# DEGRADATION OF SELECTED ENVIRONMENTAL PHARMACEUTICALS BY AQUEOUS CHLORINATION AND UV/CHLORINATION: KINETICS, BY-PRODUCTS, AND ECOTOXICITY

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Field of Study: Environmental Chemistry

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**DEGRADATION OF SELECTED ENVIRONMENTAL PHARMACEUTICALS BY AQUEOUS CHLORINATION AND UV/CHLORINATION: KINETICS, BY-PRODUCTS, AND ECOTOXICITY**

## ABSTRACT

As emerging contaminants, some pharmaceuticals in the environment have been found to produce toxic effects to the living organism. During conventional water treatment processes, untreated pharmaceuticals are often exposed to chlorination process. Recent studies have shown that the chlorination process is not effective in removing pharmaceuticals due to its slow reaction. Hence, the focus of the study was to combine UV irradiation with chlorination (UV/chlorination) to enhance the rate of degradation of pharmaceuticals. The objectives of this study were to investigate the reaction kinetics and mechanism of the degradation of selected pharmaceuticals during chlorination and UV/chlorination. Then, the ecotoxicity of selected pharmaceuticals after chlorination and UV/chlorination was determined experimentally. The efficiency of chlorination and UV/chlorination in the removal of selected pharmaceuticals in different matrices was also evaluated. The selected pharmaceuticals for this study were 5,5-diphenylhydantoin (antiepileptic), hydrochlorothiazide (antidepressants) and tolfenamic acid (nonsteroidal anti-inflammatory drugs). For chlorination, the second-order rate constant ($k_{app}$) for the reaction between selected pharmaceuticals and free available chlorine (FAC) was determined at pH 5 to 8. The result indicated that the degradation of selected pharmaceuticals by FAC was highly pH dependence at the selected pH range. At pH 5 to 8, it was found that $k_{app}$ of 5,5-diphenylhydantoin, hydrochlorothiazide and tolfenamic acid was ranged from 0.8 - 2.5 $M^{-1}$ $min^{-1}$, 1.6 - 70.6 $M^{-1}$ $min^{-1}$, and 1.0 – 41.1 $M^{-1}$ $min^{-1}$, respectively. For UV/chlorination, the effect of FAC dosage and pH on the degradation of selected pharmaceuticals was evaluated. UV/chlorination was found to be more effective in removing the selected pharmaceuticals as compared with conventional

chlorination and UV alone. The selected pharmaceuticals degradation rate was found to increase with increasing FAC concentration. On the other hand, the degradation of selected pharmaceuticals was found to be more favorable under the acidic condition. Characterization of the transformation by-products (TBPs) formed during the chlorination of the selected pharmaceuticals were carried out using gas chromatography-mass spectrometry and liquid chromatography-triple quadrupole mass spectrometer. The TBPs for chlorination process were determined after 24 h of FAC exposure. Meanwhile, for UV/chlorination process the TBPs were identified after 6-10 min of UV/chlorination treatment. The result indicated that chlorination and UV/chlorination of pharmaceuticals could produce various TBPs via hydroxylation, chlorination, and oxidation reactions. Based on computational calculation, some of the TBPs were found to be more toxic than its parent compound. The toxicity study revealed that UV/chlorination may increased the toxicity of the pharmaceuticals solution. Hence, this study showed that detail evaluation of toxicity is required when applying UV/chlorination for the treatment of pharmaceuticals.

**Keywords:** Pharmaceuticals, water treatment, advanced oxidation processes, chemical oxidation and degradation.

**DEGRADASI FARMASEUTIKAL TERPILIH MELALUI PENGKLORINAN AKUEUS DAN UV/PENGKLORINAN: KINETIK, PRODUK SAMPINGAN DAN KETOKSIKAN EKOLOGI**

## ABSTRAK

Sesetengah farmaseutikal di alam sekitar telah didapati menghasilkan kesan toksik kepada organisma hidup. Semasa proses rawatan air konvensional, farmaseutikal sering terdedah kepada proses pengklorinan. Kajian terbaru menunjukkan bahawa proses pengklorinan tidak berkesan dalam rawatan farmaseutikal kerana kadar tindakbalas yang perlahan. Oleh itu, fokus kajian ini adalah untuk menggabungkan ultralembayung (UV) dan pengklorinan (UV/pengklorinan) untuk meningkatkan keupayaan rawatan farmaseutikal. Objektif kajian ini adalah untuk mengkaji kinetik dan mekanisme tindak balas farmaseutikal dalam proses pengklorinan dan UV/pengklorinan. Kemudian, ketoksikan ekologi farmaseutikal selepas pengklorinan dan UV/pengklorinan ditentukan secara eksperimen. Kecekapan pengklorinan dan UV/pengklorinan dalam penyingkiran farmaseutikal yang terpilih dalam berbagai sampel air juga dikenalpasti. Farmaseutikal yang dipilih untuk kajian ini ialah 5,5-difenilhidantoin (antiepileptik), hidroklorothiazida (antidepresan) dan asid tolfenamik (ubat antiradang bukan steroid). Bagi eksperimen pengklorinan, pemalar kadar kedua ($k_{app}$) bagi tindak balas antara farmaseutikal yang dipilih dan klorin bebas dikaji pada pH 5 hingga 8. Hasilnya menunjukkan kadar tindak balas antara farmaseutikal yang terpilih dengan klorin bebas adalah bergantung kepada keadaan pH air. Pada pH 5 hingga 8, $k_{app}$ untuk 5,5-difenilhidantoin, hidroklorothiazida dan asid tolfenamik adalah dalam julat 0.8 - 2.5, 1.6 - 70.6, dan 1.0 – 41.1 $M^{-1}$ $min^{-1}$. Bagi eksperimen UV/pengklorinan, kesan dos klorin bebas dan pH terhadap kadar penyingkiran farmaseutikal yang terpilih telah dijalankan. UV/pengklorinan didapati lebih berkesan dalam penyingkiran farmaseutikal berbanding dengan pengklorinan dan UV sahaja. Kadar penyingkiran farmaseutikal yang terpilih didapati meningkat dengan

peningkatan dos klorin bebas. Manakala, kadar penyingkiran farmaseutikal didapati lebih berkesan pada keadaan berasid. Pencirian produk sampingan yang terbentuk semasa pengklorinan dan UV/pengklorinan bagi farmaseutikal terpilih telah dijalankan dengan menggunakan kromatografi gas-spektrometer jisim dan kromatografi cecair-spektrometer jisim catur kutub tripel. Hasil eksperimen ini menunjukkan pengklorinan dan UV/pengklorinan farmaseutikal terpilih telah menghasilkan pelbagai sebatian sampingan melalui tindak balas hidroksilasi, pengklorinan, dan pengoksidaan. Kajian ketoksikan menunjukkan bahawa UV/pengklorinan akan meningkatkan ketoksikan cecair farmaseutikal. Kajian ini mencadangkan supaya penilaian ketoksikan yang teliti perlu dilakukan apabila menggunakan UV/pengklorinan untuk rawatan farmaseutikal.

