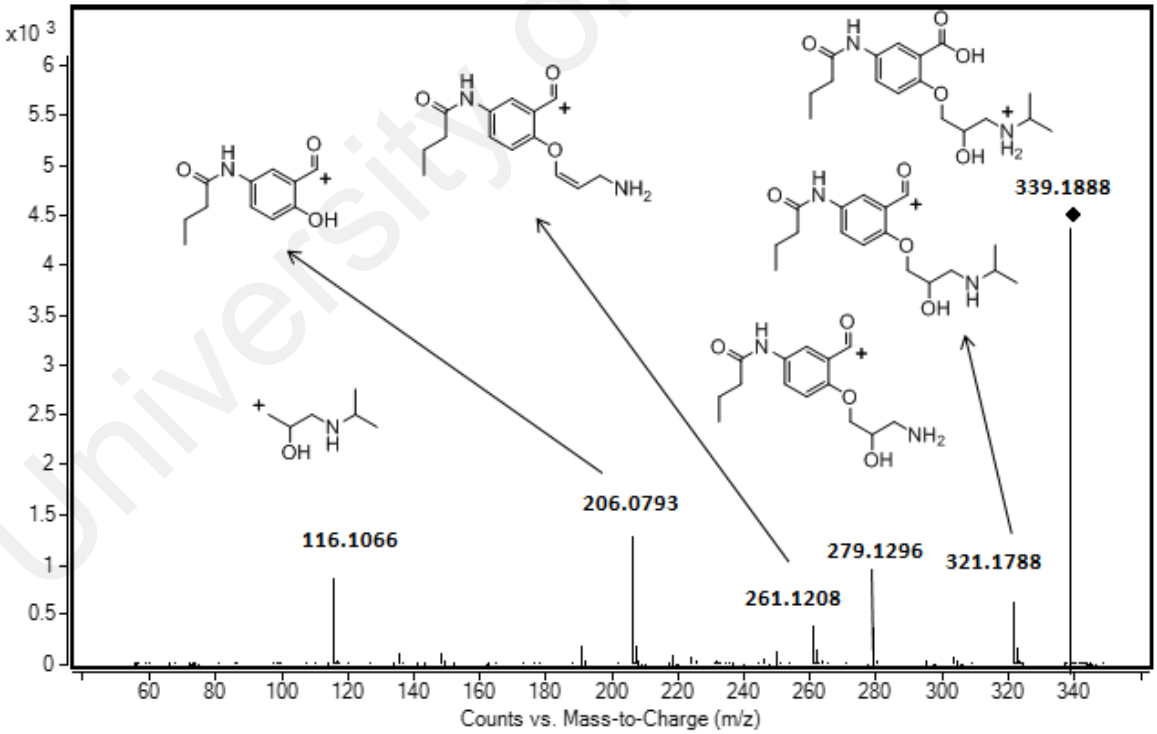


Figure 3.15 *(continued)*

During chlorination, three breakdown by-products of Abt with the $[M+H]^+$ ion lower than Abt were detected. These by-products were Abt-294, Abt-276 and Abt-265. Abt-294 was formed through the loss of isopropyl group from Abt (Figure 3.15b). This by-product has been reported during the ozonation of Abt (Tay and Madehi, 2014). The mechanism for the formation of Abt-294 through dealkylation is presented at Figure 3.16. The formation of Abt-294 is proposed to start with the reaction between the secondary amine of Abt with HOCl for the formation of chlorammonium intermediate (*I*) (Deborde and von Gunten, 2008). Intermediate *I* is a highly reactive species that could decompose through dealkylation and forms Abt-294 (Dodd et al., 2005; El Najjar et al., 2013). Abt-276 and Abt-265 are proposed to be the breakdown by-products of Abt-294. Abt-276 with the $[M+H]^+$ ion at *m/z* 277.1555 (Figure 3.15c) was produced via the removal of hydroxyl group from Abt-294. Abt-276 is also one of the ozonation by-products of Abt (Tay and Madehi, 2014). Abt-265 was produced through the removal of methylamine group from Abt-294. For Abt-265, the most dominant fragment ion in the MS/MS spectrum was *m/z* 224.0891 and this fragment ion was formed through the loss of acetyl group from Abt-265 (Figure 3.15d). The fragment ion at *m/z* 180.0646 was formed through the loss of ethyl alcohol group from the fragment ion at *m/z* 224.0891. Fragment ion at *m/z* 180.0646 is also the key ion that showed the presence of ethyl alcohol group in Abt-265.

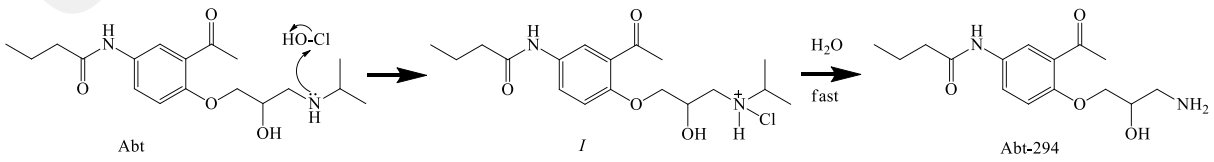


Figure 3.16: Proposed transformation pathway for the formation of Abt-294.

Two by-products with the $[M+H]^+$ ion at *m/z* 353 were detected. These by-products were labeled as Abt-352a and Abt-352b. The MS/MS spectrum for Abt-352a and Abt-352b showed almost similar fragmentation pattern (Figure 3.15e and Figure 3.15f). Abt-352a and Abt-352b were proposed to be the isomers of aromatic ring monohydroxylated Abt as these compounds showed additional 16 Da that representing one OH group. These two by-products showed unmodified 1-(isopropylamino) propan-2-ol group at *m/z* 116. Therefore, the addition of OH group to the aromatic ring of Abt is proposed. The mechanism for hydroxylation is presented at Figure 3.17. The mechanism for the hydroxylation is proposed to start with the attack of aromatic ring of Abt to the positively polarized chlorine atom of HOCl, forming a monochlorinated carbocation (*II*). Rearrangement of intermediate *II* through the elimination of one hydrogen atom forms monochlorinated Abt. Then, the nucleophilic addition of hydroxide ion (OH^-) to the aromatic ring of monochlorinated Abt forms carbanion *III*. Carbanion *III* can rearrange to form aromatic ring monohydroxylated Abt (Abt-352a and b).

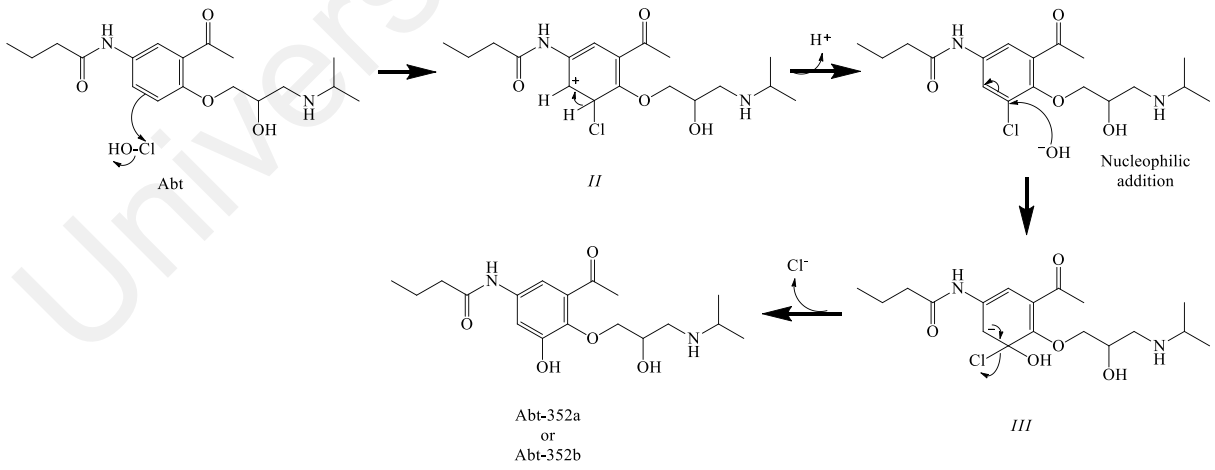


Figure 3.17: Proposed transformation pathway for the formation of Abt-352a or Abt-352b.

Abt-362 with the $[M+H]^+$ ion at *m/z* 363.0873 was the only chlorinated by-product detected for Abt. This product was formed through the addition of two chlorine atoms to the aromatic ring of Abt-294. The addition of chlorine atoms is proposed to occur at the aromatic ring of Abt-294. For Abt-362, the fragment ion at *m/z* 310.0973 was formed through the elimination of hydrogen chloride and NH_3 group from $[M+H]^+$ ion (Figure 3.15g). Fragment ion at *m/z* 310.0973 is the key ion that showing the presence of chlorine atoms at Abt-362. Further fragmentation of ion at *m/z* 310.0973 through the loss of one hydrogen chloride molecule and one water molecule formed *m/z* 260.1217. Since this by product was originated from Abt-294, the fragment ions at *m/z* 180 and 222 were also detected. The proposed mechanism for the formation of Abt-362 is presented at Figure 3.18. The mechanism for the chlorination is proposed to start with the attack of aromatic ring of Abt-294 to the positively polarized chlorine atom of HOCl, forming a monochlorinated carbocation (*IV*). Rearrangement of intermediate *IV* through the elimination of one hydrogen atom forms monochlorinated Abt-294. Then, the aromatic ring of monochlorinated Abt-294 further attacks on HOCl forming dichlorinated carbocation (*V*). Rearrangement of intermediate *V* through the elimination of one hydrogen atom forms Abt-362.

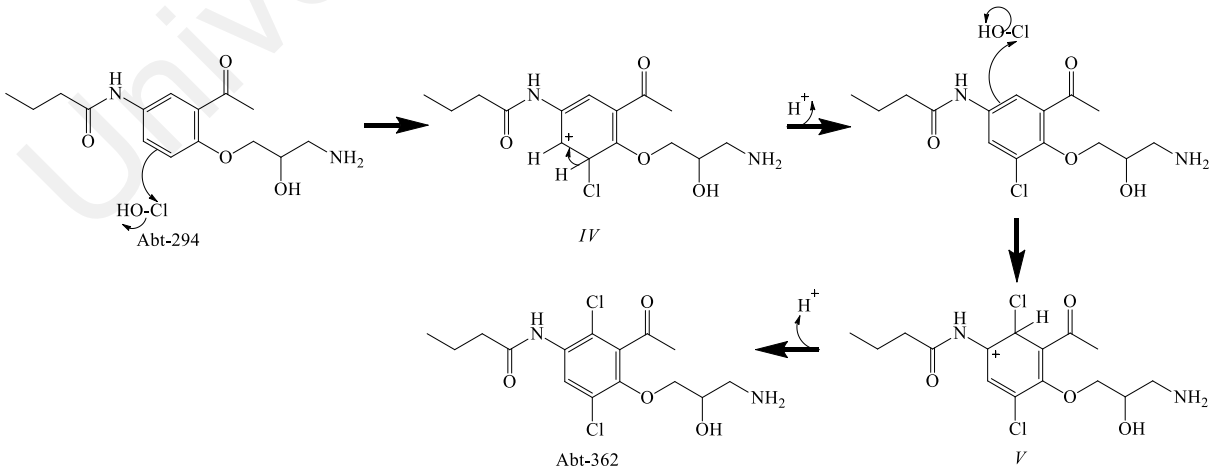


Figure 3.18: Proposed transformation pathway for the formation of Abt-362.

Abt-338 with the $[M+H]^+$ ion at *m/z* 339.1888 (Figure 3.15h) is proposed to form through the replacement of methyl group with OH group at the acetyl group of Abt. For Abt-338, fragment ion at *m/z* 321.1788 was formed through the elimination of water molecule from $[M+H]^+$ ion. Other significance product ions for this by-product were detected at *m/z* 279.1296, 261.1208, 206.0793 and 116.1066. Fragment ion at *m/z* 279.1296 was formed through the loss of propane group from the fragment ion of *m/z* 321.1788. Further fragmentation of *m/z* 279.1296 ion through the loss of water molecule produced a product ion at *m/z* 261.1208. Then, the product ion at *m/z* 261.1208 was further fragmented to form *m/z* 206.0793 through the elimination of prop-2-en-1-amine. The presence of *m/z* 116.1066 indicated that Abt-338 contains unmodified 1-(isopropylamino) propan-2-ol group. The proposed mechanisms for the formation of Abt-338 from Abt-336 are shown at Figure 3.19. Chlorination reaction with carbonyl functional groups such as acetyl group for the formation of carboxylic acid group have been reported by Deborde and von Gunten (2008). This reaction is proposed to start with the initial substitution reactions at the α-carbon. The reaction can take place by a series of hydrogen atoms replacement by chlorine atoms from HOCl to produce intermediate *VI*. Then, the attack of hydroxide ion at intermediate *VI* forms intermediate *VII* which could rearrange through the elimination of CCl_3 group to form Abt-338.

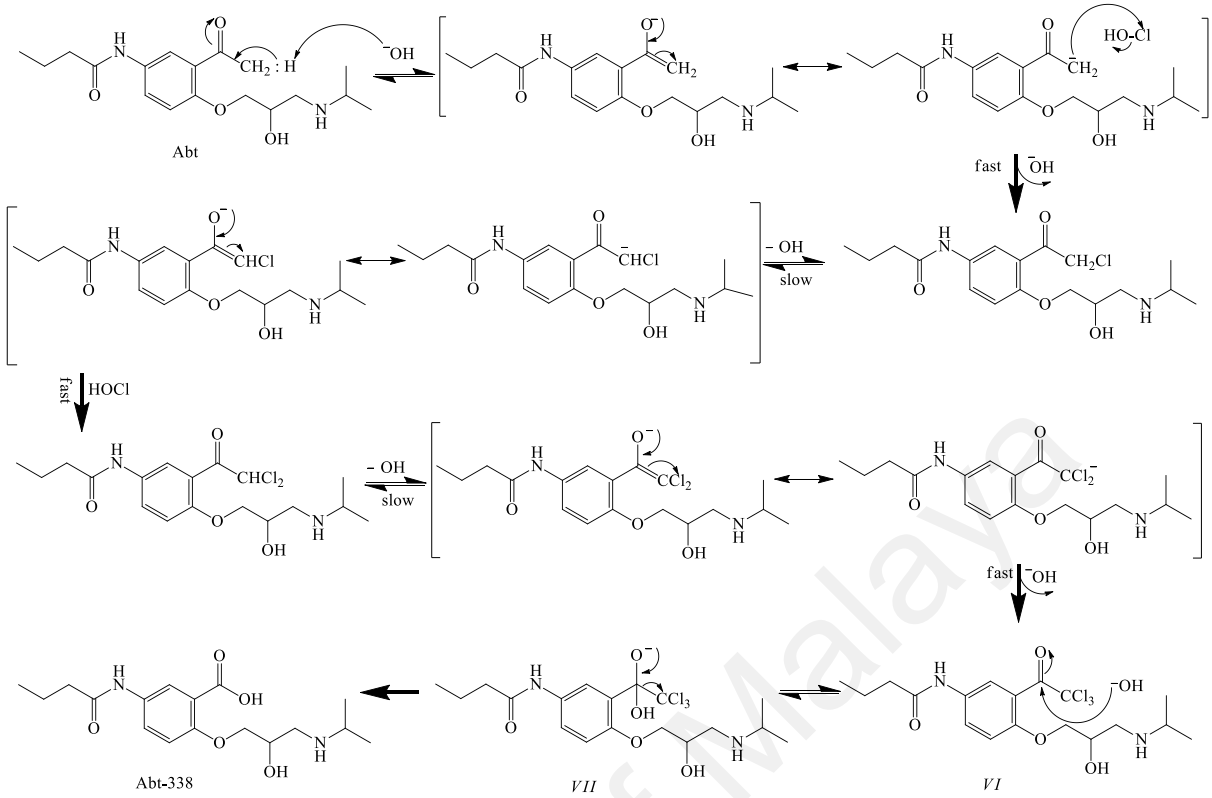


Figure 3.19: Proposed transformation pathway for the formation of Abt-338.

3.5.2 Transformation by-products of Stl

Table 3.8: Proposed transformation by-products of Stl during chlorination.