**Kata kunci:** Farmaseutikal, rawatan air, proses pengoksidaan lanjutan, pengoksidaan kimia dan degradasi.

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | | |
|---|---|---|
| AOP | : | Advanced oxidation process |
| BSTFA | : | N,O-Bis(trimethylsilyl)trifluroacetamide |
| CFU | : | Colony forming units |
| DBPs | : | Disinfection by-products |
| DES | : | Diethylstilbestrol |
| dichloro-MTX | : | 4-amino-3,5-dichloro-N 10-methylpteroylglutamic |
| DPH | : | 5,5-Diphenylhydantoin |
| EF | : | Electro-Fenton |
| *E. Coli* | : | *Escherichia coli* |
| EE | : | Ethinylestradiol |
| ED | : | Endocrine disrupting |
| ESI | : | Electrospray ionization |
| FAC | : | Free available chlorine |
| GAC | : | Granular activated carbon |
| GC-MS | : | Gas chromatography mass spectrometry |
| HAA | : | Haloacetic acids |
| HAN | : | Haloacetonitriles |
| HCTZ | : | Hydrochlorothiazide |
| HPLC | : | High performance liquid chromatography |
| $k_{app}$ | : | Second-order rate constant |
| $k_{obs}$ | : | Pseudo-first-order rate constant |
| $LC_{50}$ | : | Lethal concentration |
| LC-QqQ-MS | : | Triple quadrupole liquid chromatography mass spectrometer |
| LC-MS | : | Liquid chromatography mass spectrometer |

| | | |
|---|---|---|
| $LD_{50}$ | : | Lethal dosage 50 |
| LVF | : | Levofloxacin |
| MTX | : | Methotrexate |
| monochloro-MTX | : | 4-amino-3-chlorinated-N 10-methylpteroylglutamic |
| NAPQI | : | N-acetyl-p-benzoquinone imine |
| NaOCl | : | Sodium hypochlorite |
| NOM | : | Natural organic matter |
| NSAID | : | Non-steroidal anti-inflammatory drugs |
| PFCs | : | Perfluorinated chemicals |
| PS | : | Persulfate |
| RCS | : | Reactive chlorine species |
| SPE | : | Solid phase extraction |
| TBPs | : | Transformation by-products |
| THMs | : | Halogenated trihalomethanes |
| TMCS | : | Trimethylchlorosilane |
| TOL | : | Tolfenamic acid |
| UV | : | Ultraviolet |
| WWTP | : | Wastewater treatment plants |

# CHAPTER 1: INTRODUCTION

## 1.1 Background of the study

Pharmaceuticals are a class of emerging environmental contaminants that are extensively and increasingly being used in human and veterinary medicine. The first report on the presence of pharmaceuticals in the aquatic environment was in the 1970s (Tabak and Bunch, 1970). Today, pharmaceuticals have been detected in various water samples such as the effluents of wastewater treatment plants (WWTP), river, and lakes. These chemicals are used to improve human and animal health; hence the usage of pharmaceuticals is unstoppable. Production of pharmaceuticals around the world is increasing every year. According to Webb et al. (2003), 1 tonnes of 170 pharmaceuticals are estimated to be used in excess per year.

The presence of pharmaceuticals in the aquatic environment has gained increasing attention in recent years. Various types of pharmaceuticals have been detected in environments ranging from nanogram (ng/L) to microgram (μg/L) levels. This concentration may seem low, but pharmaceuticals are developed to trigger specific biological effects at low dosage in the human body and animal (Halford et al., 2010). Since pharmaceuticals are continuously released into the environments, other living organisms are exposed to the chemicals for their entire lifetime. Pharmaceuticals have been found to cause various negative impacts on non-targeted organisms in the aquatic and terrestrial environment (Iwanowicz et al., 2016; Örn et al., 2016). Recently, research on the identification of the ecotoxicity of environmental pharmaceuticals has become an active research area throughout the world. Well-known negative effects of pharmaceuticals to the living organisms are such as endocrine disruption (Li et al., 2015; Jung et al., 2015) and increasing the resistance of microorganisms against antibiotics (Adachi et al., 2013; Cheng et al., 2016; Masarikova et al., 2016). Oxidative stress and oxidative toxic effects on the aquatic organism are also the examples of the negative

effects of pharmaceuticals to the living organism (Correia et al., 2016; Schmidt et al., 2014). The presence of pharmaceuticals in the aquatic environment also has been found to lead to the alteration of thyroid hormonal level of fish and decelerate the growth of mud snail (Feiner et al., 2014; Saravanan et al., 2014).

Instead of potential bioaccumulation and persistence of the environmental pharmaceuticals, the pharmaceuticals that released into the environment as mixtures are also raised various concerns. This is because the environmental effects of the combination of various pharmaceuticals are remained unknown (Stackelberg et al., 2004; Tixier et al., 2003). According to Kümmerer (2004), human health may be at risk through long-term consumption of drinking water that containing trace levels of pharmaceuticals. However, the risk of adverse effects on human through the ingestion of pharmaceuticals that present in drinking water seems to be negligible (Simazaki et al., 2015). Thus, the risks posed to human from pharmaceuticals contamination seem to be more to the environmental hygiene concern rather than toxicology or pharmacology. Therefore, further study needs to be done as drinking water containing trace levels of pharmaceuticals is still an unresolved issue in many countries (Yang et al., 2017).

Most of the consumed pharmaceuticals by human are not completely metabolized, and it is excreted into the wastewater which is later treated by wastewater treatment plants. The presence of pharmaceuticals has posed a great challenge to conventional wastewater treatment plants to remove it from wastewater as the currently available conventional wastewater treatments plants are not designed for this purpose (Fatta-Kassinos et al., 2011). As a result, numerous studies have shown that pharmaceuticals are present in the effluent of conventional wastewater treatment plants (Gabet-Giraud et al., 2014). During water treatment, pharmaceuticals, which escaped from the physicochemical and biological treatments, are often exposed to the disinfection process

(Khalit and Tay, 2016a). So far, chlorination is one of the most widely used disinfection methods to kill pathogen in water treatment. Chlorine-based disinfectants can be added in the form of gas, solid or liquid (Table 1.1) during disinfection. For the safety purpose, sodium hypochlorite (NaOCl) and calcium hypochlorite ($Ca(OCl)_2$) are the most frequently used disinfectant in water treatments (Solsona and Méndez, 2003).

**Table 1.1:** Types of chlorine disinfectants.

| Chlorine disinfectants | Phase |
|---|---|
| Chlorine gas ($Cl_2$) | Gas |
| Sodium hypochlorite (NaOCl) | Liquid |
| Calcium hypochlorite ($Ca(OCl)_2$) | Solid |
| Chloramine | Solid |

Recent studies have shown that the chlorination process was not effective in removing pharmaceuticals due to its slow reaction between pharmaceuticals and free available chlorine (FAC) as compared to other chemical treatment methods such as ozonation (Gao et al., 2014; Liu et al., 2012). FAC is referring to the total concentration of hypochlorous acid (HOCl) and hypochlorite ion ($OCl^-$) that available in water during chlorination. Chlorination of pharmaceuticals was also found to produce potential toxic by-products (Nam et al., 2015). These by-products have the potential to cause the public health hazards (Gopal et al., 2007). For example, chlorination of carbamazepine and oxcarbazepine was found to produce acridine as main by-products (Yin et al., 2017a). This by-product was found to be more toxic than its parent compounds as it showed the carcinogenic properties (Postigo and Richardson, 2014).

Current studies have shown that combining UV (ultraviolet) and chlorination (UV/chlorination) could enhance the rate of degradation of organic pollutants such as atrazine (Kong et al., 2016) chlortoluron (Guo et al., 2016), and carbamazepine (Wang et al., 2016a) as compared with chlorination process alone. Exposure of HOCl and $OCl^-$ to

UV irradiation has been found to produce hydroxyl radical (•OH) and chlorine radical (•Cl) as the reactive oxidants, as shown in Eq. 1.1 and 1.2 (Wolfe, 1990). UV/chlorination has been reported as more effective advanced oxidation method to produce •OH than common UV/$H_2O_2$ due to the effectiveness of HOCl/$OCl^-$ in absorbing UV photons as compared to hydrogen peroxide ($H_2O_2$) (Duan et al., 2017; Moreira et al., 2016; Zhang et al., 2016). Formation of radicals also able to eliminate multiresistant types of microorganism at high efficiency during water treatment (Mackuľak et al., 2016). Moreover, UV/chlorination process also gives better effectiveness in removing taste and odour compounds as compared to UV/$H_2O_2$ (Boal et al., 2015; Rosenfeldt et al., 2013; Wang et al., 2015a). Hence, UV/chlorination process has the potential to be used in wastewater treatment plant (Dong et al., 2017).

$$HOCl + h\nu \rightarrow \bullet OH + Cl \qquad \text{(Eq. 1.1)}$$

$$OCl^- + h\nu \rightarrow \bullet OH + \text{other products} \qquad \text{(Eq. 1.2)}$$

The previous study has shown the efficiency of UV/chlorination method not only for the removal of micropollutant but also inactivation of water-borne pathogens in water. Some countries such as Spain have started to apply this method to treat their wastewater (Matamoros and Salvadó, 2013). Although UV/chlorination has been used in real wastewater treatment plants, however, the fate of micropollutants especially pharmaceuticals in this system are seldom reported. In oxidation process, a different organic compound often reacts with an oxidant at the different rate. Also, oxidation of organic compounds was often found to produced various by-products which can be more toxic than its parent compound. Hence, it is important to study in detail the effectiveness of UV/chlorination in pharmaceuticals removal and also the by-products formed during treatment.

In this study, the fate of three pharmaceuticals namely 5,5-diphenylhydantoin (DPH), hydrochlorothiazide (HCTZ) and tolfenamic acid (TOL) in chlorination and UV/chlorination treatment was determined. These three compounds were frequently detected in the environments. In chlorination process, the second-order rate constant for the reaction between the selected pharmaceuticals and FAC was determined. For UV/chlorination process, the effects of the different operating parameter such as chlorine dosage and pH on the degradation of selected compounds were studied. The transformation by-products of selected pharmaceuticals that produced from chlorination and UV/chlorination were determined using gas chromatography-mass spectrometry (GC-MS) and triple quadrupole (QqQ) liquid chromatography-mass spectrometry (LC-MS). The ecotoxicity of selected pharmaceuticals after treating with chlorination and UV/chlorination was also evaluated.

## 1.2 Objectives of study

The objectives of this research were to study the fate of DPH, HCTZ, and TOL during chlorination and UV/chlorination process. The specific objectives are as below:

- To determine the kinetics of the degradation of the selected pharmaceuticals during chlorination.
- To study the effects of operating parameters of UV/chlorination on the degradation of selected pharmaceuticals.
- To identify the degradation of by-products of the selected pharmaceuticals during chlorination and UV/chlorination.
- To access the ecotoxicity of the treated DPH, HCTZ and TOL in aqueous solution.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Pharmaceuticals as Emerging Contaminants

Emerging pollutants are defined as compounds that are not currently recorded in existing water-quality regulations and are thought to cause potential threats to environmental ecosystems, human health and safety (La Farre et al., 2008). Pharmaceuticals are one of the major groups of emerging contaminants. These compounds are not regulated yet either due to the lack of information regarding their occurrence and environmental effects, or the lack of appropriate analytical methods for their determination in complex environmental samples (Yan et al., 2014). In the 1950s, products made from perfluorinated chemicals (PFCs), such as fast food packaging, paper plates, stain-resistant carpets and pharmaceuticals were used around the world but were not widely recognized as contaminants until the early of the 21st century. These emerging contaminants become a major concern in recent years due to the introduction of high resolution and high sensitivity instruments, such as LC-MS which enable the measurement of these contaminants because most of emerging contaminants are occurred at trace level and required higher sensitivity instruments (Lei et al., 2015).