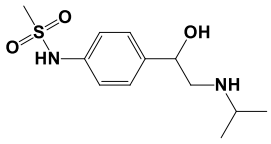
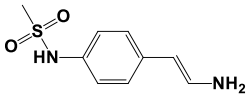
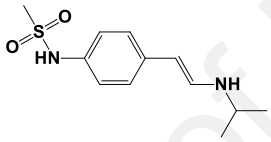
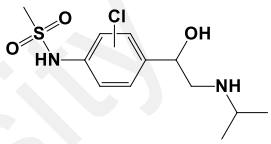
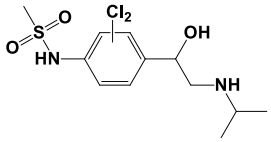
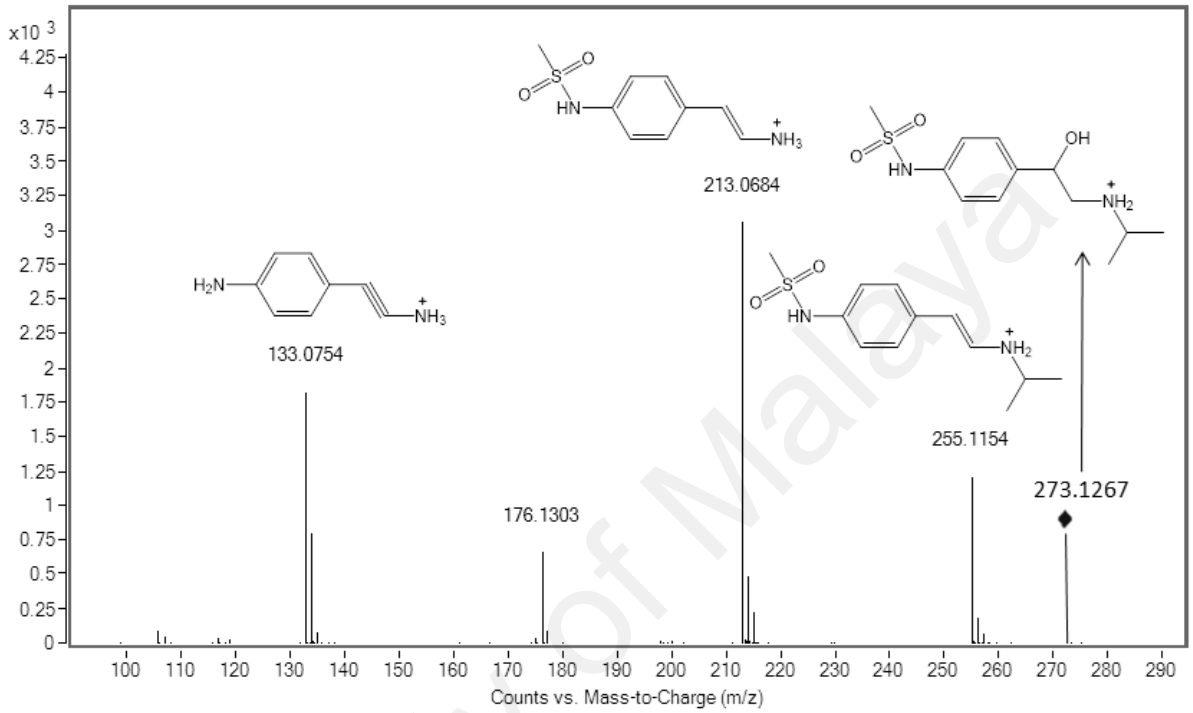
Quasi-molecular ion $[M+H]^+$	Chemical formulae $[M+H]^+$	Proposed structure (label-retention time)	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Reaction pH		
						6	7	8
273.1267	$C_{12}H_{21}N_2O_3S^+$	 **Stl (2.648 min)**	272.1192	272.1195	1.11	√	√	√
213.0692	$C_9H_{13}N_2O_2S^+$	 **Stl-212 (2.418 min)**	212.062	212.0619	-0.43	√	√	√
255.1162	$C_{12}H_{19}N_2O_2S^+$	 **Stl-254 (2.688 min)**	254.1096	254.1089	-2.56	√	x	x
307.0878	$C_{12}H_{20}ClN_2O_3S^+$	 **Stl-306 (3.361 min)**	306.0802	306.0805	1.07	√	√	√
341.0488	$C_{12}H_{19}Cl_2N_2O_3S^+$	 **Stl-340 (3.917 min)**	340.0426	340.0415	-3.18	√	√	√

Table 3.8 shows the structure of Stl and its proposed transformation by-products. For Stl, four transformation by-products were identified. For Stl a fragment ion at *m/z* 255.1154 was formed through the loss of H_2O molecule from the $[M+H]^+$ ion (Figure 3.20a). Then, further fragmentation of the fragment ion at *m/z* 255.1154 through the loss of isopropyl group formed the fragment ion at *m/z* 213.0684. The fragment ion at *m/z*

133.0754 was formed through the loss of methanesulfonate group and two hydrogen atoms from *m/z* 213.0684.

a) Stl



b) Stl-254

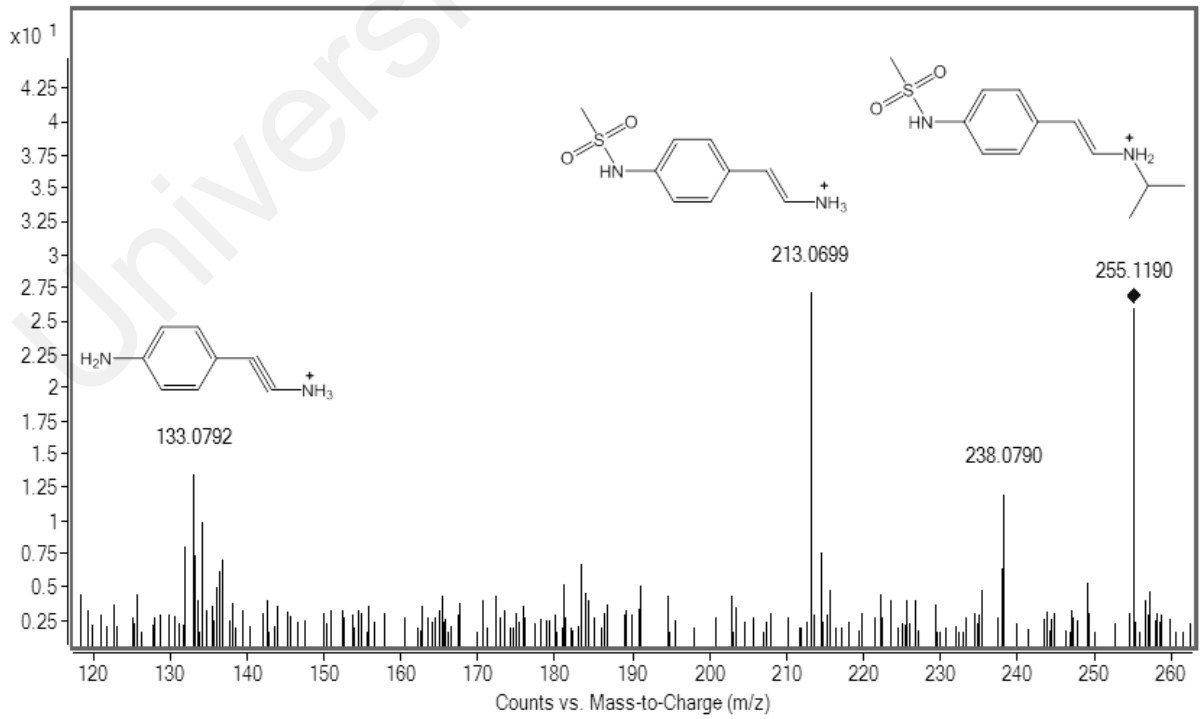
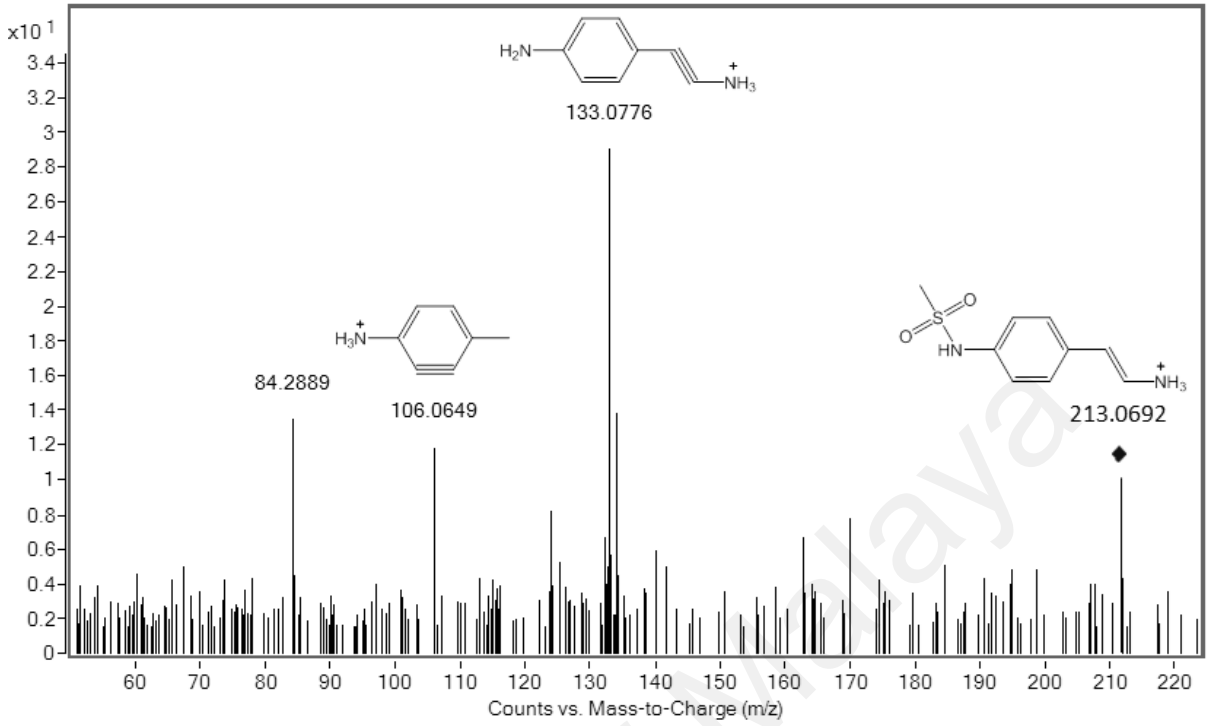


Figure 3.20: MS/MS spectrum of Stl and its by-products.

c) Stl-212



d) Stl-306

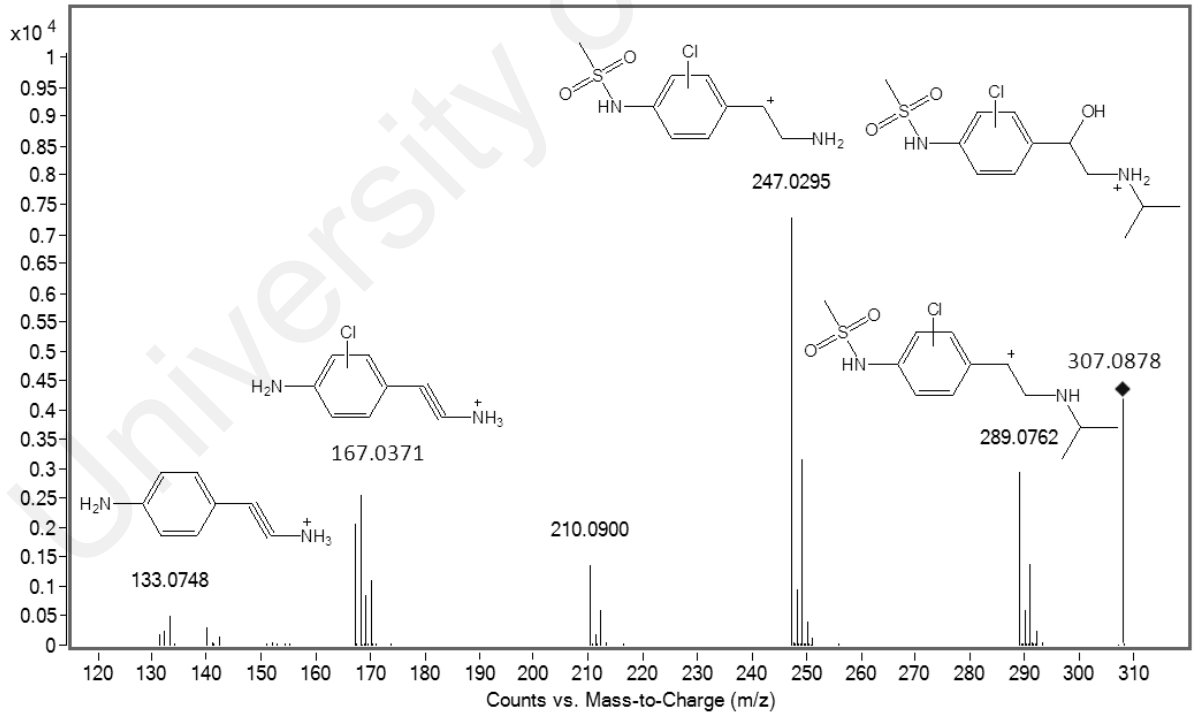


Figure 3.20 *(continued)*

e) Stl-340

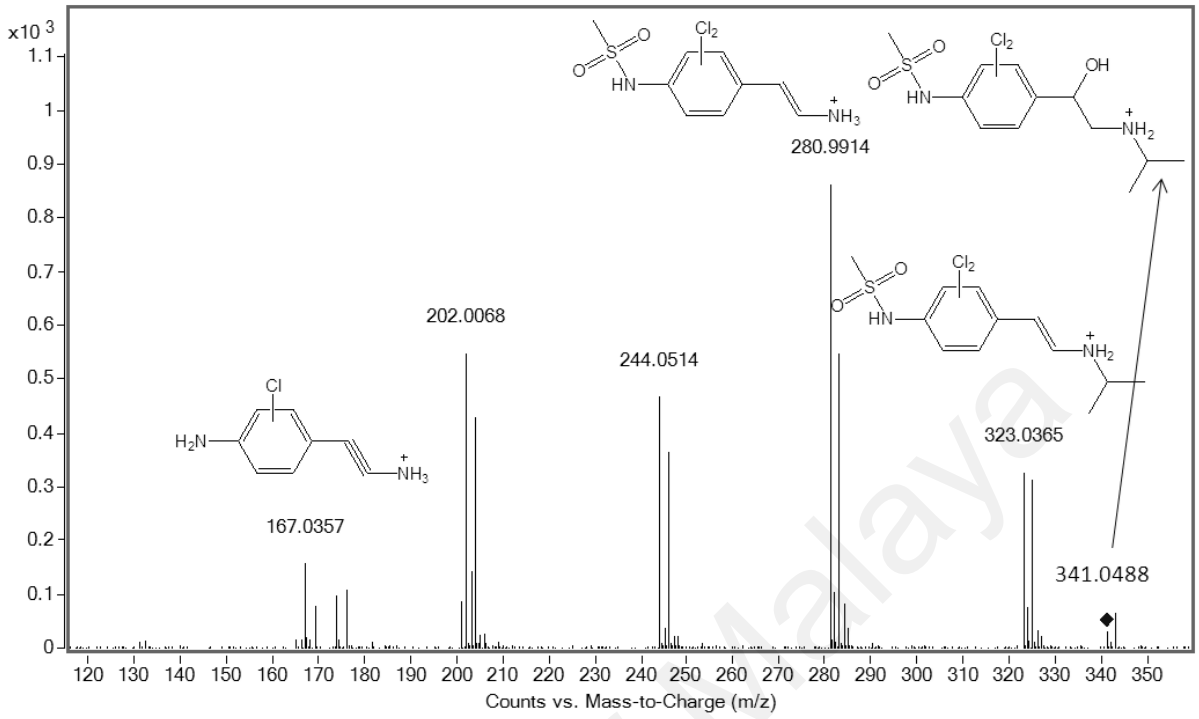


Figure 3.20 (*continued*)

During chlorination, two breakdown by-products of Stl with the $[M+H]^+$ ion lower than Stl were detected. These by-products were Stl-254 and Stl-212. Stl-254 (Figure 3.20b) was proposed to be the breakdown by-products of Stl that formed through the removal of one H_2O group. The most dominant fragment ion in the MS/MS spectrum of Stl-254 was *m/z* 213.0699 which was formed through the loss of isopropyl group from $[M+H]^+$ ion. The product ion at *m/z* 133.0792 was formed through the loss of methanesulfonate group and two hydrogen atoms from *m/z* 213.0699 (Figure 3.20b). The formation of Stl-254 is proposed to start with the protonation of the OH group of Stl to form intermediate (*II*) (Figure 3.21). Then, the removal of water molecule from intermediate *II* forms carbocation *III*. Rearrangement of *III* leads to the formation of C=C.

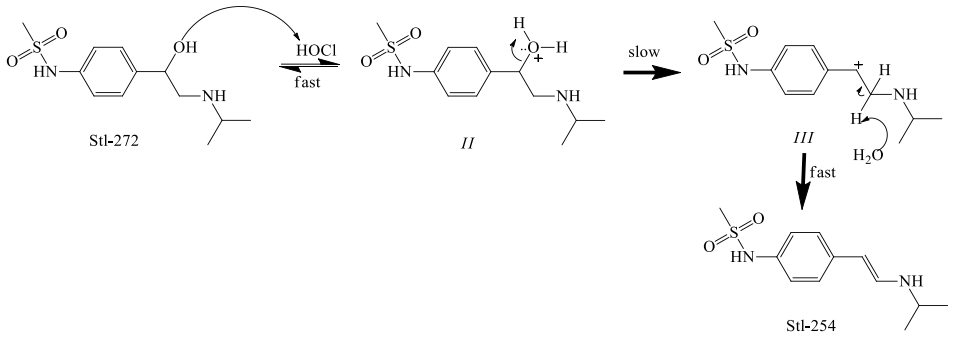


Figure 3.21: Proposed transformation pathway for the formation of Stl-254.

Stl-212 (Figure 3.20c) was proposed to be the breakdown by-products of Stl-254 (Figure 3.20b) which was formed through the loss of isopropyl group from Stl-254 (Figure 3.22). For Stl-212, the most dominant fragment ion in the MS/MS spectrum was *m/z* 133.0776 by removal of methanesulfinate group from *m/z* 213.0692 (Figure 3.20c). Further fragmentation of fragment ion at *m/z* 133.0776 through the loss of methenamine group formed the fragment ion at *m/z* 106.0649. The formation of Stl-212 is proposed to start from the reaction between the secondary amine group of Stl-254 with HOCl for the formation of chlorammonium intermediate (*I*) (Deborde and von Gunten, 2008). Intermediate *I* is a highly reactive species that could decompose through dealkylation to form Stl-212 (Dodd et al., 2005; El Najjar et al., 2013).

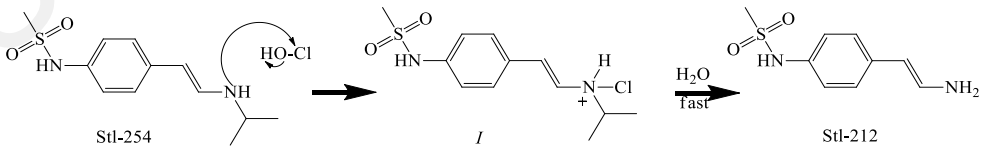


Figure 3.22: Proposed transformation pathway for the formation of Stl-212.

Stl-306 and Stl-340 are mono and dichlorinated Stl. Stl-306 and Stl-340 showed the $[M+H]^+$ ion of 33.9611 and 67.9221 amu higher than the Stl, indicating the addition of one and two chlorine atoms to the Stl, respectively. For Stl-306, the addition of chlorine atom was proposed at the aromatic ring. The significance product ions for this by-product were detected at *m/z* 289.0762, *m/z* 247.0295, *m/z* 167.0371 and *m/z* 133.0748 (Figure 3.20d). Fragment ion at *m/z* 289.0762 was formed through the loss of H_2O molecule from $[M+H]^+$ ion and the fragment ion at *m/z* 247.0295 was formed through the loss of isopropyl group of the secondary amine of *m/z* 289.0762. Then, further fragmentation of *m/z* 247.0295 formed ion at *m/z* 167.0371 through the loss of methanesulfinate group. The product ion at *m/z* 133.0748 was formed through the loss of one chlorine atom at aromatic ring from *m/z* 167.0371. The presence of ion at *m/z* 133.0748 was further confirmed the addition of chlorine atom at aromatic ring. For Stl-340, the addition of two chlorine atoms was also proposed to occur at the aromatic ring. For this by-product, the fragment ion at *m/z* 323.0365 was formed through the elimination of H_2O group from $[M+H]^+$ ion (Figure 3.20e). Further fragmentation of *m/z* 323.0365 ion through the loss of isopropyl group at secondary amine formed the fragment ion at *m/z* 280.9914. Fragment ion at *m/z* 167.0357 was formed through the elimination of methanesulfinate group and one chlorine atom from *m/z* 280.9914. The proposed mechanism for the formation of Stl-340 is presented at (Figure 3.23). The mechanism for the chlorination is proposed to start with the attack of aromatic ring of Stl to the positively polarized chlorine atom of HOCl, forming a monochlorinated carbocation (*IV*). Rearrangement of intermediate *IV* through the elimination of one hydrogen atom forms monochlorinated Stl-306. Then, the aromatic ring of monochlorinated Stl-306 further attacks on HOCl forming dichlorinated carbocation

(*V*). Rearrangement of intermediate *V* through the elimination of one hydrogen atom forms Stl-340.

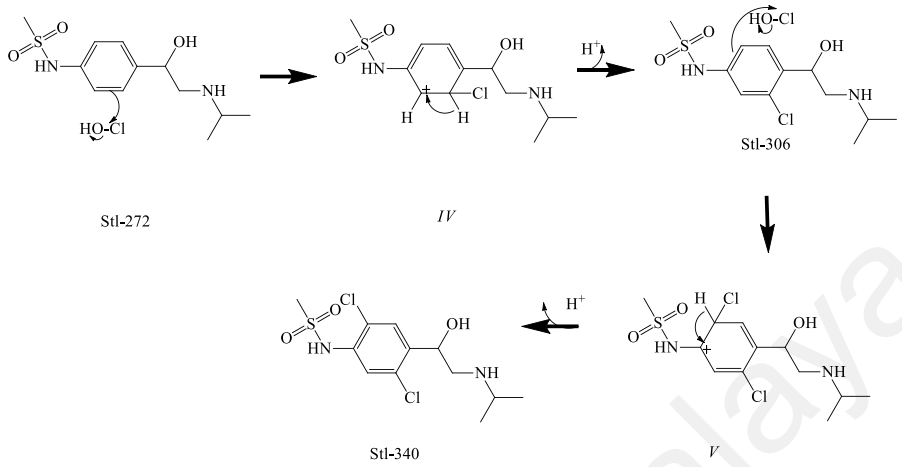
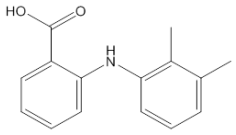
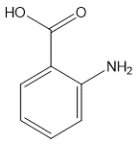
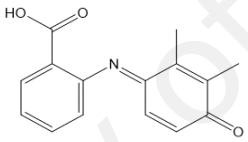
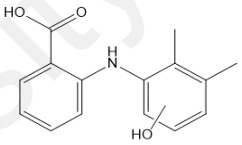
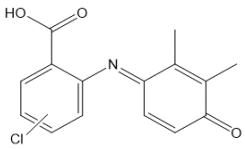


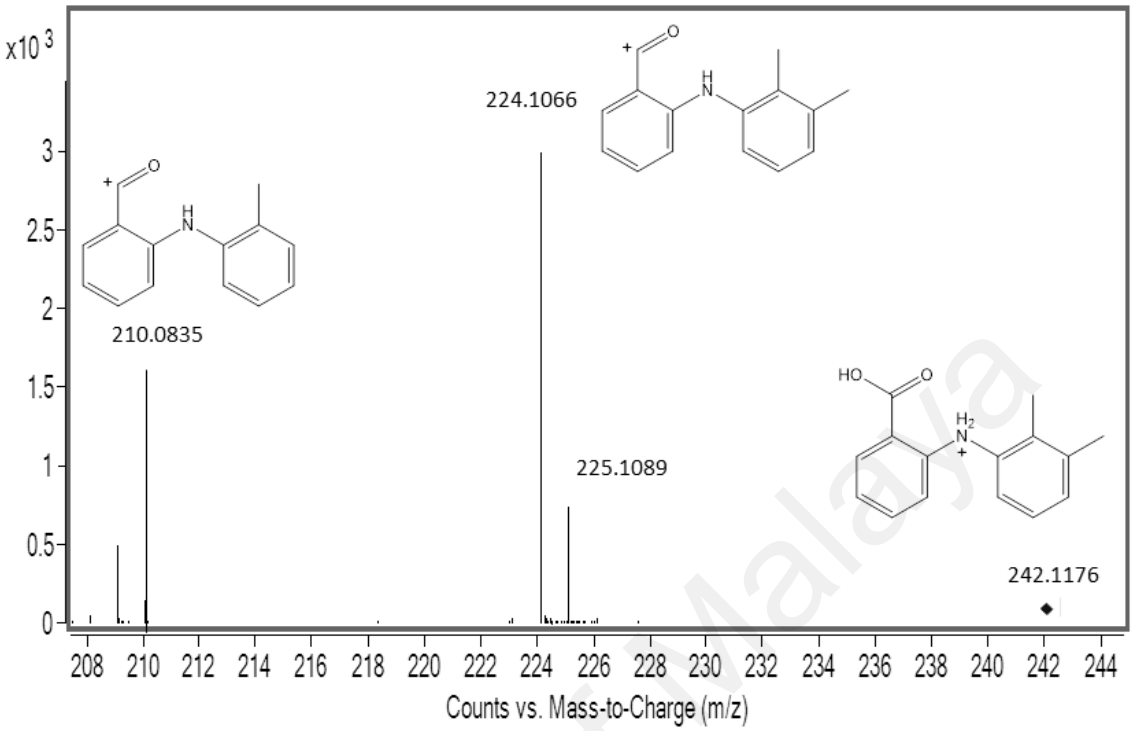
Figure 3.23: Proposed transformation pathway for the formation of Stl-340.

3.5.3 Transformation by-products of Mfe

Table 3.9: Proposed transformation by-products of Mfe during chlorination.

Quasi-molecular ion $[M+H]^+$	Chemical formulae $[M+H]^+$	Proposed structure (label-retention time)	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Reaction pH		
						6	7	8
242. 1176	$C_{15}H_{16}NO_2^+$	 **Mfe (10.102 min)**	241. 1100	241.1103	1.2	√	√	√
138.055	$C_7H_8NO_2^+$	 **Mfe-138 (7.378 min)**	137.0486	137.0477	-6.65	√	√	√
256.0968	$C_{15}H_{14}NO_3^+$	 **Mfe-256 (8.976 min)**	255.0894	255.0895	0.38	√	√	√
258.1125	$C_{15}H_{16}NO_3^+$	 **Mfe-258(9.327 min)**	257.1035	257.1052	6.77	√	x	x
290.0578	$C_{15}H_{13}ClNO_3^+$	 **Mfe-290(9.400 min)**	289.0508	289.0506	-0.87	√	√	√

a) Mfe



b) Mfe-138

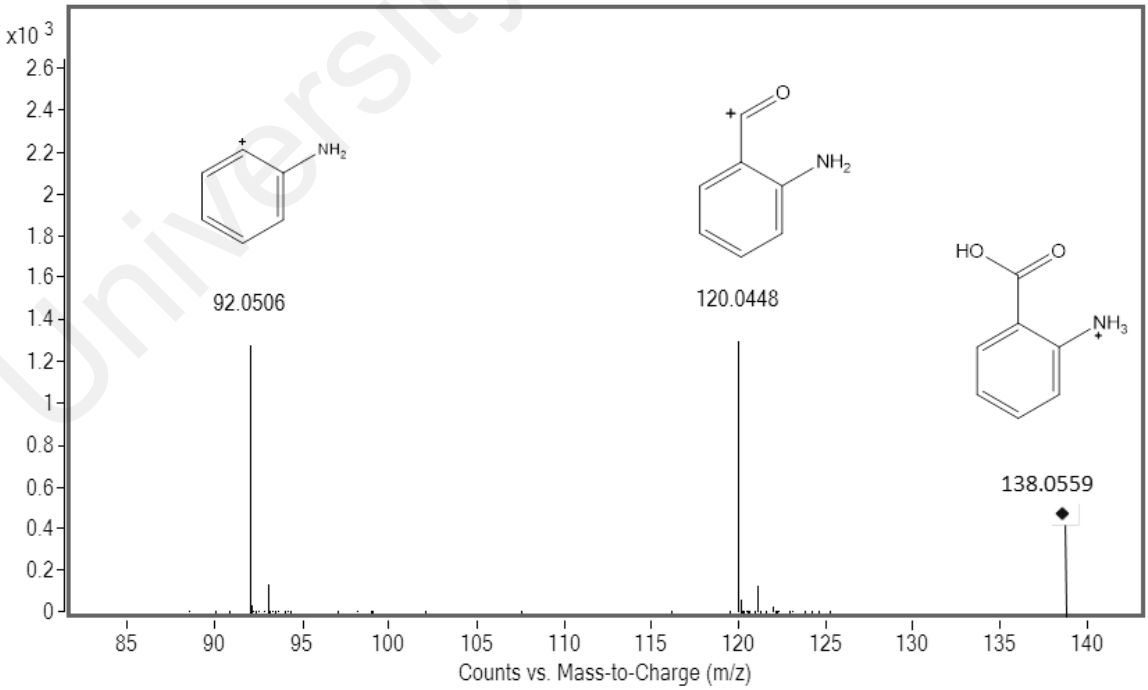
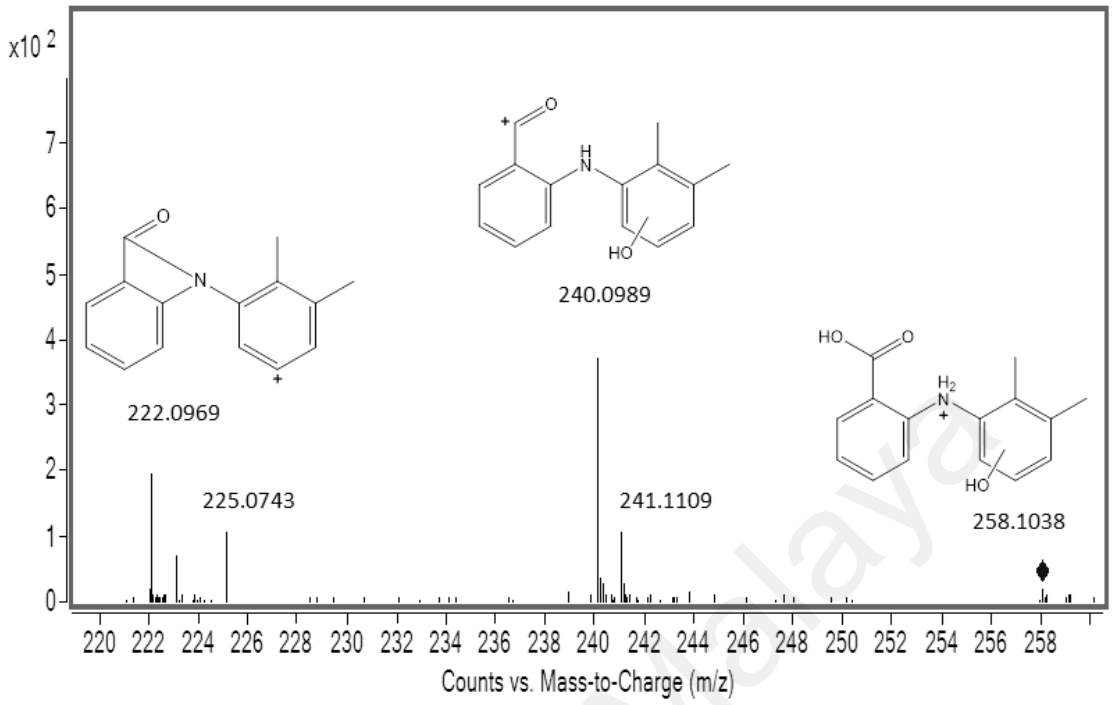


Figure 3.24: MS/MS spectrum of Mfe and its by-products.

c) Mfe-258



d) Mfe- 256

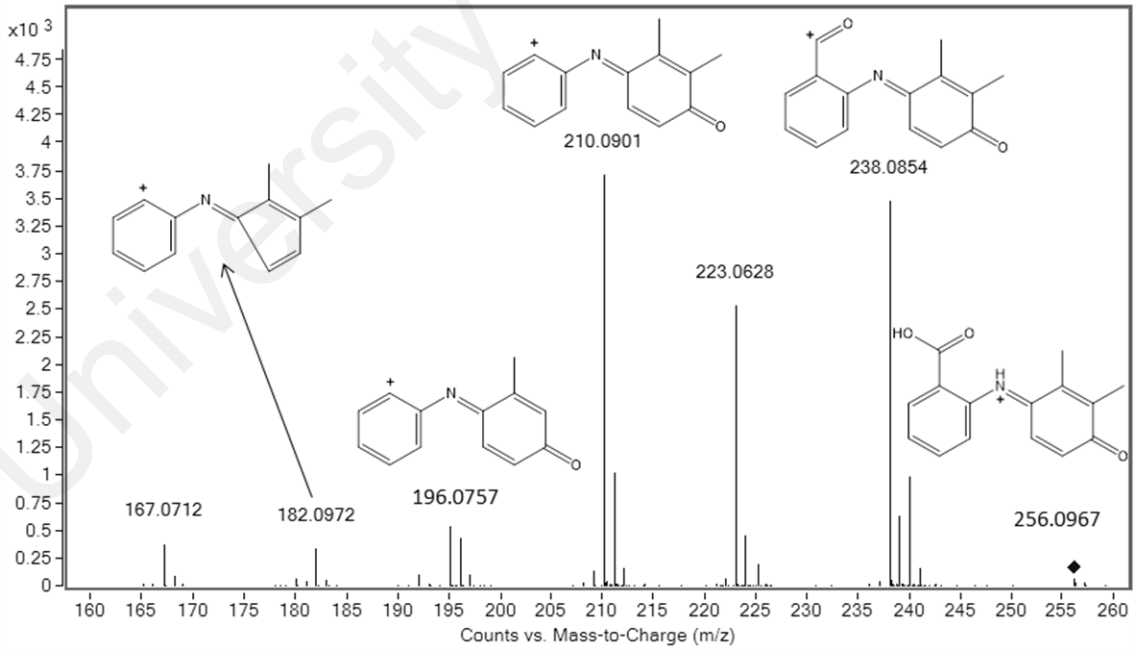


Figure 3.24 *(continued)*

e) Mfe-290

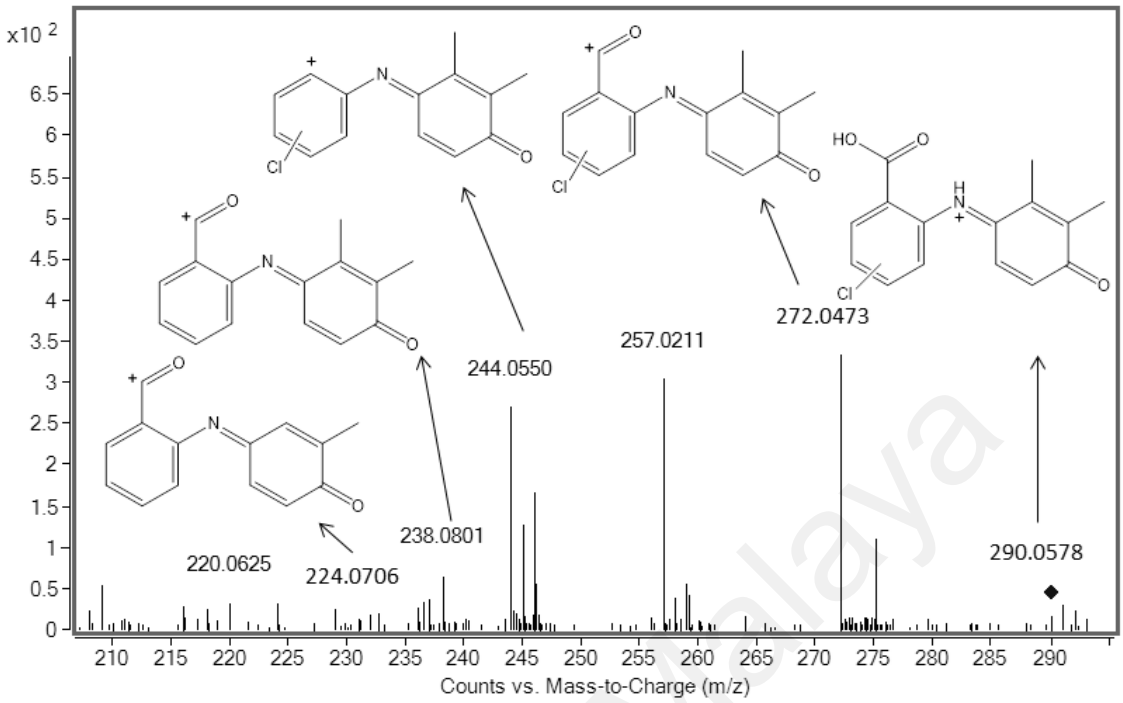


Figure 3.24 *(continued)*

Table 3.9 shows the structure of Mfe and the proposed transformation by-products. For Mfe, four transformation by-products were identified. The MS/MS spectrum of Mfe is presented in Figure 3.24a. The MS/MS spectrum of Mfe with $[M+H]^+$ ion of *m/z* 242.1176 showed two significant fragment ions at *m/z* 224.1066 and 210.0835 (Figure 3.24a). Fragment ions at *m/z* 224.1066 was produced from the loss of a H_2O molecule from $[M+H]^+$ ion. Further fragmentation of *m/z* 224.1066 ion through the loss of one methyl group formed fragment ion at *m/z* 210.0835. Mfe-138 (Figure 3.24b) is 2-aminobenzoic acid formed through the loss of o-xylene group from Mfe. For Mfe-138, the fragment ion of *m/z* 120.0448 was formed through the loss of H_2O group from $[M+H]^+$ ion. Further fragmentation of *m/z* 120.0448 through the loss of carbonyl group formed *m/z* 92.0506. The mechanism for the formation of Mfe-138 is proposed to start from the formation of highly reactive chloramine intermediate *I* (Dodd et al., 2005; El

Najjar et al., 2013) (Figure 3.25). Chloramine intermediate *I* is unstable and it tend to decomposed to form Mfe-138.