Pharmaceuticals are chemicals which are used in human and veterinary medicine, and in the agricultural practice (Zhao, 2006). Pharmaceuticals are one of the emerging contaminants which have frequently been detected in the environment, and it has become the growing concerns in recent years since these compounds are released into the environment at an accelerative rate. Human consumes pharmaceuticals to improve, cure, prevent and treat diseases. The ingested pharmaceuticals are often excreted in urine and feces as a mixture of unchanged compounds, metabolites or conjugated substances which will be reaching the wastewater treatment plants. According to Rivera-Utrilla et al. (2013), many pharmaceuticals in the environment are pharmacologically active, resistant

to degradation, highly persistent in water, and have the potential to cause the negative impact to the aquatic organisms and human health.

### 2.1.1 Pharmaceuticals in environments

Manufacturing residues from pharmaceuticals industry, effluents from the hospitals and sewage treatment facilities, and landfill leachates are identified as the sources of pharmaceuticals in the environment (Figure 2.1). Sources of pharmaceuticals can be divided into two: human and veterinary medicine. Among various sources, the effluent of WWTP and landfill leaching has been identified as major sources of pharmaceuticals in the environment (Li, 2014; Luo et al., 2014). During wastewater treatment process, pharmaceuticals are often not completely removed. This is because most of the municipal sewage treatment plants are not engineered specifically for pharmaceuticals removal (Luo et al., 2014; USEPA, 2010). Hence, these pharmaceuticals are frequently detected in aquatic systems that receiving the effluents of WWTP (Al Qarni et al., 2016; Chen et al., 2013; Guan et al., 2016).

The landfill is a site for the final disposal of most municipal solid waste, including unwanted or expired household medicines. Hence, the presence of pharmaceuticals was frequently reported at the landfill (Masoner et al., 2014; Peng et al., 2014; Qi et al., 2018; Sui et al., 2017). A study from China investigated the distribution of pharmaceuticals in four municipal landfill leachate samples 15 pharmaceuticals including naproxen, atenolol, gemfibrozil, and ketamine were detected in the range of 16.2 to 8102.5 ng/L (Lu et al., 2016). Sui et al. (2017) also reported the presence of pharmaceuticals such as carbamazepine, metoprolol, trimethoprim, and gemfibrozil in the landfill leachates collected from Shanghai. The concentration of these pharmaceuticals was ranged from 0.39 to 349 μg/L. According to Buszka et al. (2009), the presence of hormones and pharmaceuticals are not only detected in landfill leachate but also in the groundwater

around that area. Barnes et al. (2004) also reported the presence of pharmaceuticals in the groundwater near the landfill and concentration of pharmaceuticals in the groundwater was ranged from 360 to 3110 ng/L. These pharmaceuticals were found to be persistent in groundwater.

According to Cardoso et al. (2014), China and India produce half of the world production of pharmaceuticals and the first-ever contamination by pharmaceuticals from industrial was detected in these two countries. In 1988, the wastewaters of a pharmaceutical factory in China were found to contain oxytetracycline and oxalic acid at the concentration of 600 mg/L and 9100 mg/L, respectively. After 30 years, Li et al. (2008) reported that 250 μg/L of oxytetracycline was detected at 20 km downstream from this factory. Another study at the pharmaceutical factories located at Taiwan and Korea showed that pharmaceutical such as non-steroidal anti-inflammatory drugs (NSAID) and antibiotics was detected at a high concentration ranging from 1 μg/L to 43.9 mg/L (Lin and Tsai, 2009; Sim et al., 2011).

Sewage sludge is also one of the sources of pharmaceuticals in the environment (Boix et al., 2016; Koba et al., 2018; Lin et al., 2018; Olarinmoye et al., 2016). A study by Lin et al. (2018) reported that 14 over 37 pharmaceuticals investigated were detected in the sludge. The concentration of pharmaceutical detected was in the range of 0.85 to 2900 μg/kg dry weight. According to Jelic et al. (2011), the frequency of detection of diclofenac, bezafibrate, carbamazepine, and hydrochlorothiazide in sludge sample was 100%. A study from New York (USA), showed that the percent adsorption of antidepressants and antihypertension to the sludge was ranged from 1.9 to 37%. Sludge collected from wastewater treatment plant is usually applied as fertilizer to agricultural land with the liquid effluent discharged directly into the freshwater environment (Ebele et al., 2017).

The veterinary drugs also may enter the environments through agricultural activity. Based on the study by Bártíková et al. (2016), Wei et al. (2011) and Zhao et al. (2016), veterinary drugs have been detected in soil, surface water and groundwater. These veterinary drugs are released into the environment through aquaculture and poultry of livestock (Ebele et al., 2017). According to La Farre et al. (2008), veterinary drugs are released into the environment when animal wastes are used for agricultural purpose. Consequently, these animal drugs together with their metabolites polluted the soil and surface water.