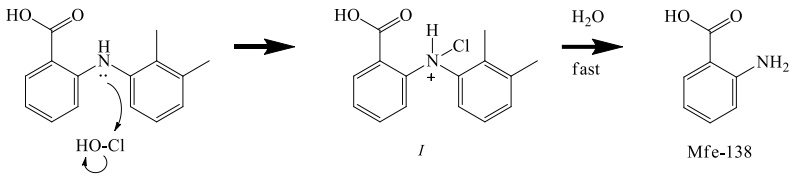


Figure 3.25: Proposed transformation pathway for the formation of Mfe-138.

Mfe-258 with $[M+H]^+$ ion at *m/z* 258.1038 (Figure 3.24c) was formed through the hydroxylation of Mfe. For this transformation by-product, the fragment ion at *m/z* 240.0989 was formed through the removal of one H_2O molecule from $[M+H]^+$ ion. Further fragmentation of ion at *m/z* 240.0989 through the removal of another H_2O molecule formed ion at *m/z* 222.0969. The formation of hydroxylated by-products during chlorination has been frequently reported (Dodd and Huang, 2007; Quintana et al., 2010; Radil et al., 2012; Negreira et al., 2015; Guo et al., 2016). For Mfe, methyl group is an electron donating group that activates the aromatic ring whereas carboxylic acid group is an electron withdrawing group that deactivates the aromatic ring of Mfe (Wade, 2010). Therefore, the addition of OH group was proposed at the more reactive o-xylene moiety of Mfe (Figure 3.26). This reaction could start from the reaction between the aromatic ring and the positively polarized chlorine atom of HOCl for the formation of monochlorinated Mfe (*II*). Monochlorinated Mfe was not detected in this study suggesting that this by-product is unstable under the selected condition. Monochlorinated Mfe (*II*) can further react with hydroxide ion through the nucleophilic addition for the formation of intermediate *III*. Intermolecular rearrangement of *III* through the elimination of chlorine atom forms Mfe-258. HOCl can oxidize the organic

molecule with aminophenol moiety to form a product, Mfe-256 with quinone-imine moiety as shown in (Figure 3.26) (O'Brien et al., 1991; Wen and Nelson 2011). Mfe-256 (Figure 3.24d) with $[M+H]^+$ ion at *m/z* 256.0967 shows three major fragments at *m/z* 238.0854, 210.0901 and 196.0757. The ion fragment at *m/z* 238.0854 was formed through the loss of H_2O molecule from $[M+H]^+$ ion. Then, further fragment ion at *m/z* 238.0854 through the loss of CO group formed *m/z* 210.0901 ion. The fragment ion at *m/z* 196.0757 was formed through the loss of methyl group from fragment ion at *m/z* 210.0901.

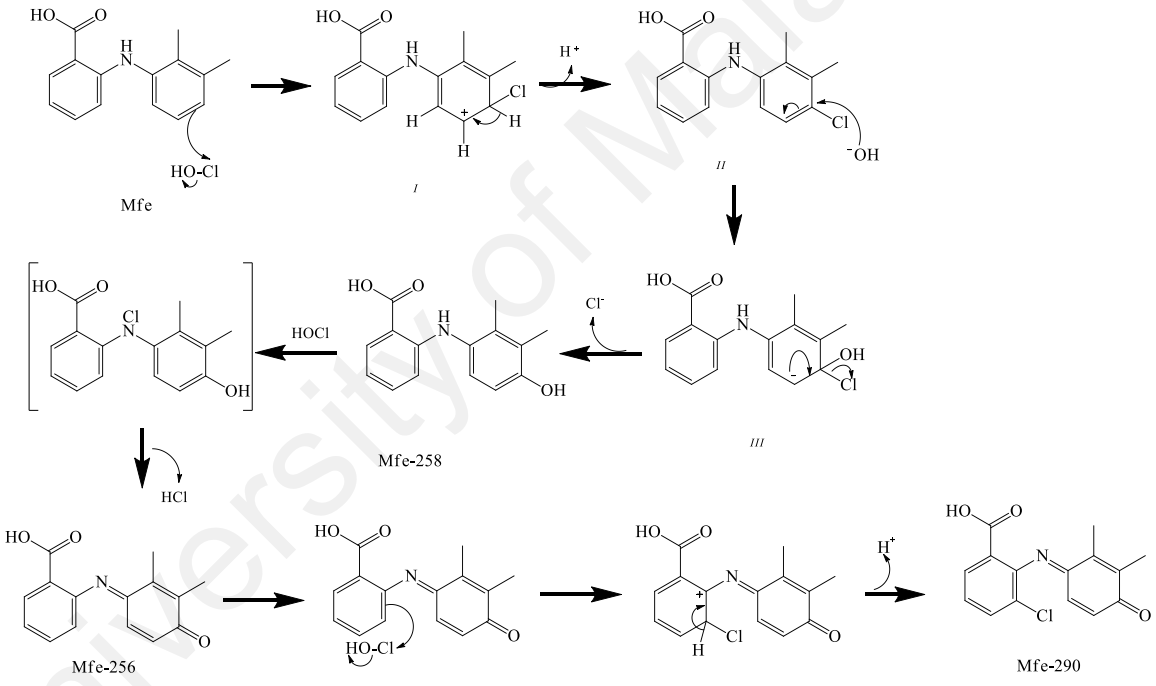


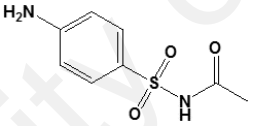
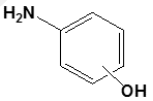
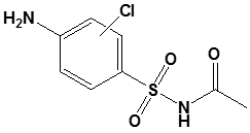
Figure 3.26: Proposed transformation pathway for the formation of Mfe-258, Mfe-256, Mfe-290.

Mfe-290 (Figure 3.24e) with the $[M+H]^+$ ion at *m/z* 290.0578 was the only detected chlorinated by-product. It was formed by the addition of one chlorine atom to Mfe-256 (Figure 3.24d). Mfe-290 shows two significant fragment ions at *m/z* 272.0473 and *m/z* 244.0550. Fragment ion at *m/z* 272.0473 was formed from $[M+H]^+$ ion through the removal of one H_2O molecule. Then, further fragmentation of fragment ion at *m/z* 272.0473 formed ion at *m/z* 244.0550 through the elimination CO group. The fragment ion at *m/z* 238.0801 was formed from fragment ion at *m/z* 272.0473 through the removal of hydrogen chloride group. Then, the fragment ion at *m/z* 224.0706 was formed through the removal of methyl group from fragment ion at *m/z* 238.0801. For Mfe-290, chlorine atom addition was proposed at the benzoic acid moiety of Mfe due to the electrophilic nature of quinone-imine moiety (O'Sullivan et al., 2013) (Figure 3.24e).

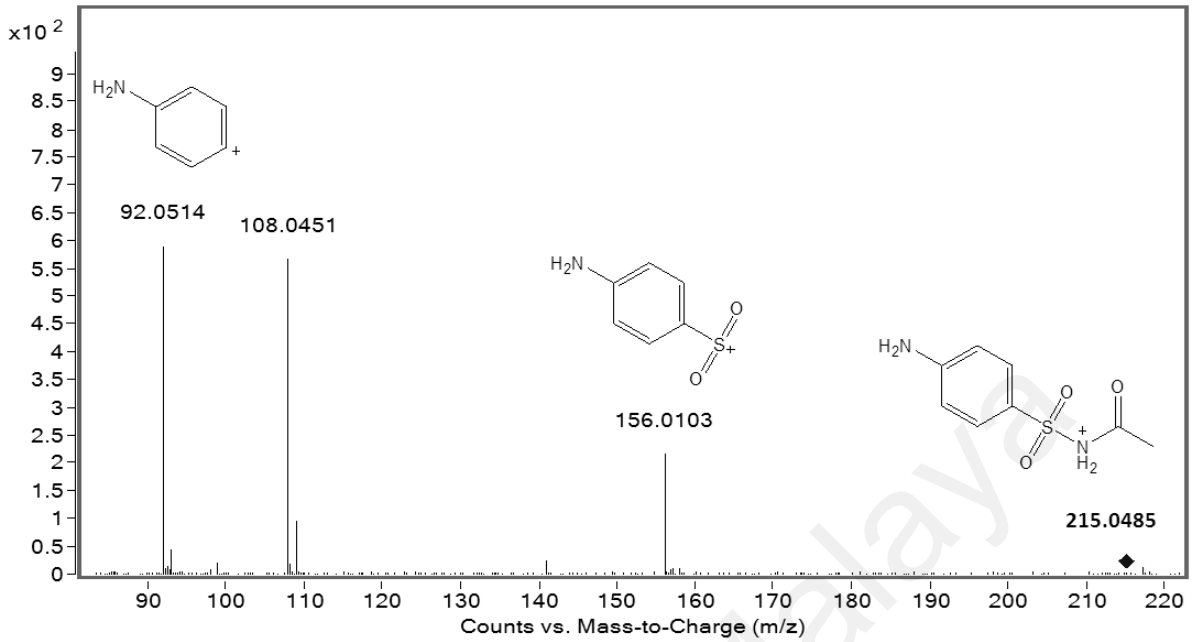
3.5.4 Transformation by-products of Sfa

For Sfa, two transformation by-products, Sfa-109 and Sfa-248 were identified (Table 3.10). The MS/MS spectrum of Sfa with $[M+H]^+$ ion of *m/z* 215.0485 (Figure 3.27a) showed two significant fragment ions at *m/z* 156.0103 and *m/z* 92.0514. The fragment ion at *m/z* 156.0103 was produced from the loss of acetamide molecule from the $[M+H]^+$ ion. Further fragmentation of the fragment ion at *m/z* 156.0103 through the loss of sulfur dioxide group formed the fragment ion at *m/z* 218.1169.

Table 3.10: Proposed transformation by-products of Sfa during chlorination.

Quasi-molecular ion $[M+H]^+$	Chemical formulae $[M+H]^+$	Proposed structure (label-retention time)	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Reaction pH		
						6	7	8
215.0485	$C_8H_{11}N_2O_3S^+$	 **Sfa (2.173 min)**	214.0418	214.0412	-2.65	√	√	√
110.0600	$C_6H_8NO^+$	 **Sfa-109 (1.402 min)**	109.0540	109.0528	-11.29	√	√	√
249.0095	$C_8H_{10}ClN_2O_3S^+$	 **Sfa-248 (6.655 min)**	248.0027	248.0022	-1.98	√	x	x

a) Sfa-214



b) Sfa-109

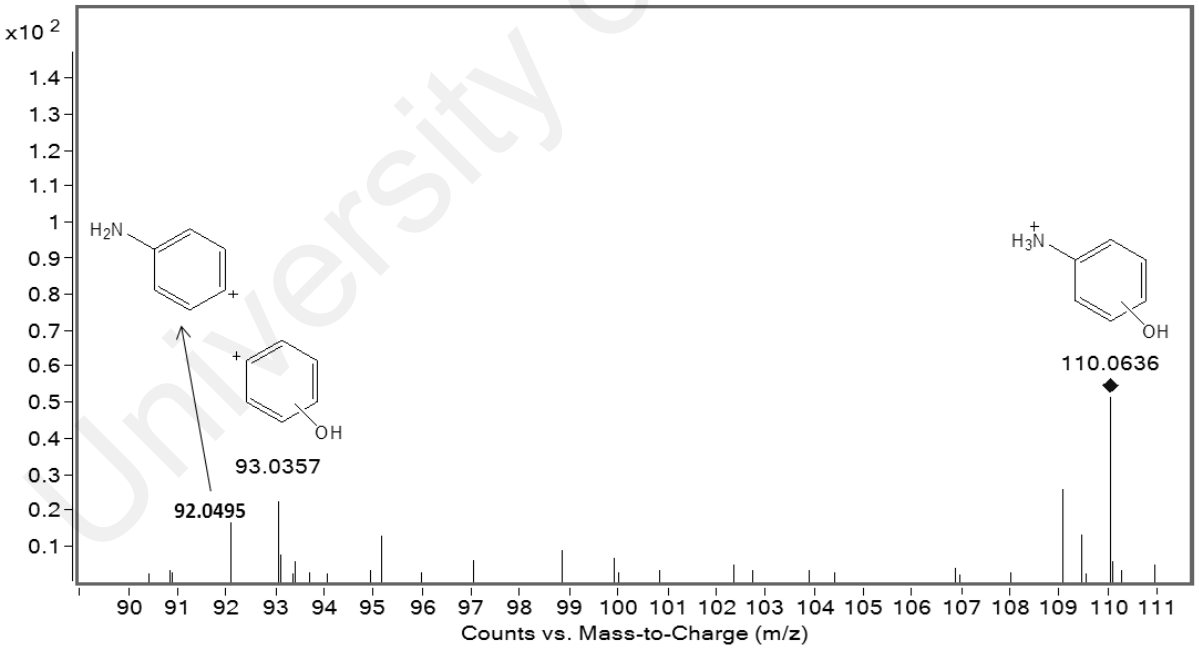


Figure 3.27: MS/MS spectrum of Sfa and its by-products.

c) Sfa-248

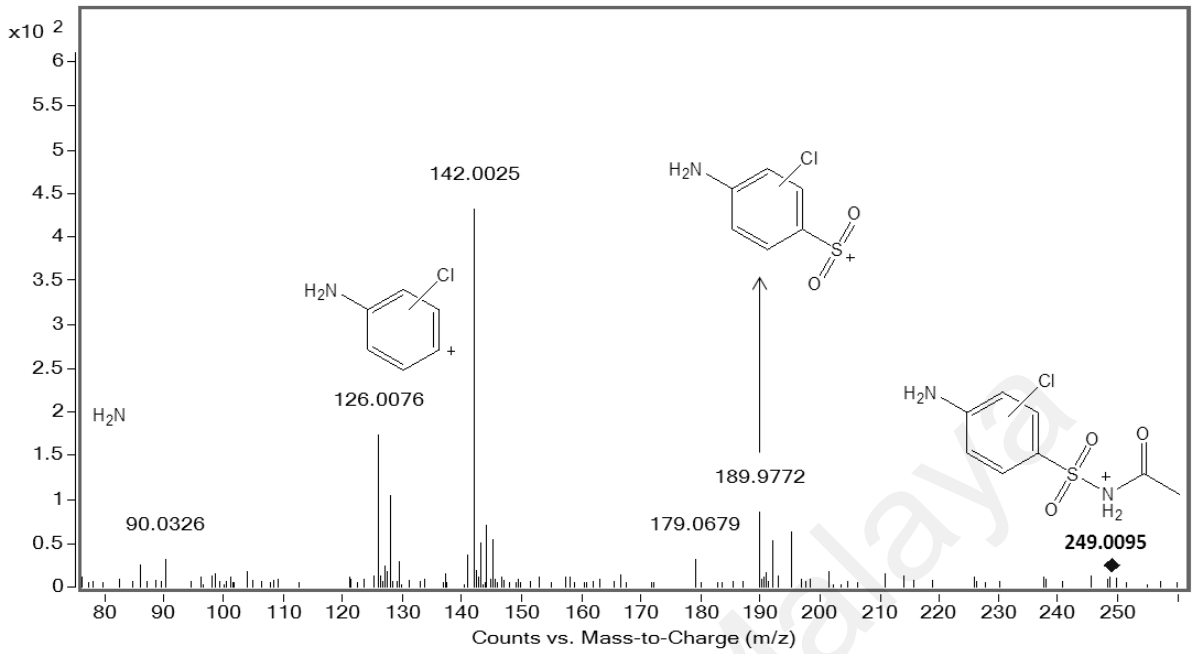


Figure 3.27 (*continued*)

During chlorination, two transformation by-products of Sfa were detected. These by-products were Sfa-109 (Figure 3.27b) with $[M+H]^+$ ion of *m/z* 215.0485 and Sfa-248 (Figure 3.27c) with $[M+H]^+$ ion of *m/z* 110.0636. For Sfa-109, the MS/MS spectrum showed two significant fragment ions at *m/z* 93.0357 and *m/z* 92.0495 which represent the carbocation of phenol and aniline, respectively. Sfa-248is a monochlorinated Sfa. These by-products showed two significance fragment ions at *m/z* 189.9772 and *m/z* 126.0076. The fragment ion at *m/z* 189.9772 was produced through the loss of acetamide molecule from the $[M+H]^+$ ion of Sfa-248. Further fragmentation of the fragment ion at *m/z* 189.9772 through the loss of sulfur dioxide group formed the fragment ion at *m/z* 126.0076 which represents the monochlorinated aniline. *m/z* 126.0076 ion confirmed the addition of chlorine atom at the aromatic ring. The proposed mechanism for the formation of Sfa-248 is presented at Figure 3.28. The

mechanism for the chlorination is proposed to start with the attack of aromatic ring of Sfa-214 to the positively polarized chlorine atom of HOCl, forming a monochlorinated carbocation (*I*). Rearrangement of intermediate *I* through the elimination of one hydrogen atom forms monochlorinated Sfa-248.

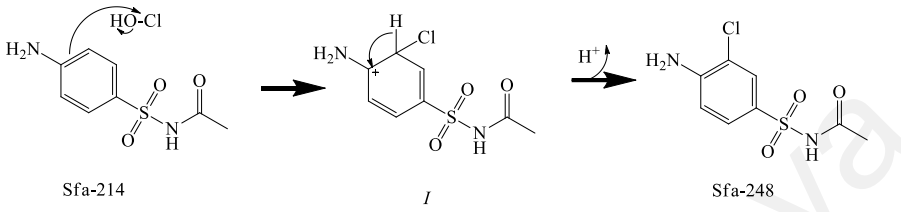


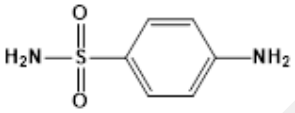
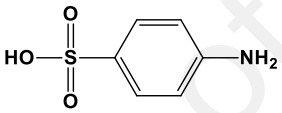
Figure 3.28: Proposed transformation pathway for the formation of Sfa-248.

3.5.5 Transformation by-products of Sfn

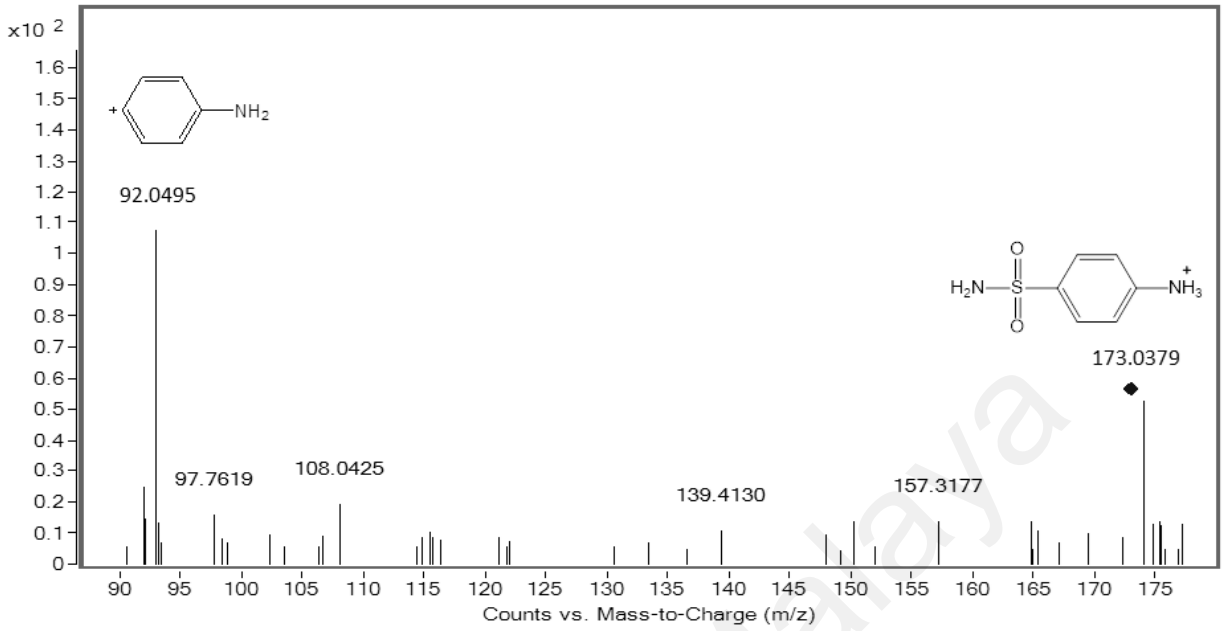
For Sfn, one transformation by-products identified (Table 3.11). The MS/MS spectrum of Sfn with $[M+H]^+$ ion of *m/z* 173.0379 (Figure 3.29a) showed one significant fragment ions at *m/z* 92.0495. The fragment ion at *m/z* 92.0495 was produced from the loss of sulfonic amide molecule from the $[M+H]^+$ ion. During chlorination, one breakdown by-products of Sfn was detected. These by-products was Sfn-173 (Figure 3.29b) which showed higher $[M+H]^+$ ion as compared to parent compound. For Sfn-173, the MS/MS spectrum with $[M+H]^+$ ion of *m/z* 174.0219 showed two significant fragment ions at *m/z* 92.0495 and *m/z* 77.0387. The fragment ion at *m/z* 92.0495 was produced by elimination of sulfonic acid group. Further fragmentation of fragment ion at *m/z* 92.0495 produced fragment ion at *m/z* 77.0387 by elimination of amine group. The mechanism for the chlorination is proposed to start with the attack of sulfoamines

of Sfn-173 to the positively polarized chlorine atom of HOCl, forming a chloramine (*I*). Rearrangement of intermediate *I* through the elimination of chloramine group forms intermediate *II*. Then, the nucleophilic addition of hydroxide ion (OH^-) to the intermediate *II* form monohydroxylated Sfn-173 (Figure 3.30).

Table 3.11: Proposed transformation by-products of Sfn during chlorination.

Quasi-molecular ion $[M+H]^+$	Chemical formulae $[M+H]^+$	Proposed structure (label-retention time)	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Reaction pH		
						6	7	8
173.0379	$C_6H_9N_2O_2S^+$	 **Sfn (1.912 min)**	172.0303	172.0306	2.27	√	√	√
174.0219	$C_6H_8NO_3S^+$	 **Sfn-173 (1.871min)**	173.0168	173.0147	-12.28	√	√	√

a) Sfn-172



b) Sfn-173

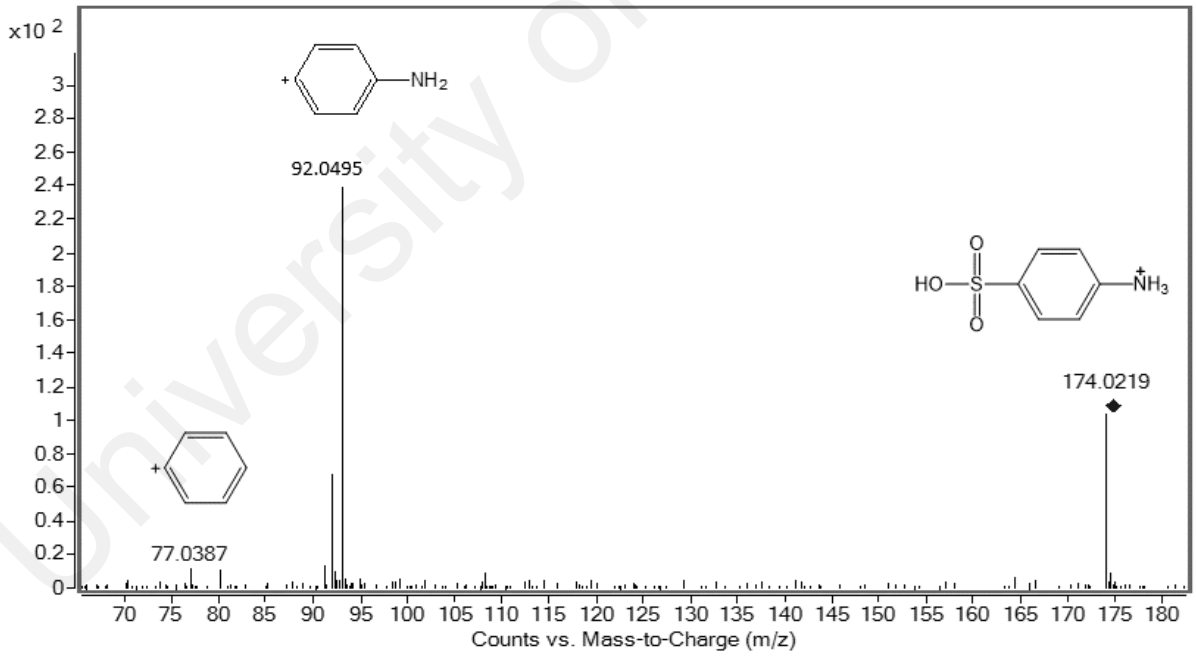


Figure 3.29: MS/MS spectrum of Sfn and its by-products.

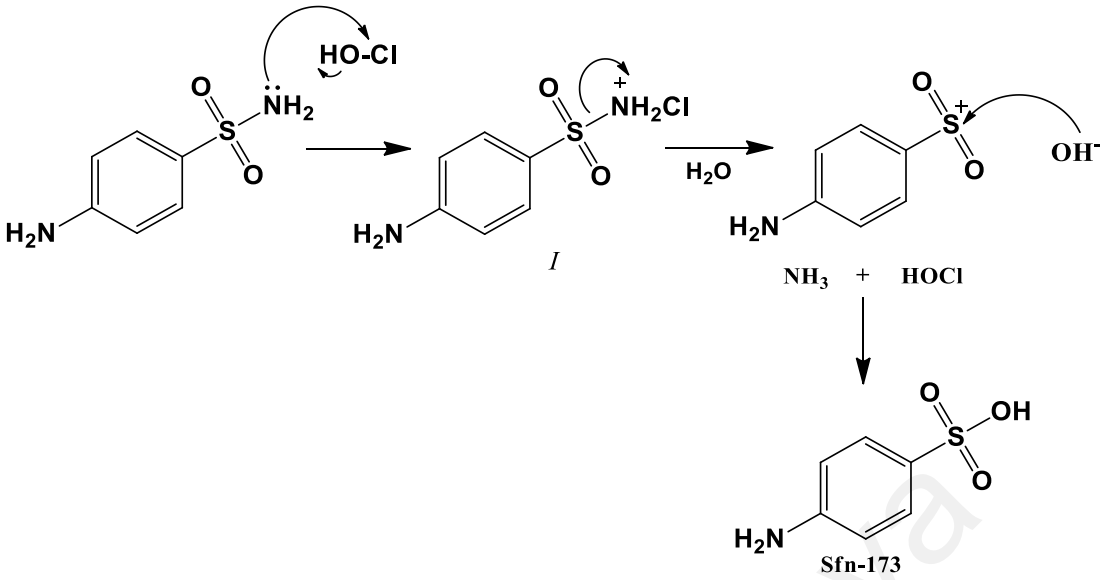


Figure 3.30: Proposed transformation pathway for the formation of Sfn-173.

CHAPTER 4

CHAPTER 4

4.1 Conclusion

In this study, the k_{app} for the reaction between Abt, Stl, Mfe, Sfa and Sfn with FAC were determined using pseudo-first order kinetic model. The k_{app} values were determined at pH ranging from 6 to 8. The selected pH range represents the pH condition for most of the environmental water samples and it was found to influence the concentration of reactive HOCl, In general, three different trends in the variation of k_{app} with pH were observed. For Abt, k_{app} was found to increase with pH. The increased of k_{app} with increasing pH for Abt (0.03 – 0.19 $M^{-1}s^{-1}$) revealed that the neutral form of Abt was more reactive toward chlorination as compared to its protonated form. On the other hand, the k_{app} for both Stl and Mfe was found to decrease with increasing pH. The decreased of k_{app} for Stl and Mfe with pH was due to the decreased of α_{HOCl}. For Sfa and Sfn, k_{app} was found to increase from pH 6 to 7 and started to decrease from pH 7 to 8. For Sfa, the increased of k_{app} mainly attributed by the increase of more reactive deprotonated Sfa. Then, the decreased of k_{app} was mainly attributed to the decreased of available HOCl. For Sfn, the increased of k_{app} mainly due to the increased of the molar fraction of more reactive neutral Sfn. However, as Sfa, the decreased of k_{app} of Sfn was due to decreased of α_{HOCl}.

Chlorination of selected pharmaceuticals were also conducted in real water samples which included lake water, ground water and secondary effluent. The purpose of this study was to assess the efficiency of chlorination in pharmaceuticals removal in more realistic condition. For ground and lake water,

4.1 ppm of FAC was sufficient to remove more than 90% of the selected pharmaceuticals. However, the effectiveness of chlorination in the removal of pharmaceuticals was relative poor in the secondary effluent due to high COD and TOC concentrations. This results also showed that the presence of higher amount of organic matter compete with pharmaceuticals for the reaction with FAC. Therefore, secondary effluent required higher amount of FAC to remove pharmaceuticals as compared to ground and lake water that had low COD value. Generally, the trend of the removal of some selected pharmaceuticals in cleaner water matrices can be predicted using the calculated $t_{1/2}$. For example, at 10 μM FAC dosage, the percentage removal of Stl with $t_{1/2}$ of 9.0 min was 12% and the percentage of removal for Sfn with the shortest $t_{1/2}$ of 0.3 min was 98%. Therefore, it can be concluded that the chlorination could remove some of the pharmaceuticals in water however its efficiency can be retarded by organic matter in water samples.

The chlorination of selected pharmaceuticals was found to produce various transformation by-products. Both side chain and aromatic ring of the selected pharmaceuticals were found to react with HOCl which led to the formation of aliphatic chain degraded, hydroxylated, and chlorinated by-products. For β-blockers group (Abt and Stl), 11 transformation by-products ware identified and these transformation by-products were formed through dealkylation, hydroxylation and chlorination, reactions. For NSAIDs group (Mfe), there were four transformation by-products were identified and instead of hydroxylated and chlorinated by-products, additional oxidation by-products was detected. These by-products were formed through oxidation of o-xylene moiety. For Sfa, two identified transformation by-products were aminophenol (Sfa-109)

and monochlorinated Sfa (Sfa-248). For Sfn, only 4-aminobenzenesulfonic acid (Sfn-173) was detected as transformation by-products. The chlorinated by-products that detected in this study were Abt-362, Stl-306, Stl-340, Mfe-290, and Sfa-248. Chlorinated by-products are often reported as more toxic chemicals as compared to their parent compound. Therefore, this research proved that the chlorinated by-products can be produced during chlorination.

4.2 Future Works

In the future research, the following topics are recommended:

- Assessment of the ecotoxicity of the transformation by-products that formed during chlorination of pharmaceuticals. Studies have proven some of the transformation by-products were more toxic than its parent compound. Therefore, the ecotoxicity of the transformation by-products should be assessed.
- Assessment on the fate of pharmaceuticals in the real disinfection process of water treatment plants.
- Monitoring of pharmaceuticals in finished drinking water.

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APPENDIX

Aqueous chlorination of acebutolol: kinetics, transformation by-products, and mechanism

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RESEARCH ARTICLE

Aqueous chlorination of acebutolol: kinetics, transformation by-products, and mechanism

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Abstract This study investigated the reaction kinetics and the transformation by-products of acebutolol during aqueous chlorination. Acebutolol is one of the commonly used β-blockers for the treatment of cardiovascular diseases. It has been frequently detected in the aquatic environment. In the kinetics study, the second-order rate constant for the reaction between acebutolol and chlorine (k_{app}) was determined at 25 ± 0.1 °C. The degradation of acebutolol by free available chlorine was highly pH dependence. When the pH increased from 6 to 8, it was found that the k_{app} for the reaction between acebutolol and free available chlorine was increased from 1.68 to 11.2 M^{-1} min^{-1}. By comparing with the reported k_{app} values, the reactivity of acebutolol toward free available chlorine was found to be higher than atenolol and metoprolol but lower than nadolol and propranolol. Characterization of the transformation by-products formed during the chlorination of acebutolol was carried out using liquid chromatography-quadrupole time-of-flight high-resolution mass spectrometry. Seven major transformation by-products were identified. These transformation by-products were mainly formed through dealkylation, hydroxylation, chlorination, and oxidation reactions.

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Keywords Beta-blockers · Chlorination · Water treatment · Degradation by-products · Pharmaceuticals · QTOF

Introduction

The presence of pharmaceuticals as emerging micropollutants has attracted much attention due to their potential impact on the environment (Sharma 2008; Soufan et al. 2013; Garcia et al. 2014). Due to the massive usage, various pharmaceuticals have been frequently detected in surface water, groundwater, drinking water, influent, and effluent of wastewater treatment plants (Szabo and Minamyer 2014; Osachoff et al. 2014; Sun et al. 2014) with the concentration ranging from nanogram per liter to microgram per liter (Lahti and Oikari 2011; Tian et al. 2014). The presence of pharmaceuticals in various environmental water samples has become a challenge for the conventional water treatment facilities to remove these emerging pollutants during water treatment processes (Tay et al. 2010; Gabet-Giraud et al. 2014). In addition, occurrence of pharmaceuticals in the effluent of wastewater treatment plants (Sun et al. 2014) and drinking water (Padhye et al. 2014; Szabo and Minamyer 2014) has showed that pharmaceuticals are not completely removed during wastewater and drinking water treatment.

Disinfection in water treatment is a process to remove, deactivate, or kill pathogenic microorganisms (Cai et al. 2013). Among various disinfection methods, chlorination is the most commonly used method due to its low operational cost. Owing to the ineffectiveness of coagulation-flocculation in the removal of pharmaceutical during water treatment (Kim et al. 2007; Kosma et al. 2010; Luo et al. 2014), pharmaceuticals are often exposed to the chlorination process. For chlorination, free available chlorine (FAC) is the most commonly used disinfectant (Cai et al. 2013; Zhang et al. 2015). FAC is

known to react with organic pollutants forming variety of harmful transformation by-products (Glassmeyer and Shoemaker 2005; Zarelli et al. 2012; Zhang et al. 2013). Therefore, it is essential to study the fate of organic pollutants in the chlorination process.

This study focused on the aqueous chlorination of acebutolol (Abt). Abt is a commonly used β-blockers for the treatment of cardiovascular diseases, and it has been frequently detected in various water samples such as influent and effluent wastewater treatment plant (Gabet-Giraud et al. 2010; Salem et al. 2012; Tay and Madehi 2014), surface water (Gabet-Giraud et al. 2014), and lake water (Daneshvar et al. 2010). Based on literature review, aqueous chlorinations of β-blockers such as metoprolol, propranolol, and atenolol have been widely reported (Bedner and MacCrehan 2006; Quintana et al. 2012). However, the chlorination of Abt has not been reported elsewhere. Therefore, the main objective for the present study was to investigate the reaction kinetics and the transformation by-products of Abt during chlorination. First, the k_{app} for reaction between FAC with Abt was determined at different pHs. Then, the transformation by-products of Abt were identified and the mechanisms of transformation of Abt during chlorination were elaborated.

Materials and methods

Materials and stock solutions

Acebutolol hydrochloride was purchased from Sigma (St. Louis, USA). Sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate were obtained from Merck (Darmstadt, Germany). Sodium hypochlorite solution with available chlorine 4.00–4.99 %, sodium thiosulfate, and formic acid were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile was obtained from RCI Lab Solution (Bangkok, Thailand). All chemicals were used without further purification.

Acebutolol stock solution was prepared by dissolving appropriate amount of acebutolol hydrochloride in ultrapure water (Elga, Buckinghamshire, UK). Stock solution of sodium hypochlorite was prepared daily by diluting it with ultrapure deionized water. The concentration of FAC stock solution was standardized using iodometric method (Adam and Gordon 1995).

Kinetics experiments

All kinetic experiments were performed in 50-mL jacketed beaker at 25±0.1 °C. For the determination of k_{app}, chlorination experiments were performed under pseudo-first-order kinetic conditions ([HOCl] ≫ [Abt]). The pH of the reaction mixtures was adjusted using phosphate buffer (50 mM) for 6 to 8 pH range. The chlorination was initiated by injecting an aliquot of sodium hypochlorite solution to the Abt solution. The mixture was stirred with magnetic bar throughout the experiment. One milliliter of the reaction mixture was withdrawn every 30 min, and the reaction was quenched using sodium thiosulfate solution. The reaction mixture was then subjected for HPLC analysis.

Reaction by-products

For transformation by-product identification, chlorination was performed on 53.6 μM of Abt solution at pH 6, 7, and 8. The molar ratios of HOCl to Abt were kept at 0.5:1, 1:1, and 2:1. The samples were shook for 24 h. Then, 2 mL of the samples was filtered through a 0.2-μm syringe filter before liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) analysis.