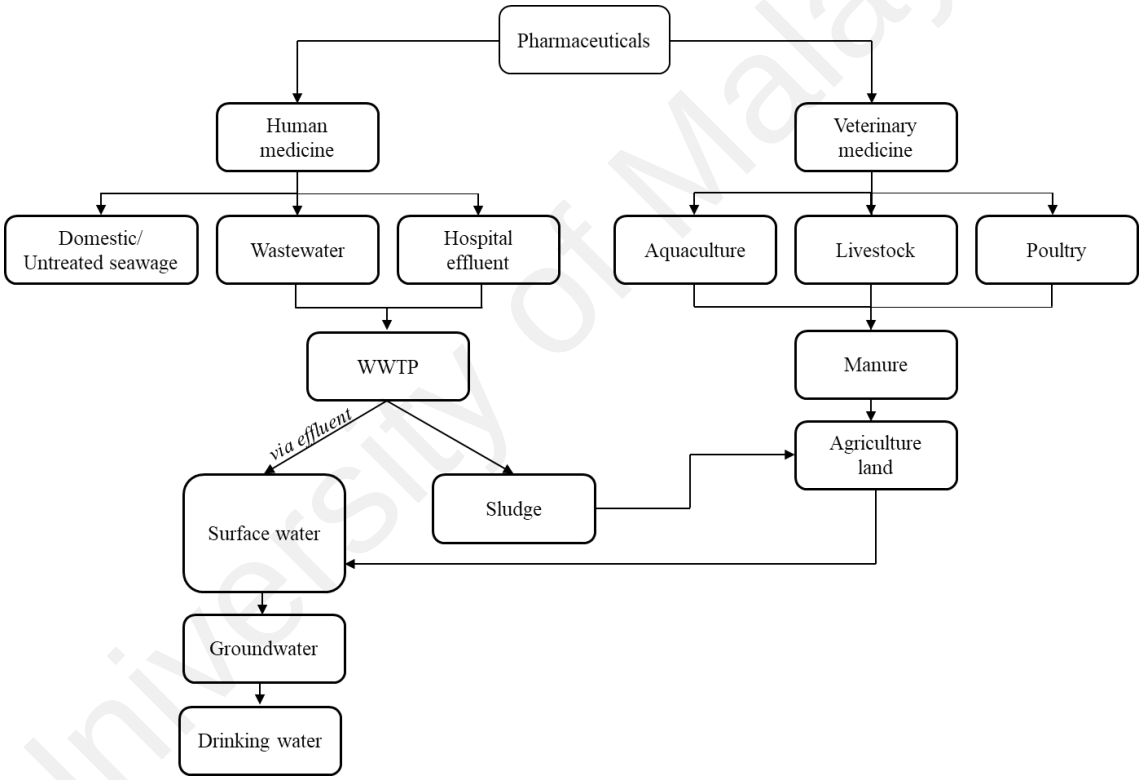



**Figure 2.1:** The possible sources of pharmaceuticals in the environment.

Table 2.1 shows the concentration of pharmaceutical residues in several environmental matrices such as surface water (Dai et al., 2015), river water (Tamura et al., 2017), ground water (López-Serna et al., 2013) and drinking water (Huerta-Fontela et al., 2011; Simazaki et al., 2015), as well as in hospital effluent (Kosma et al., 2014; Santos et al., 2013) and sea water (Moreno-González et al., 2015). The detected pharmaceuticals

in the aquatic environments are a wide variety of classes such as antiepileptics, diuretics, synthetic steroids, antidepressants, NSAID, antibiotics and beta-blockers (Table 2.1). Pharmaceuticals are designed specifically to produce biological activity at the minimum dosage (Ebele et al., 2017). Therefore, at very low concentrations, pharmaceuticals can affect the aquatic organisms upon exposure (Fent et al., 2006). Research on the eco-toxicological effects of pharmaceuticals on aquatic organism has been actively carrying out. Various negative effects of pharmaceuticals have been reported. For example, fluoxetine, one of anti-depressant, which has been frequently detected in the aquatic environment at the concentration of 34.8 to 62.1 ng/L (Moreno-González, 2015; Santos et al., 2013) resulted in a change of the physiological functions, including reproduction, metabolism, and locomotion in mussels at the concentrations approaching or even below the environmental levels (Ford and Fong, 2016). Ethinylestradiol (EE) and diethylstilbestrol (DES) are examples of endocrine disrupting pharmaceuticals (Cook et al., 2016; Wang et al., 2015b). Endocrine disrupting (ED) is an exogenous substance or mixture that alters the function of the endocrine system and consequently causes adverse health effects to living organism (Bergman et al., 2013). One of the major effects of ED is their ability to interfere with the endocrine system to produce undesired effects or disruption of homeostasis. It may affect the developments of the pregnant woman or wild animal and the developments of her offspring for over several generations (Fowler et al., 2012). The presence of ED in the environment also causes the rose of breast, prostate and testicular cancer (Brophy et al., 2012; Knower et al., 2014). Furthermore, exposure of ED can disrupt glucose and lipid homeostasis and may result in obesity and diabetes mellitus (Gore et al., 2015).

**Table 2.1:** Concentration of different classes of pharmaceuticals detected in different water bodies.

| Compound | Concentration (ng/L) | | | | | |
|---|---|---|---|---|---|---|
| | Hospital effluent | Surface water | Ground water | River water | Drinking water | Sea water |
| ***Antiepileptics*** | | | | | | |
| Carbamazepine | 41 [j] | 21.2-189 [c] | 115 [d] | | 0.4 [f] | |
| DPH | | | | | 0.02 [g] | |
| ***Diuretics*** | | | | | | |
| HCTZ | 239-997 [a] | | | | 1 [g] | 102.6 [h] |
| Furosemide | 435-9953 [a] | | | | | |
| ***NSAID*** | | | | | | |
| Diclofenac | 63.2 [b] | 7.8-150 [c] | 225 [d] | 63.5 [e] | 2.5 [f] | |
| Ibuprofen | | | 61 [d] | 184 [e] | 1.1 [f] | |
| Ketoprofen | | | 97.7 [d] | 110 [e] | | 39.1 [h] |
| Naproxen | 128 [b] | | 0.43 [d] | 22.8 [e] | | |
| TOL | 311 [I] | | | 462 [i] | | |
| ***Lipid regulators*** | | | | | | |
| Gemfibrozil | 347.1 [b] | 8.1-63.4 [c] | 209 [d] | | | 60 [h] |
| Bezafibrate | 429.8 [b] | 12.3-70.6 [c] | 6.64 [d] | 628 [e] | | |
| Fenofibrate | 93 [b] | | 14.6 [d] | | 0.2 [f] | |
| ***Antidepresants*** | | | | | | |
| Fluoxetine | | 16 [n] | | | | |
| Paroxetine | | 13 [n] | | | | |
| Venlafaxine | | 73 [n] | | 1.9 [n] | | |
| ***Synthetic steroids*** | | | | | | |
| Ethinyl Estradiol | | 4100 [k] | | 44.2 [m] | | |
| Diethylstilbestrol | | 11.1 [k] | | 14.4 [m] | | |
| ***Antibiotics*** | | | | | | |
| Sulfamethoxazole | 132 [j] | | | 396 [e] | | 70.4 [h] |
| Trimetoprim | 186.7 [b] | | | 127 [e] | | |
| Triclosan | | 1–11 [r] | | 16.8 [q] | | |
| Oxytetracycline | | 90.3 [p] | | 0.6–18.6 [o] | | |
| ***Beta-blockers*** | | | | | | |
| Atenolol | 46 [j] | | | 139 [e] | 1 [g] | |
| Propranolol | 4.28-10.6 [a] | 37 [c] | | 3.3 [e] | 4 [g] | 40.4 [h] |
| Metoprolol | 5.5-18.4 [a] | 11.3-353 [c] | 191 [d] | 5.6 [e] | 0.01 [g] | 31.4 [h] |
| Sotalol | 83.1-186 [a] | | 4.34 [d] | | 0.1 [g] | 59.8 [h] |