Instrumental analysis

For kinetic study, the concentration of acebutolol was monitored using HPLC system (Shimadzu, Japan) consisted of a LC-20AT pump, a SPD-M20A diode array detector, a SIL-20AHT auto sampler, and a CTO-10AS column oven. A reversed-phase Chromolith RP-18 monolithic column (100 mm×4.6 mm; Merck, Germany) was used for separation. A mixture of acetonitrile-0.1 % formic acid (10/90 *v*/*v*) was used as mobile phase, with a flow rate of 1 mL min^{-1}. Abt was monitored at the wavelength of 240 nm.

The analysis of transformation by-products was analyzed using 6500 Accurate Mass quadrupole time-of-flight mass spectrometer bearing with electrospray ionization (ESI) source coupled to 1200 Series Rapid Resolution LC system (LC-QTOF-MS, Agilent Technologist, Santa Clara, USA) as reported by Tay and Madehi (2014) with slight modification on the solvent gradient. In this work, 0.1 % of formic acid (A) and acetonitrile (B) was used as mobile phase at a flow rate of 0.25 mL min^{-1}. The initial solvent system was 10 % acetonitrile and hold for 1 min, followed by 5-min ramp to 100 % acetonitrile and hold for 3 min.

Results and discussion

Kinetics reaction between acebutolol with FAC

The rate of reaction for the chlorination of organic and inorganic compounds in water is a second-order reaction: first order to the FAC and first-order to organic or inorganic compound (Acero et al. 2010). Consequently, in the presence of

excess FAC, the degradation of Abt in water followed the pseudo-first-order reaction as indicated by Eq. (1):

$$-\frac{d[\mathrm{Abt}]t}{dt} = k_{\mathrm{app}}[\mathrm{Abt}]t[\mathrm{HOCl}]t = k_{\mathrm{obs}}\,[\mathrm{Abt}]t \quad (1)$$

$$\ln\left(\frac{[\mathrm{Abt}]}{[\mathrm{Abt}]_{\mathrm{o}}}\right) = -k_{\mathrm{obs}}t \quad (2)$$

where k_{obs} is the pseudo-first-order rate constant and $[Abt]_t$ and $[HOCl]_t$ are the concentrations of Abt and FAC at different reaction times, t. By integrating Eq. (1), Eq. (2) was obtained. Then, pseudo-first-order rate constant (k_{obs}) can be determined experimentally by monitoring the concentration of Abt versus time as indicated by Eq. (2). Figure 1 shows the plot of $\ln([Abt]_t/[Abt]_o)$ as a function of reaction time, where $[Abt]_o$ is the initial concentration of Abt. The k_{app} value was calculated by dividing the obtained k_{obs} with the initial concentration of chlorine. Under this pseudo-first-order condition, the concentration of initial chlorine was presumed to be unchanged throughout the experiments.

The effect of pH on k_{app} for the reaction between FAC and Abt was investigated in detail since pH can affect the speciation of both FAC and Abt. When NaOCl is dissolved in water, it forms HOCl and OCl^- (Deborde and Gunten 2008). The formation of OCl^- through the dissociation of HOCl which has the pK_a of 7.5 (at 25 °C) is a pH-dependent process (Ge et al. 2008). According to Chamberlain and Adams (2006), the mole fraction of HOCl and OCl^- representing by α_{HOCl} and $\alpha_{OCl\text{-}}$ can be estimated using Henderson-Hasselbalch equation (Eqs. (3) and (4)). HOCl possessing a redox potential of 1.49 V is a stronger oxidant as compared to OCl^- (Ge et al. 2008). Consequently, pH of water will influence the reactivity of chlorination toward the degradation of organic pollutants.

$$\alpha_{\mathrm{HOCl}} = \frac{1}{1+\left(10^{-7.5}\times 10^{\mathrm{pH}}\right)} \quad (3)$$

$$\alpha_{\mathrm{OCl-}} = 1-\alpha_{\mathrm{HOCl}} \quad (4)$$

The pK_a of Abt is 9.21 (Babic et al. 2007). Abt with secondary amine functional group can appear in neutral, protonated, and deprotonated forms in water. As HOCl, the ionization of Abt is also influenced by pH of the solution (Kibbey et al. 2007). In this study, the pH ranging from 6 to 8 was selected to study the influence of pH on the k_{app}. This selected pH range represents most of the pH condition for environmental water samples (Deborde and Gunten 2008), and it can significantly influence the speciation of FAC (Fig. 2). Figure 2 shows that k_{app} was found to increase with increasing pH from 6 to 8. This result indicated that the k_{app} was clearly pH dependent as shown in most of the chlorination reaction during water treatment (Gallard and Gunten 2002). HOCl is the predominant species during chlorination when pH is in the range of 3 to 7.5 (Zhang et al. 2013). However, when the pH increases from 7.5 to 8, the molar fraction of OCl^- increases from 0.03 to 0.76. In most cases, the reaction between organic compounds with OCl^- can be considered as insignificant due to its low reactivity (Deborde and Gunten 2008). Therefore, the obtained result indicated that the degradation of Abt was not influenced by the increase of OCl^- concentration. The selected pH conditions for chlorination were lower than the pK_a of Abt. Consequently, Abt can only appear as neutral or protonated form during chlorination. When the pH increased from 6 to 8, the molar fraction of neutral Abt was increased (Fig. 3). In this case, the ratio of $\frac{[\mathrm{Abt}]}{[\mathrm{Abt}^+]}$ was estimated using Henderson-Hasselbalch equation. Therefore, the increase of k_{app} with increasing pH showed that the neutral form of Abt is more reactive toward chlorination as compared to its protonated form. As reported by Abia et al. (1998), secondary amine species that appeared in neutral form reacted favorably with HOCl under basic condition to form chloramine as compared to acidic condition. Therefore, Abt is proposed to degrade via the formation of chloramine species. Chloramine is not stable, and it tends to decompose via base-catalyzed decomposition and loss its aliphatic hydrocarbon group (Abia et al. 1998). In this case, chloramine of Abt was not detected. However, the by-products of chloramine decomposition, Abt-294, were detected.

The $t_{½}$ of Abt in chlorination process at different pH was calculated based on the k_{app} by using Eq. (5). The result indicated that $t_{½}$ of Abt decreased significantly with increasing pH (Table 1). Table 2 shows the $t_{½}$ of different β-blockers during chlorination at pH 7.

$$t_{1/2} = \frac{1}{k_{\mathrm{app}}[Cl]_o} \quad (5)$$

Based on the k_{app} values reported by Pinkston and Sedlak (2004), the $t_{½}$ of β-blockers was compared. Abt was found to be more reactive toward FAC as compared with atenolol and metoprolol. However, Abt was less reactive toward FAC as compared with propranolol and nadolol. Therefore, it can be concluded that the reactivity of β-blockers toward FAC is as follows:

Atenolol = Metoprolol < Acebutolol < Nadolol < Propranolol

Transformation by-products of Abt

In this study, structure elucidation of the transformation by-products was performed using LC-QTOF-MS. This instrument produces MS and MS/MS with high mass accuracy. The proposed transformation by-products of Abt are shown in Table 3. Analysis was performed by comparing mass spectrometric data of the initial Abt solution as control sample with the data collected from the chlorinated Abt solution. The MS/

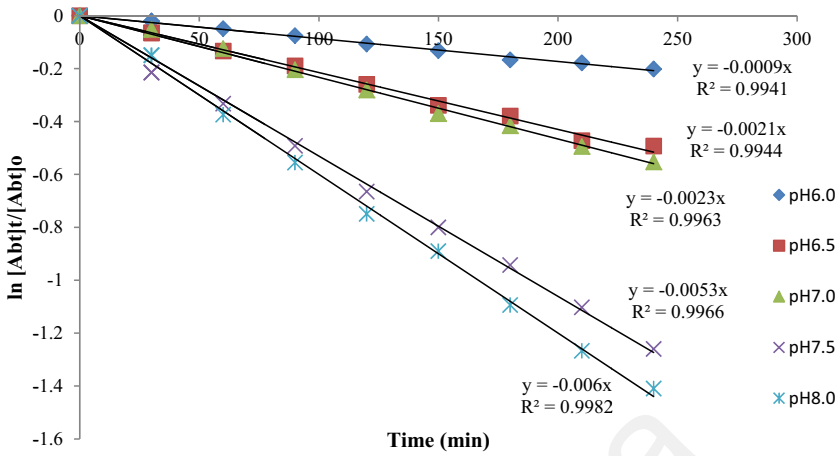


Fig. 1 Pseudo-first-order kinetics plot for the degradation of Abt via chlorination at pH 6 to 8 [temperature=25±0.1 °C, $[Abt]_o=2.68\times10^{-5}$ M, $[HOCl]_o=5.37\times10^{-4}$M]

MS spectrum of Abt with quasi-molecular ion $[M+H]^+$ of *m/z* 337.2119 is shown in the Fig. S1a (in Supplementary Material). For Abt, a fragment ion at *m/z* 319.2009 and 260.1279 was formed through the loss of H_2O molecule and propan-2-amine group from the $[M+H]^+$ ion, respectively (Tay and Madehi 2014). Further fragmentation of the fragment ion at *m/z* 260.1279 through the loss of acetaldehyde group formed the fragment ion at *m/z* 218.1169.

During chlorination, three breakdown by-products of Abt with the $[M+H]^+$ ion lower than Abt were detected. These by-products were Abt-294, Abt-276, and Abt-265. Abt-294 was formed through the loss of isopropyl group from Abt (Fig. S1b). This by-product has been reported during the ozonation of Abt (Tay and Madehi 2014). The mechanism for the formation of Abt-294 through dealkylation is presented in Fig. 4a. The formation of Abt-294 is proposed to start with the reaction between the secondary amine of Abt with HOCl for the formation of chloramine intermediate (*I*) (Deborde and Gunten 2008). Intermediate *I* is a highly reactive species that could decompose through dealkylation and forms Abt-294 (Dodd et al. 2005; El Najjar et al. 2013). Abt-276 and Abt-265 are proposed to be the breakdown by-products of Abt-294. Abt-276 with the $[M+H]^+$ ion at *m/z* 277.1555 (Fig. S1c) was produced via the removal of hydroxyl group from Abt-294. Abt-276 is also one of the ozonation by-products of Abt (Tay and Madehi 2014). Abt-265 was produced through the removal of methylamine group from Abt-294. For Abt-265, the most dominant fragment ion in the MS/MS spectrum was *m/z* 224.0891, and this fragment ion was formed through the loss of acetyl group from Abt-265 (Fig. S1d). The fragment ion at *m/z* 180.0646 was formed through the loss of ethyl alcohol group from the fragment ion at *m/z* 224.0891. Fragment ion at *m/z* 180.0646 is the key ion that showed the presence of ethyl alcohol group in Abt-265.

Two by-products with the $[M+H]^+$ ion at *m/z* 353 were detected. These by-products were labeled as Abt-352a and Abt-352b. The MS/MS spectrum for Abt-352a and Abt-352b showed almost similar fragmentation pattern (Fig. S1e, f). Abt-352a and Abt-352b were proposed to be the isomer of aromatic ring monohydroxylated Abt, as these compounds showed additional 16 Da that representing one OH group. These two by-products showed unmodified 1-(isopropylamino)propan-2-ol group at *m/z* 116. Therefore, the addition of OH group to the aromatic ring of Abt is

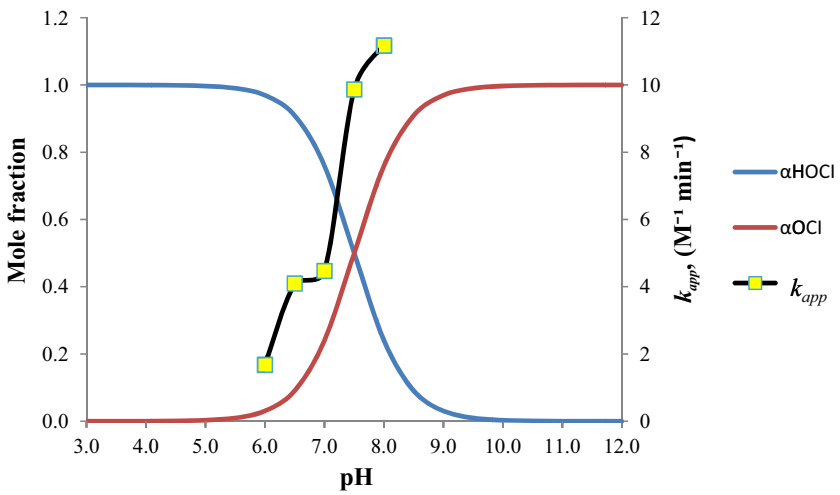


Fig. 2 The variation of k_{app} with pH

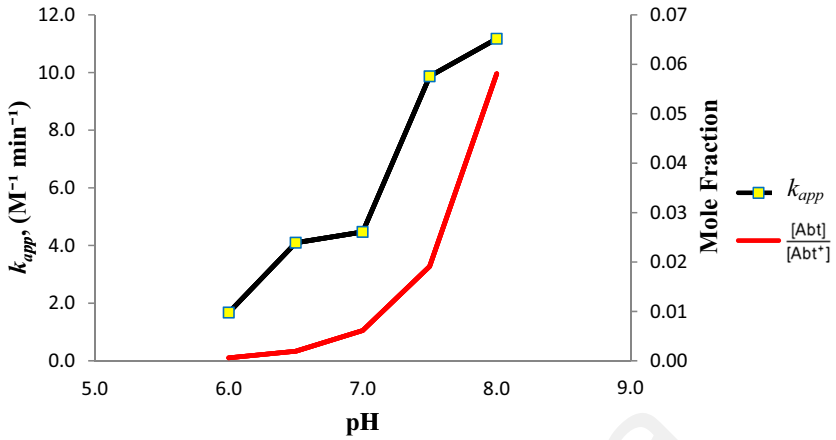


Fig. 3 The variation of k_{app} and $\frac{[Abt]}{[Abt^+]}$ with pH

proposed. The mechanism for hydroxylation is presented in Fig. 4b. The mechanism for the hydroxylation is proposed to start with the attack of aromatic ring of Abt by the positively polarized chlorine atom of HOCl, forming a monochlorinated carbocation (*II*). Rearrangement of intermediate *II* through the elimination of one hydrogen atom forms monochlorinated Abt. Then, the nucleophilic addition of hydroxide ion (OH^-) to the aromatic ring of monochlorinated Abt forms carbanion *III*. Carbanion *III* can rearrange to form aromatic ring monohydroxylated Abt.

Abt-362 with the $[M+H]^+$ ion at *m/z* 363.0873 was the only chlorinated by-product of Abt. This product was formed through the addition of two chlorine atoms to the aromatic ring of Abt-294. The addition of chlorine atoms is proposed to occur at the aromatic ring of Abt-294. For Abt-362, the fragment ion at *m/z* 310.0973 was formed through the elimination of hydrogen chloride and NH_3 group from $[M+H]^+$ ion (Fig. S1g). Fragment ion at *m/z* 310.0973 is the key ion that showing the presence of chlorine atoms at Abt-362. Further fragmentation of ion at *m/z* 310.0973 through the loss of one hydrogen chloride molecule and one water molecule formed *m/z* 260.1217. Since this by-product was originated from Abt-294, the fragment ions at *m/z* 180 and 222 were also detected. The proposed mechanism for the formation of Abt-362 is presented in Fig. 4c. The mechanism for the chlorination is proposed to start with the attack of aromatic ring of Abt-294 by the positively polarized chlorine atom of HOCl, forming a monochlorinated carbocation (*IV*). Rearrangement of intermediate *IV* through the elimination of one hydrogen atom forms monochlorinated Abt-294. Then, the aromatic ring of monochlorinated Abt-294 is further attacked by HOCl forming dichlorinated carbocation (*V*). Rearrangement of intermediate *V* through the elimination of one hydrogen atom forms Abt-362.

Abt-338 with the $[M+H]^+$ ion at *m/z* 339.1888 (Fig. S1h) is proposed to form through the replacement of methyl group with OH group at the acetyl group of Abt. For Abt-338, fragment ion at *m/z* 321.1788 was formed through the elimination of water molecule from $[M+H]^+$ ion. Other significant product ions for this by-product were detected at *m/z* 279.1296, 261.1208, 206.0793, and 116.1066. Fragment ion at *m/z* 279.1296 was formed through the loss of propane group from the fragment ion of *m/z* 321.1788. Further fragmentation of *m/z* 279.1296 ion through the loss of water molecule produced a product ion at *m/z* 261.1208. Then, the product ion at *m/z* 261.1208 was further fragmented to form *m/z* 206.0793 through the elimination of prop-2-en-1-amine. The presence of *m/z* 116.1066 indicated that Abt-338 contains unmodified 1-(isopropylamino) propan-2-ol group. The proposed mechanisms for the formation of Abt-338 from Abt-336 are shown in Fig. 4d. Chlorination reaction with carbonyl functional groups such as acetyl group for the formation of carboxylic acid group has been reported by Deborde and Gunten (2008). This reaction is proposed to start with the initial substitution

Table 1 The rate constants and $t_{½}$ for Abt during chlorination

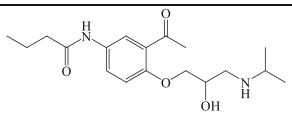
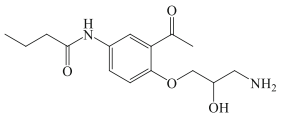
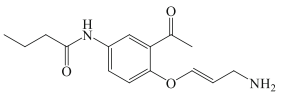
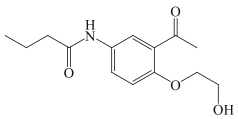
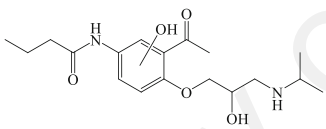
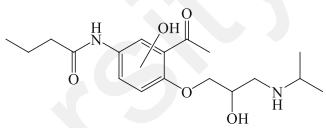
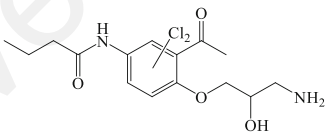
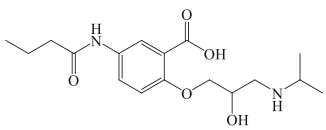
pH	k_{obs} (min^{-1})	k_{app} (M^{-1} min^{-1})	$^*t_{½}$(min)
6.0	0.0009±0.0001	1.68±0.05	1111
6.5	0.0022±0.0002	4.10±0.08	455
7.0	0.0024±0.0001	4.47±0.03	417
7.5	0.0053±0.0004	9.87±0.15	189
8.0	0.0060±0.0001	11.2±0.05	167

Table 2 $t_{½}$ of different β-blockers in the chlorination process

β-Blocker	pH	Temperature (°C)	k_{app} (M^{-1} min^{-1})	$t_{½}$ ($\times 10^2$ min)
Abt	7	25±0.1	4.47	4.17
Atenolol[a]	7	23±2	1.02	18.3
Metoprolol[a]	7	23±2	1.02	18.3
Nadolol[a]	7	23±2	10.8	1.72
Propranolol[a]	7	23±2	450	0.04

[a] Pinkston and Sedlak (2004)

Table 3 Proposed transformation by-products of Abt during chlorination

Quasi-molecular ion $[M+H]^+$	Elemental composition $[M+H]^+$	Proposed structure (label-retention time)	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Reaction pH		
						pH 6	pH 7	pH 8
337.2119	$C_{18}H_{29}N_2O_4^+$	 **Abt-336 (6.91 min)**	336.2052	336.2049	-0.75	√	√	√
295.1643	$C_{15}H_{23}N_2O_4^+$	 **Abt-294 (4.68 min)**	294.1581	294.1580	-0.43	√	√	√
277.1555	$C_{15}H_{21}N_2O_3^+$	 **Abt-276 (3.79 min)**	276.1470	276.1474	1.54	√	√	√
266.1358	$C_{14}H_{20}NO_4^+$	 **Abt-265 (2.49 min)**	265.1302	265.1314	4.65	x	x	√
353.2094	$C_{18}H_{29}N_2O_5^+$	 **Abt-352a (3.11 min)**	352.1994	352.1998	1.06	√	x	x
353.2047	$C_{18}H_{29}N_2O_5^+$	 **Abt-352b (3.12 min)**	352.2000	352.1998	-0.59	√	x	x
363.0873	$C_{15}H_{21}CI_2N_2O_4^+$	 **Abt-362 (8.76 min)**	362.0800	362.0800	-0.01	√	√	x
339.1888	$C_{17}H_{27}N_2O_5^+$	 **Abt-338 (6.35 min)**	338.1830	338.1842	3.34	√	x	x

reactions at the α-carbon. The reaction can take place by a series of hydrogen atom replacement by chlorine atom from HOCl to produce intermediate *VI*. Then, the attack of hydroxide ion at intermediate *VI* forms intermediate *VII* which could rearrange through the elimination of CCl_3 group to form Abt-338.