[a]Santos et al., (2013) [b]Kosma et al., (2014) [c]Dai et al., (2015) [d]López-Serna et al., (2013) [e]Tamura et al., (2017) [f]Simazaki et al., (2015) [g]Huerta-Fontela et al., (2011) [h]Moreno-González et al., (2015) [i]Guan et al., (2016) [j]Al Qarni et al., (2016) [k]Zhou et al., (2012) [m]Wang et al., 2015b [n]Giebułtowicz and Nałęcz, (2014) [o]Chen and Zhou, 2014 [p]Kolar et al., (2014) [q]Zhao et al., (2010) [r]Bottoni and Caroli, (2015)

Other major concern of the presence of pharmaceuticals in the environment is the presence of the complex mixture of pharmaceuticals in the aquatic environment which

may lead to synergistic interactions and the increased of the toxicity of the aquatic environments (Backhaus, 2014). A study by Thomas and Klaper (2012) reported that the mixture of psychoactive pharmaceuticals, venlafaxine, fluoxetine, and carbamazepine at environmentally relevant concentrations induced autism-like gene expression in fathead minnows. Another study showed that the exposure of fish and benthic invertebrates to psychoactive drugs altered the behavioral responses of the species (Brodin et al., 2014; Rosi-Marshall et al., 2015). Pomati et al. (2006) study the effect of a mixture of 13 human pharmaceuticals (atenolol, bezafibrate, carbamazepine, cyclophosphamide, ciprofloxacin, furosemide, HCTZ, ibuprofen, lincomycin, ofloxacin, ranitidine, salbutamol, and sulfamethoxazole) on human embryonic cells. The result showed that the cell growth was significantly inhibited.

The extensive use of antibiotics in human and animal husbandry, result in their continuous released into the environment (Carvalho and Santos, 2016; Lin et al., 2016). Most of the antibiotics are only partially metabolized in the range of 30 - 90% by living organism. Meanwhile, the remaining antibiotics are excreated as unchanged parent compounds and biologically active metabolites (Puckowsk et al., 2016). Hence, the unmetabolized antibiotics are reported to promote the development of antibiotic resistance among bacteria (Fiorentino et al., 2015; He et al., 2016). According to Rowe et al. (2017), hospital effluents contributed to high levels of antibiotic resistance genes in the aquatic environment. Sivaraman et al. (2004) found that one of the enzymes in *Escherichia coli* (*E. coli*), known as Fabl, that regulates the synthesis of fatty acid was inhibited by the presence of triclosan. Another study also showed the mixture of antibiotics that increased the resistance of two natural bacterial strains found in the receiving waters of WWTP effluents (Costanzo et al., 2005). Moreover, the presence of antibiotics could have a detrimental effect on naturally occurring bacteria that present in the environment. A study by Davies et al. (2006) showed that even at sub-inhibitory

concentrations, antibiotics still exert the biological impact on natural microbial communities by influencing transcription in microbes. Robinson et al. (2005) reported on the adverse effects of oxolinic acid on *Daphnia magna*. Oxolinic acid is used as a feed additive in fish farms and it is commonly found to adsorb to the sediment of marine origin. According to Hektoen et al. (1995), oxolinic acid is a persistent antibacterial substance that enhanced unfavorable environmental effects. Oxytetracycline is a veterinary antibiotic, which is commonly used to treat infections caused by gram-positive and gram-negative bacteria, mycoplasma and viruses (Awad et al., 2014; Reemtsma and Jekel, 2006). Previous studies have shown that oxytetracycline was frequently detected in the aquatic environment (Garbono et al., 2017; Liyanage and Manage, 2014; Song et al., 2016). The presence of oxytetracycline in the environment was found to inhibit the growth of several aquatic species such as *Tetraselmis chuii* (Microalgae), *Selenastrum capricornutum* (Freshwater green algae) and *Lemna minor* (Duckweed) (Eguchi et al., 2004; Ferreira et al., 2007; Pro et al., 2003).

A study by Schwaiger et al. (2004) investigated the effect of diclofenac on the brown trout, a salmonid species. The results showed the adverse effect of diclofenac on rainbow trout, which included the pathogenic alternation in gills and kidney after exposure to 5-50 µg/L of diclofenac for 28 days. The effect of gemfibrozil on goldfish was reported by Mimeault et al. (2005). The plasma testosterone in goldfish was reduced to half after 14 days of exposure to waterborne gemfibrozil. The results also indicated that the exposure to the environmental levels of gemfibrozil leads to bioconcentration of the drug in plasma has the potential to disrupt the endocrine system of fish.

## 2.2 Behavior of Pharmaceuticals in Conventional WWTP

**Table 2.2:** Concentrations of pharmaceuticals in the influents and effluents of wastewater treatment plants.

| Pharmaceuticals | Influent (ng/L) | Effluent (ng/L) | % Removal | Country | References |
|---|---|---|---|---|---|
| ***Antiepileptics*** | | | | | |
| Carbamazepine | 1032 | 923 | 10.6 | Mexico | Estrada-Arriaga et al., (2016) |
| DPH | 157 | 78.5 | 50 | USA | Dong et al., (2015) |
| Primidone | 129 | 83.85 | 35 | USA | Dong et al., (2015) |
| ***Antidepressants*** | | | | | |
| Bupropion | 110 | 67.4 | 38.7 | USA | Subedi and Kannan, (2015) |
| Norsertraline | 71.1 | 54.4 | 23.5 | USA | Subedi and Kannan, (2015) |
| Sertraline | 80.8 | 62.8 | 22.3 | USA | Subedi and Kannan, (2015) |
| ***NSAID*** | | | | | |
| Ibuprofen | 1607.8 | 341.4 | 78.8 | Algeria | Kermia et al., (2016) |
| Naproxen | 1219.7 | 33.7 | 72.6 | Algeria | Kermia et al., (2016) |
| Mefenamic acid | 10 | 7.3 | 27.0 | China | Sun et al., (2016) |
| Ketoprofen | 77 | 71 | 7.8 | China | Sun et al., (2016) |
| Diclofenac | 49 | 40 | 18.4 | China | Sun et al., (2016) |
| ***Antibiotics*** | | | | | |
| Sulfamethoxazole | 25 | 9.1 | 63.6 | China | Sun et al., (2016) |
| Trimetoprim | 132.1 | 59.8 | 54.7 | Greece | Kosma et al., (2014) |
| ***Beta-blockers*** | | | | | |
| Propanolol | 92.6 | 83 | 10.4 | Spain | Rubirola et al., (2014) |
| Sotalol | 231 | 136 | 41.1 | Spain | Rubirola et al., (2014) |
| Nadolol | 56.2 | 11 | 80.4 | Spain | Rubirola et al., (2014) |
| Atenolol | 277 | 88.2 | 68.2 | Mexico | Estrada-Arriaga et al., (2016) |
| Metoprolol | 228 | 205 | 10.0 | Mexico | Estrada-Arriaga et al., (2016) |

Table 2.2 shows the concentration of pharmaceuticals in the influents and effluents of wastewater treatment plants in several countries. The removal of pharmaceuticals by these WWTPs was varied from 7.8 to 80.4%. The difference in removal of pharmaceuticals during wastewater treatment was mainly due to the different chemical and physical properties of pharmaceuticals (Ternes and Joss, 2007). Hence, resulting in different behaviors during treatment processes, which can explain why compounds that belong to the same therapeutic class do not exhibit similar removal efficiencies.

Sewage is defined as wastewater that produced by the community (Mijinyawa and Lawal, 2008). Wastewater is known for content suspended solid, dissolved organic

matter, nutrient (nitrogen and phosphorus) and pathogens. Therefore, WWTP plays an important role to ensure the wastewater content is safe as regulated by the water quality standard for the effluent. WWTP is a multi-stage process which begins with preliminary and primary treatment for the removal of solids (chemical and physical) and then, it follows by secondary treatment for the removal of dissolved organic matter (biological). The last treatment in WWTP is the disinfection process, which is important to kill disease-causing pathogens, such as bacteria, viruses, and protozoans. But, to control the eutrophication of receiving water, some WWTP implemented tertiary treatment before disinfection process of wastewater.

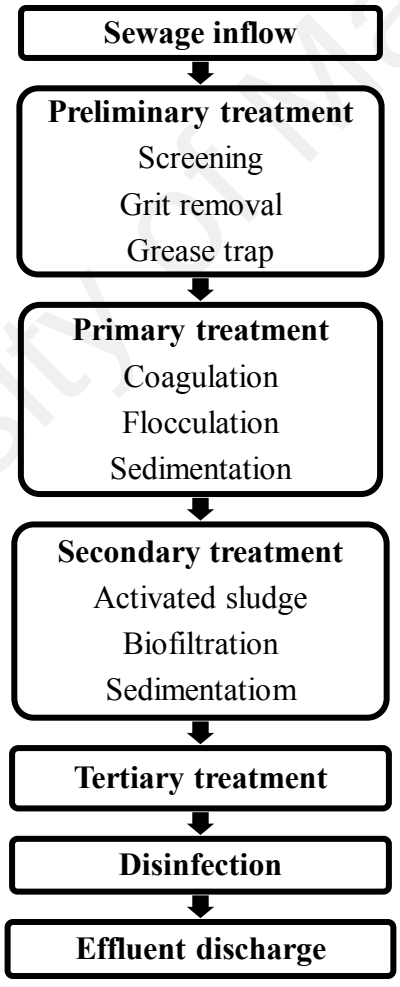



**Figure 2.2:** Flow diagram for wastewater treatment plants (WWTP).

Primary (physicochemical) treatment consisted of coagulation, flocculation and sedimentation process. Coagulation is the destabilization process of suspended solid by the addition of coagulant such as ferric chloride into wastewater (Amuda and Amoo, 2007). During flocculation, the destabilized suspended solid agglomerates into settleable flocs, and the flocs are then settled to the bottom of water by sedimentation process. In this treatment process, pharmaceuticals that are more likely to partition onto the suspended solid can exhibit higher removal efficiency. Consequently, the efficiency of the primary treatment process highly depends on the hydrophobicity of the pharmaceuticals. Unfortunately, some of the pharmaceuticals are polar. As a result, the removals of pharmaceuticals in physicochemical treatment are relatively poor, and this has been proven by various studies (Matamoros and Salvadó, 2013). In the real case study, Roberts et al. (2016) reported that the removal of carbamazepine, venlafaxine, atenolol, sotalol, propranolol, diphenhydramine and triclosan by the primary treatment of Australia's largest inland sewage treatment plant were ranged from -110.6 to 20.5%. A study from Taihu Lake in China showed that the effectiveness of primary treatment in the removal of NSAIDs and antibiotics was ranged from 10 – 40% (Lin et al., 2016). Yuan et al. (2015) showed the percentage removal of pharmaceuticals such as lomefloxacin, carbamazepine, norfloxacin, bezafibrate, atenolol, ofloxacin and tetracycline in WWTP was in range –25.9 to 58.1%.