Conclusion

In this study, the k_{app} for the reaction between FAC and Abt was increased from 1.68 to 11.2 M^{-1} min^{-1} with increasing pH from 6 to 8. The increase of k_{app} was mainly due to the increase of the

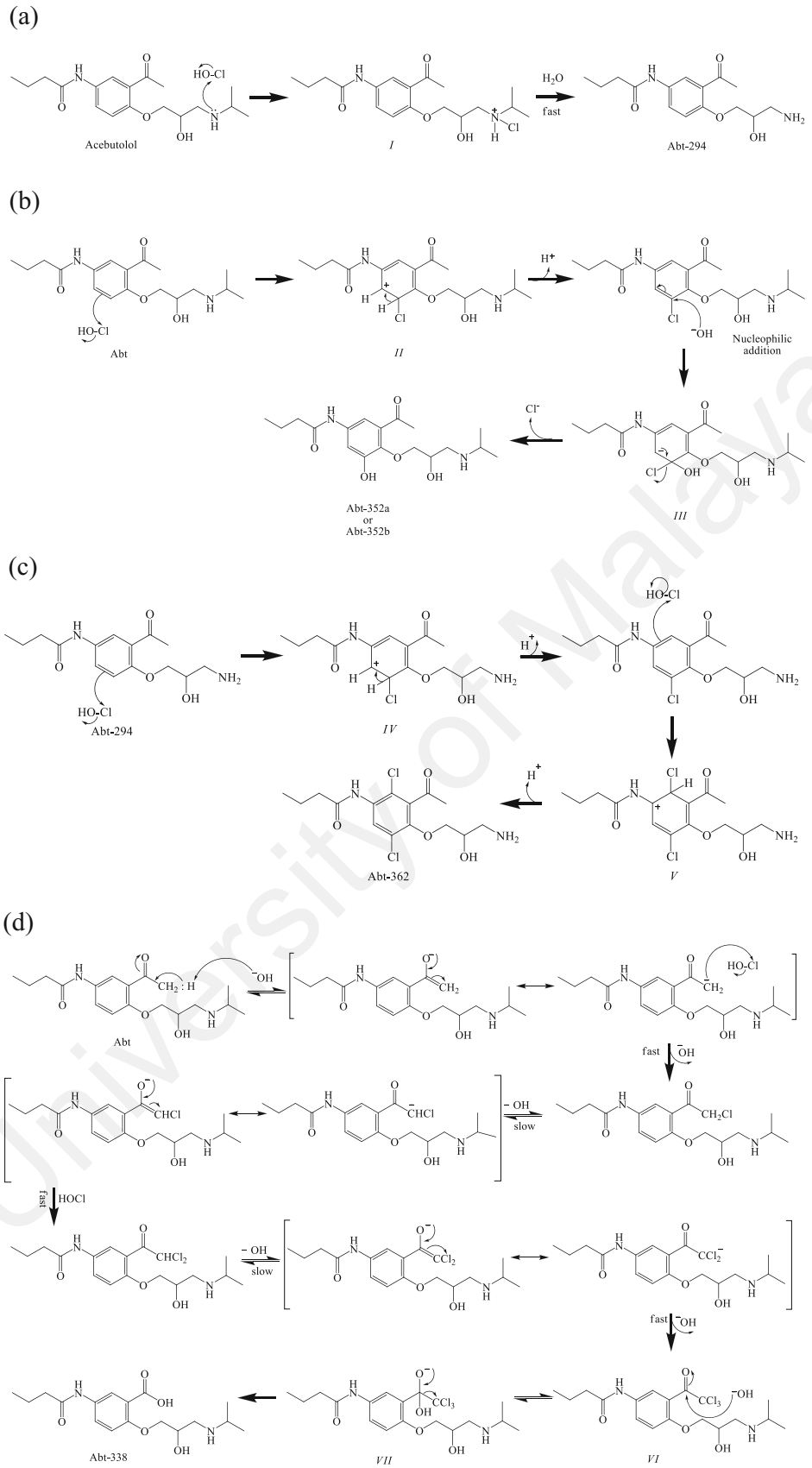


Fig. 4 Proposed transformation pathway for the formation of **a** Abt-294, **b** Abt-352a or Abt-352b, **c** Abt-362, and **d** Abt-338

amount of neutral Abt with increasing pH. Therefore, neutral form of Abt is more reactive toward FAC as compared to the protonated Abt. The result also indicated that the effect of HOCl speciation on k_{app} was negligible. The reactivity of Abt toward FAC was found to be higher than atenolol and metoprolol but lower than propranolol and nadolol. For the transformation by-product identification, seven transformation by-products were detected using LC-QTOF-MS. According to the detected transformation by-products, the transformation of Abt during chlorination involved dealkylation, hydroxylation, chlorination, and oxidation reactions.

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Aqueous chlorination of mefenamic acid: kinetics, transformation by-products and ecotoxicity assessment†

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Mefenamic acid (Mfe) is one of the most frequently detected nonsteroidal anti-inflammatory drugs in the environment. This study investigated the kinetics and the transformation by-products of Mfe during aqueous chlorination. The potential ecotoxicity of the transformation by-products was also evaluated. In the kinetic study, the second-order rate constant (k_{app}) for the reaction between Mfe and free available chlorine (FAC) was determined at 25 ± 0.1 °C. The result indicated that the degradation of Mfe by FAC is highly pH-dependent. When the pH was increased from 6 to 8, it was found that the k_{app} for the reaction between Mfe and FAC was decreased from 16.44 to 4.4 M^{-1} s^{-1}. Characterization of the transformation by-products formed during the chlorination of Mfe was carried out using liquid chromatography-quadrupole time-of-flight accurate mass spectrometry. Four major transformation by-products were identified. These transformation by-products were mainly formed through hydroxylation, chlorination and oxidation reactions. Ecotoxicity assessment revealed that transformation by-products, particularly monohydroxylated Mfe which is more toxic than Mfe, can be formed during aqueous chlorination.

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Environmental impact

This study investigated the fate of mefenamic acid (Mfe) in the chlorination process by focusing on the kinetics of the reaction, transformation by-products and ecotoxicity assessment. Mfe is one of the nonsteroidal anti-inflammatory drugs. It has been frequently detected in domestic wastewater. Water treatment plants rely on physico-chemical processes and biological treatment for the removal of pharmaceuticals. Unfortunately, these processes are not effective and incompletely treated pharmaceuticals will be exposed to disinfection processes such as chlorination. During chlorination, hypochlorous acid (HOCl) is the main oxidant and it was found to react with organic compounds and produce chlorinated by-products which may be toxic. Therefore, the determination of the fate of pharmaceuticals in the chlorination process is essential.

1. Introduction

The presence of pharmaceuticals in the environment has been a growing concern due to its negative impacts on the ecosystem and living organisms.[1,2] The effluent of wastewater treatment plants (WWTPs) has been identified as one of the major sources of pharmaceuticals in the environment.[3–5] Ingested pharmaceuticals are often not completely metabolized by humans. These unmetabolized pharmaceuticals can be excreted *via* urine or faeces and reach municipal and hospital WWTPs.[6] Most of the conventional WWTPs rely on physico-chemical processes and biological treatment for the removal of pharmaceuticals.[7,8] Unfortunately, these processes do not effectively remove pharmaceuticals[9] and incompletely treated pharmaceuticals can be exposed to disinfection processes such as chlorination and ozonation.

Among various disinfection methods, chlorination has been frequently applied in water treatment plants due to its cost effectiveness.[10–13] During chlorination, hypochlorous acid (HOCl) is the main oxidant. HOCl was found to react with numerous organic molecules producing different chlorinated by-products which may be more toxic than their parent compound.[14,15] Therefore, the determination of the fate of pharmaceuticals in the chlorination process is essential. The identification of transformation by-products of chlorination is also an important consideration.[16]

This study focused on the aqueous chlorination of mefenamic acid (Mfe). Mfe is one of the nonsteroidal anti-inflammatory drugs that have been used to relieve mild to moderate pain such as menstrual pain.[17,18] It works by stopping the body's production of a substance that causes pain, fever, and inflammation. This compound with the concentration up to μg L^{-1} has been frequently detected in domestic wastewater

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and the effluent of municipal WWTPs.[19–21] The degradation of Mfe by various chemical oxidation processes such as photodegradation, ozone/UV oxidation and ClO_2 oxidation has been reported.[22,23] Ozone/UV oxidation was reported as an effective method for the removal of Mfe from water.[22] In the photodegradation study, Mfe was found to react with hydroxyl radicals at a magnitude of 10^{10} M^{-1} s^{-1} through various reactions which include hydroxylation and oxidation.[23] Hey *et al.*[24] reported that Mfe removal *via* ClO_2 oxidation was highly dependent on the dosage of ClO_2. 90–95% of Mfe can be removed from water with a ClO_2 dosage of 1.25 mg L^{-1}. Based on the literature review, chlorination of Mfe using free available chlorine (FAC) has not been reported elsewhere. Therefore, the main objectives of this study were to study the kinetics of the reaction between FAC and Mfe, to identify the transformation by-products of Mfe during chlorination and to assess the ecotoxicity of the proposed transformation by-products of Mfe.

2. Materials and methods

2.1 Materials

Mfe (purity higher than 98%) was obtained from Sigma. Sodium hypochlorite with available chlorine 4.00–4.99% was obtained from Sigma-Aldrich. Sodium dihydrogen phosphate monohydrate and sodium hydrogen phosphate dihydrate with purity higher than 99.5% were obtained from Merck. HPLC grade methanol was purchased from RCI Labscan. Sulfuric acid and HPLC grade acetonitrile (96%) were purchased from Merck. Formic acid (98%) was obtained from Fluka. Ascorbic acid (99%) was obtained from HmbG Chemicals. All other chemicals were of reagent grade and used without further purification.

Mfe stock solution was prepared by dissolving an appropriate amount of Mfe in 50% of methanol in ultrapure water (Elga, Buckinghamshire, UK). A stock solution of sodium hypochlorite was prepared freshly by diluting it with ultrapure water. The concentration of the FAC stock solution was standardized using the iodometric method.[25]

2.2 Kinetic experiments

All kinetic experiments were performed at 25 ± 0.1 °C in a 50 mL jacketed beaker. For the determination of the second-order rate constant (k_{app}), chlorination experiments were performed under pseudo first-order kinetic conditions ([HOCl] : [Mfe] = 20 : 1). The pH of the reaction mixtures was adjusted using phosphate buffer (50 mM). The final methanol concentration in each 50 mL jacketed beaker was 0.25% (v/v). The chlorination was initiated by injecting an aliquot of sodium hypochlorite solution to the Mfe solution. The mixture was stirred with a magnetic bar throughout the experiment. 1 mL of the reaction mixture was withdrawn every 15 s and the reaction was quenched using ascorbic acid solution. The reaction mixture was then subjected to HPLC analysis. The determination of k_{app} was performed in three replicates.

2.3 By-product analysis

For transformation by-product identification, chlorination was performed on 82.9 μM of Mfe solution at pH 6, 7 and 8. The molar ratios of HOCl to Mfe were kept at 0.5 : 1, 1 : 1 and 2 : 1. The samples were shaken for 24 h. Then, 2 mL of the samples were filtered through a 0.2 μm syringe filter before LC-QTOFMS analysis. The samples were analysed without quenching. Controls were prepared using a similar method without the addition of HOCl. The controls were also shaken for 24 h.

2.4 Instrumental analysis

For the kinetic study, the concentration of Mfe was monitored using a HPLC system (Shimadzu, Japan) consisting of a LC-20AT pump, a SPD-M20A diode array detector, a SIL-20AHT autosampler, and a CTO-10AS column oven. A reversed-phase Chromolith RP-18 monolithic column (100 mm × 4.6 mm; Merck, Germany) was used for separation. Analyte peaks were resolved using isocratic elution with 10% acetonitrile and 90% of 0.1% formic acid in water. The flow rate was 1 mL min^{-1}. Mfe was monitored at a wavelength of 209 nm.

The analysis of transformation by-products was performed using a 6500 accurate mass quadrupole time-of-flight mass spectrometer bearing an electrospray ionization (ESI) source coupled to a 1200 series rapid resolution LC system (LC-QTOFMS, Agilent Technologies, Santa Clara, USA) as reported in a previous study.[26] Briefly, 0.1% formic acid in water (A) and acetonitrile (B) were used as the mobile phase at a flow rate of 0.25 mL min^{-1}. The gradient started at 10% acetonitrile, followed by 10 min ramp to 100% acetonitrile and hold for 3 min. A QTOF-MS system was operated in a high resolution mode at 4 GHz. Ions were generated using an electrospray ion source using Agilent Jet Stream Technology. The Agilent Jet Stream Technology uses superheated nitrogen sheath gas at a temperature of 300 °C and a flow rate of 11 L min^{-1}. Analyses were performed in the ESI positive ion mode using the following settings: nebulizer at a pressure of 35 psig, V_{cap} voltage of 3500 V, fragmentor voltage of 125 V, skimmer voltage of 65 V, nozzle voltage of 1000 V, and the collision energy was fixed at 20 V. A sprayer with a reference solution was used as continuous calibration in positive ion using the following reference masses: m/z 121.0509 and 922.0098. The recorded full-scan and MS/MS data were processed using Agilent MassHunter Workstation Software.

2.5 Ecotoxicity assessment

The ecotoxicity assessment of Mfe and its transformation by-products was conducted as described by Tay and Madehi.[27] Briefly, ECOSAR software (Version 1.11) was used to estimate the hazard indices of Mfe and its transformation by-products on green algae, daphnia and fish. The acute toxicity values of Mfe and its transformation by-products were presented as LC_{50} (concentration of the tested compound that is lethal to half of fish and daphnia population after 96 and 48 h of exposure, respectively) and EC_{50} (concentration of the tested

compound that inhibits the growth of 50% of green algae after 96 h of exposure). The chronic toxicity values (ChV) of Mfe and its transformation by-products on green algae, daphnia and fish were also calculated. ChV represents the repeated doses of substances causing the development of adverse effects.

3. Results and discussion

3.1 Kinetic reaction between Mfe and FAC

The reaction between the organic compound and FAC in aqueous solution follows the second order kinetic model, that is, first order to the selected organic pollution and first order to FAC as shown by eqn (1):[11]

$$-\frac{d[C]}{dt} = k_{app}[C]_t[FAC]_t \quad (1)$$

where k_{app} is the apparent second order rate constant and [C] is the concentration of the selected organic pollutant and [FAC] is the total concentration of HOCl and ClO^-. In order to determine the second order rate constant for the reaction between Mfe and FAC, the experiments were conducted with FAC in excess ([FAC] $\gg$ [Mfe]) in order to achieve the pseudo-first order condition. Therefore, eqn (1) can be simplified as eqn (2):

$$-\frac{d[Mfe]}{dt} = k_{obs}[Mfe]_t \quad (2)$$

where k_{obs} is the pseudo-first-order rate constant representing $k_{app} \times [FAC]_t$ and $[Mfe]_t$ is the concentration of Mfe. Integration of eqn (2) yields eqn (3).

$$\ln\frac{[Mfe]_t}{[Mfe]_0} = -k_{obs}t \quad (3)$$

where $[Mfe]_0$ is the initial concentration of Mfe and t is the reaction time. Fig. 1 shows a linear plot for $\ln\frac{[Mfe]_t}{[Mfe]_0}$ *versus* t, obtained from experimental results at different pH values. Under the selected conditions, the concentration of FAC was found to remain unchanged throughout the kinetic study. In this study, the rate constant was determined in the pH range of 6 to 8. This selected pH range represents most of the pH conditions for environmental water samples.[11]

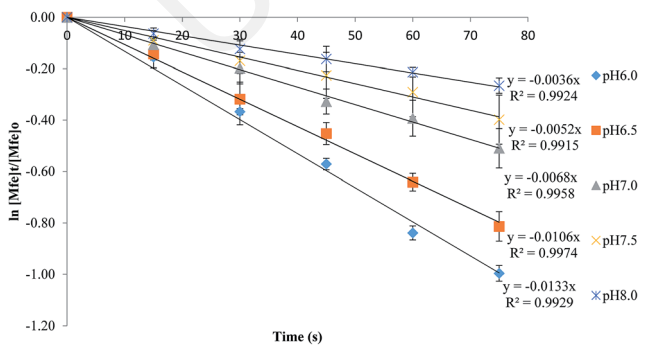


Fig. 1 Determination of the pseudo-first-order kinetic plot for the chlorination of Mfe at pH 6 to 8. [Temperature = 25 ± 0.1 °C, $[Mfe]_o$ = 4.14 × 10^{-5} M $[HOCl]_o$ = 8.27 × 10^{-4} M].

Table 1 shows the k_{app} obtained from pH 6 to 8. The values of k_{app} were obtained by dividing k_{obs} with $[FAC]_0$. The result indicated that k_{app} was highly pH-dependent and it decreased with increasing pH. In order to investigate the reason for the variation of k_{app} with pH, the k_{app} was plotted with the molar fraction of HOCl, OCl^-, neutral and ionized forms of Mfe. In aqueous solution, HOCl with a pK_a of 7.54 dissociates to form OCl^-.[16,28,29] The degree of dissociation of HOCl depends on the pH of the solution.[30] Hence, the molar fraction of HOCl and OCl^- can be estimated by using the Henderson–Hasselbalch equation (eqn (4)). OCl^- has a low reactivity toward the reaction with organic compounds as compared with HOCl. Therefore, the reactions between OCl^- and organic compounds were assumed to be negligible and only the reactions with HOCl have been considered during chlorination.[11,15,31] Fig. 2 shows that the k_{app} decreases with decreasing molar fraction of HOCl. As HOCl, Mfe with a pK_a of 4.20 (ref. 32) can appear as neutral (Mfe) or anion (Mfe^-) forms in solution under different pH conditions (Fig. 2). In this study, the molar fraction (α) of neutral and anion forms of Mfe was also estimated using the Henderson–Hasselbalch equation as shown in eqn (4). Under the selected pH conditions, Mfe appeared dominantly as an anion (Fig. 2). Therefore, the effect of the degree of deprotonation of Mfe on the degradation of Mfe is negligible under the selected conditions. Consequently, it can be concluded that the decrease of k_{app} with increasing pH of solution is mainly attributed to the decrease of the available HOCl in the solution. HOCl can degrade Mfe *via* the reaction with the aromatic ring and the secondary amine group and this result was proven by the identified degradation by-products of Mfe.

Table 1 The variation of k_{app} as a function of pH (n = 3)

pH	k_{app} (M^{-1} s^{-1})
6.0	16.44 ± 0.03
6.5	12.94 ± 0.04
7.0	8.2 ± 0.1
7.5	6.17 ± 0.07
8.0	4.4 ± 0.8

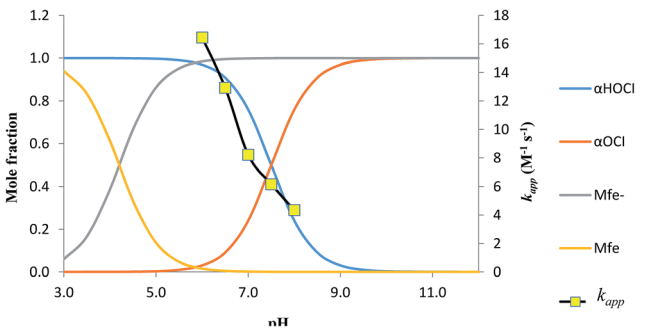


Fig. 2 Apparent second-order rate constant for chlorination of Mfe from pH 6 to 8.

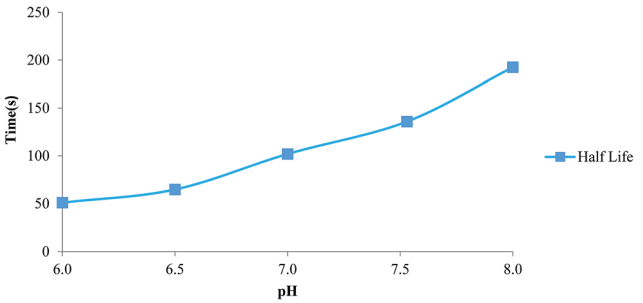


Fig. 3 The variation of $t_{1/2}$ as a function of pH for Mfe.

$$\mathrm{pH} = \mathrm{p}K_a - \log_{10}\frac{[\mathrm{Mfe}]}{[\mathrm{Mfe}^-]} \quad (4)$$

The half-lives ($t_{1/2}$) of Mfe under different pH conditions were calculated using eqn (5). According to Fig. 3, the $t_{1/2}$ of Mfe was found to increase from 51 to 193 s when the pH was increased from 6 to 8.

$$t_{1/2} = \frac{\ln 2}{k_{obs}} = \frac{\ln 2}{k_{app}[\mathrm{Cl}]_0} \quad (5)$$

3.2 Transformation by-products of Mfe

In order to obtain the transformation by-products of Mfe, chlorination was performed by allowing Mfe to be exposed to FAC for 24 h. Under such conditions, the transformation by-products which are resistant against chlorination can be determined. In this study, structural elucidation of the transformation by-products was performed using LC-QTOFMS. This instrument produces MS and MS/MS with high mass accuracy. The proposed transformation by-products of Mfe are presented in Table 2 and the MS/MS spectrum in Fig. S1 (ESI†). Analysis was performed by comparing mass spectrometric data of the controls with the data collected from the chlorinated Mfe solutions. In this study, four transformation by-products of Mfe were identified. These transformation by-products were labelled as Mfe-138, Mfe-256, Mfe-258 and Mfe-290.

The MS/MS spectrum of Mfe with a quasi-molecular ion, $[M + H]^+$, of m/z 242.1176 showed two significant fragment ions at m/z 224.1066 and 210.0835 (Fig. S1a†). The fragment ion at m/z 224.1066 was produced from the loss of a H_2O molecule from the $[M + H]^+$ ion. Further fragmentation of the m/z 224.1066 ion through the loss of one methyl group formed a fragment ion at m/z 210.0835. Mfe-138 (Fig. S1b†) is 2-aminobenzoic acid formed through the loss of the *o*-xylene group from Mfe. For Mfe-138, the fragment ion of m/z 120.0448 was formed through the loss of the H_2O group from the $[M + H]^+$ ion. Further fragmentation of m/z 120.0448 through the loss of the carbonyl group formed m/z 92.0506. The mechanism for the formation of Mfe-138 is proposed to start from the formation of highly reactive chloramine intermediate I.[33,34] Chloramine intermediate I is unstable and it tends to decompose to form Mfe-138 (Fig. 4a).

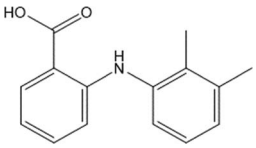
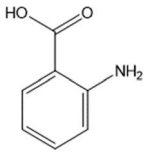
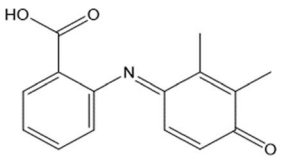
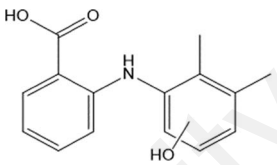
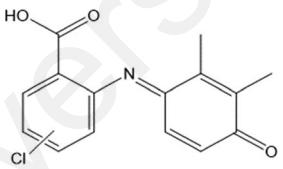
Mfe-258 (Fig. S1c†) with the $[M + H]^+$ ion at m/z 258.1038 was formed through the hydroxylation of Mfe. For this transformation by-product, the fragment ion at m/z 240.0989 was formed through the removal of one H_2O molecule from the $[M + H]^+$ ion. Further fragmentation of the ion at m/z 240.0989 by the removal of another H_2O molecule formed an ion at m/z 222.0969. The formation of hydroxylated by-products during chlorination has been frequently reported.[14,35–37] For Mfe, the methyl group is an electron donating group that activates the aromatic ring whereas the carboxylic acid group is an electron withdrawing group that deactivates the aromatic ring of Mfe.[38] Therefore, the addition of the OH group was proposed at the more reactive *o*-xylene moiety of Mfe (Fig. 4b). This reaction could start from the reaction between the aromatic ring and the positively polarized chlorine atom of HOCl for the formation of monochlorinated Mfe (II). Monochlorinated Mfe was not detected in this study suggesting that this by-product is unstable under the selected conditions. Monochlorinated Mfe (II) can further react with the hydroxide ion through the nucleophilic addition for the formation of intermediate III. Intermolecular rearrangement of III through the elimination of the chlorine atom forms Mfe-258. HOCl can further oxidize Mfe-258 that contains an aminophenol moiety to form a product with a quinone-imine moiety (Mfe-256) as shown in Fig. 4b.[39,40] Mfe-256 is an oxidation product of Mfe-258. Mfe-256 (Fig. S1c†) with the $[M + H]^+$ ion at m/z 256.0967 shows three major fragments at m/z 238.0854, 210.0901 and 196.0757. The ion fragment at m/z 238.0854 was formed through the loss of the H_2O molecule from the $[M + H]^+$ ion. Then, further fragmentation of the ion at m/z 238.0854 through the loss of the CO group formed m/z 210.0901. The fragment ion at m/z 196.0757 was formed through the loss of the methyl group from the fragment ion at m/z 210.0901.

Mfe-290 (Fig. S1e†) with the $[M + H]^+$ ion at m/z 290.0578 was the only detected chlorinated by-product. It was formed by the addition of one chlorine atom to Mfe-256 (Fig. 4b). Mfe-290 shows two significant fragment ions at m/z 272.0473 and m/z 244.0550. The fragment ion at m/z 272.0473 was formed through the removal of one H_2O molecule from the $[M + H]^+$ ion. Then, further fragmentation of the fragment ion at m/z 272.0473 formed an ion at m/z 244.0550 through the elimination the CO group. The fragment ion at m/z 238.0801 was formed from the fragment ion at m/z 272.0473 through the removal of the hydrogen chloride group. Then, the fragment ion at m/z 224.0706 was produced through the removal of the methyl group from the fragment ion at m/z 238.0801. For Mfe-290, chlorine atom addition was proposed at the benzoic acid moiety of Mfe due to the electrophilic nature of the quinone-imine moiety which is less likely to attack the positively polarized chlorine atom of HOCl.[41]

3.3 Ecotoxicity of Mfe and its transformation by-products

The formation of toxic transformation by-products during chemical oxidation has been frequently described by many studies.[23,27,42–44] Recently, Chen *et al.*[23] have shown that the intermediate photo-products of Mfe were more toxic than their

Table 2 Proposed transformation by-products of Mfe during chlorination

Measured quasi-molecular ion $[M + H]^+$	Molecular formulae $[M + H]^+$	Proposed structure (label-retention time)	Measured exact mass (m/z)	Calculated exact mass (m/z)	Error (ppm)	Reaction pH		
						pH 6	pH 7	pH 8
242.1176	$C_{15}H_{16}NO_2^+$	 Mfe-242 (10.102 min)	241.1100	241.1103	1.2	✓	✓	✓
138.055	$C_7H_8NO_2^+$	 Mfe-138 (7.378 min)	137.0486	137.0477	−6.65	✓	✓	✓
256.0968	$C_{15}H_{14}NO_3^+$	 Mfe-256 (8.976 min)	255.0894	255.0895	0.38	✓	✓	✓
258.1125	$C_{15}H_{16}NO_3^+$	 Mfe-258 (9.327 min)	257.1035	257.1052	6.77	✓	✗	✗
290.0578	$C_{15}H_{13}ClNO_3^+$	 Mfe-290 (9.400 min)	289.0508	289.0506	−0.87	✓	✓	✓

parent compound. So far, only the acute lethal concentrations of Mfe on *Thamnocephalus platyurus* and *Oryzias latipes* have been reported. The available ecotoxicity data showed that 24 h LC_{50} of *Thamnocephalus platyurus* and 96 h LC_{50} of *Oryzias latipes* were 3.95 mg L^{-1} and 8.04 mg L^{-1}, respectively.[45,46] In this study, the LC_{50}, EC_{50} and ChV of the proposed transformation by-products were estimated using the ECOSAR computer program. The ECOSAR program has been frequently used to screen the toxicity of chemicals by various agencies such as environmental assessors, chemical manufacturers, chemical suppliers, *etc.*[27] ECOSAR calculates the toxicity of chemicals using Structure Activity Relationships (SARs) and Quantitative Structure Activity Relationships (QSARs) that estimate a chemical's acute toxicity and chronic toxicity.[47] Since most of the identified transformation by-products have not been reported, the ECOSAR program was used to predict the aquatic toxicity of transformation by-products. Although this aquatic toxicity is a predicted value, the main purpose of this study was to compare the ecotoxicity of proposed transformation by-products with that of Mfe.

The predicted LC_{50}, EC_{50} and ChV of Mfe and its transformation by-products, as related to the baseline toxicity provided by the neutral form of organic species, are presented in Table 3. The result indicated that the predicted LC_{50}, EC_{50} and ChV of Mfe-290, Mfe-256 and Mfe-138 were higher than those of Mfe, whereas, the LC_{50}, EC_{50} and ChV of Mfe-258 were

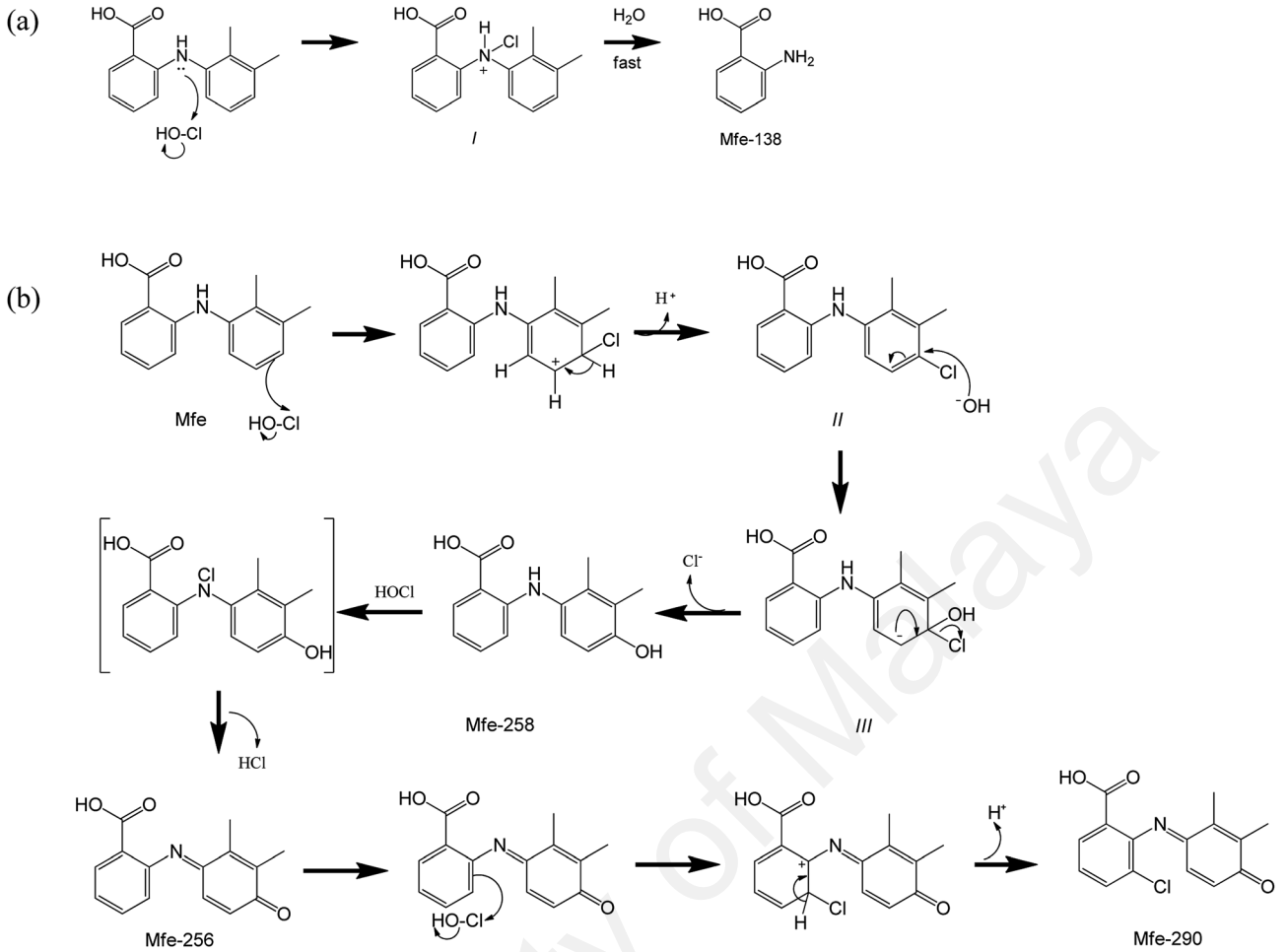


Fig. 4 Proposed transformation pathway for the formation of (a) Mfe-138 (b) Mfe-258, Mfe-256, Mfe-290.

Table 3 Predicted acute toxicity and chronic toxicity of Mfe and its chlorination by-products

	Acute toxicity (mg L^{-1})			Chronic toxicity (mg L^{-1})		
Compound	Fish (LC_{50})	Daphnids (LC_{50})	Algae (EC_{50})	Fish	Daphnids	Algae
Mfe	2.25	1.73	4.51	0.315	0.392	2.32
Mfe-258	1.34	0.954	1.81	0.171	0.174	0.783
Mfe-290	8.87	5.83	7.98	1.03	0.856	2.90
Mfe-256	29.6	18.4	19.6	3.21	2.28	6.25
Mfe-138	421	225	132	38.4	18.7	30.2

lower than those of Mfe. This result suggested that Mfe-258 was more toxic than its parent compound. Therefore, it can be concluded that a chlorination by-product which is more toxic than Mfe can be formed during chlorination.

4. Conclusion

In this study, the k_{app} for the reaction between Mfe and FAC was found to decrease from 16.44 to 4.4 M^{-1} s^{-1} with increasing pH from 6 to 8. This result indicated that k_{app} was highly pH dependent. The decrease of k_{app} was mainly attributed to the decrease of the available HOCl in the solution. For the transformation by-product identification, four transformation by-products were detected using LC-QTOFMS. The information from the transformation by-products indicated that HOCl degrades Mfe *via* the reaction with the aromatic ring through oxidation, hydroxylation and chlorination. HOCl also degrades Mfe through the reaction at the secondary amine group of Mfe. The result from the ecotoxicity assessment showed that transformation by-products which are more toxic than Mfe can be formed during aqueous chlorination of Mfe.

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