After primary treatment, incompletely treated pharmaceuticals are exposed to the secondary treatment which is also called biological treatment. In this process, microorganisms in the form of activated sludge are mixed into the primary treated effluent. Microorganisms decomposed the biodegradable materials into more simple molecules. Studies have shown that the efficiency of conventional biological treatment in the removal of pharmaceuticals was relatively low. As reported by Roberts et al. (2016), removal of carbamazepine, metoprolol, fluoxetine, atenolol, sotalol, chlorpheniramine,

and propranolol by biological treatment was ranged from 0.81 to 74.7 %. Another study by Subedi and Kannan (2015) showed the removal percent of psychoactive pharmaceuticals in WWTP ranged from 0.3 to 87%. In the same study, lorazepam, carbamazepine, and norsertraline showed a negative removal in WWTP indicating the persistence of these pharmaceuticals toward biological treatment.

In the tertiary treatment, the remaining nitrogen and phosphorus in the wastewater will be removed before discharge to the environment (Feng et al., 2016). This process is essential because the excessive released of nutrients can encourage the growth of algae and weed which lead to the eutrophication process. To enhance the micropollutants removal various advanced tertiary treatment such as granular activated carbon (GAC) filter (Oneby et al., 2010; Yang et al., 2011) has been introduced. GAC is frequently used in water treatment plant for the removal of micropollutants (Kennedy et al., 2015). The bench scale experiment showed that pharmaceuticals such as clofibric acid, carbamazepine, naproxen and diclofenac were also poorly (< 60%) removed by GAC (Bo et al., 2016). In a real case study reported by Yang et al. (2011), various pharmaceuticals such as sulfamethoxazole, erythromycin, trimethoprim, ciprofloxacin, carbamazepine, primidone, and caffeine were still detected in the GAC effluent. Drinking water purification plant is treating the surface water where the water matrices are less complicated as compared with WWTP. However, pharmaceuticals were also detected in drinking water produced from water purification plant that consisted of chemical coagulation, sedimentation, rapid sand filtration, ozonation, GAC filtration, and chlorination (Simazaki et al., 2015). As a result, the remaining or incompletely treated pharmaceuticals are exposed to the oxidation reaction when it enters the chemical treatment and disinfection processes.

### 2.2.1 Conventional WWTP: Chlorination

One of the essential processes in water treatment plant is disinfection process. Disinfection process kills the pathogen, including harmful bacteria and viruses in water before releasing into the environment (Li et al., 2013). There are several types of disinfectants which have been used in water treatment. The most commonly used disinfectants are ozone, UV irradiation, and FAC. Among these disinfectants, FAC is one the most frequently used disinfectant in WWTP due to its high efficiency in the pathogen removed and cost effectiveness (Nam et al., 2015; Li and Zhang, 2012). It also controls taste, color, and odor of water (Xiang et al., 2016). Recently, FAC has been reported as the active oxidant which can react with various kinds of micropollutants, including pharmaceuticals waste (Khalit and Tay, 2016a; Li et al., 2013).

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \qquad \text{(Eq. 2.1)}$$

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad \text{(Eq. 2.2)}$$

Chlorine-based disinfectant is available in several forms such as chlorine gas and hypochlorite solution. When chlorine gas or hypochlorite salt is added to the water, hydrolysis, and ionization take place to form HOCl and $OCl^-$ (Eq 2.1). The mixture of HOCl and $OCl^-$ are classified as FAC. HOCl is a weak acid. Hence, it tends to ionize or dissociate to form $H^+$ and $OCl^-$ (Eq. 2.2). HOCl and $OCl^-$ have different disinfecting properties. HOCl has higher disinfectant properties due to its high oxidizing potential and smaller molecular size which are favorable to penetrate through bacterial cells. Meanwhile, $OCl^-$ is a weaker disinfectant as compared to HOCl because of its low oxidizing potential and negative charge which causes it difficult to penetrate through bacteria cells. After chlorination process, FAC can persist in the treated water. Hence, it is often crucial to reduce the chlorine residue. Chlorine residue may cause the detrimental effects on aquatic life in the receiving water (Bagchi and Kelley, 1991). Basch et al. (1971), reported that the presence of chlorine residue (0.014 to 0.029 mg/L) causes 50%

of the rainbow trout died within 96 hr (96-hr LC50). Therefore, to ensure the limit of FAC residue is below 0.01 mg $Cl_2$/L in the effluent of wastewater, the FAC is often scavenged using sulfur dioxide, sulfites, activated carbon, or $H_2O_2$ (Sathasivan et al., 2017). FAC is stable and capable of providing necessary residual protection in water distribution systems. For these reasons, chlorination has become a commonly used disinfection method for the foreseeable future, despite the formation of harmful by-products (Tian et al., 2014) such as halogenated trihalomethanes (Jiang et al., 2017), haloacetic acids (Roccaro et al., 2014) and haloacetonitriles (Xue et al., 2014).

## 2.3 Transformation By-products (TBPs) Formation during chlorination

Chlorination has been successfully used to control waterborne infectious diseases for more than a century. But, one major concern in the application of chlorination process is the formation of intermediates or by-products, which could be toxic (Jahan et al., 2008). In wastewater treatment plant, FAC reacts with several naturally occurring organic compounds and forms numbers of disinfection by-products (DBPs) with harmful long-term effects. A study by Becher (1999) showed that during chlorination of water that containing natural organic matter (NOM), a complex mixture of chlorinated DBPs was formed. So far, more than 300 different types of DBPs have been identified. Halogenated trihalomethanes (THMs), and haloacetic acids (HAA) are two major classes of DBPs which are commonly found in waters after chlorination. Organic nitrogen in wastewater tends to form nitrogen-containing DBPs in the effluent. According to Sathasivan et al. (2017), this nitrogen-containing DBPs are persistent. Examples of nitrogen-containing DBPs are haloacetonitriles (HAN) haloacetamides, halonitromethanes, and nitrosamines (Chu et al., 2010; Krasner et al., 2006). These DBPs shows higher cytotoxicity and genotoxicity properties than regulated THM and HAA (Lipscomb et al., 2008; Muellner et al., 2007).

During chlorination process, oxidation or substitution reactions of pharmaceuticals can lead to the formation variety of transformation by-products (TBPs) with different toxicity (Negreira et al., 2015). El Najjar et al. (2013) performed a bioassay using *Vibrio fisheri* to study the variation of toxicity of water sample during chlorination of levofloxacin (LVF). The results showed increased toxicity after chlorination of LVF. They suggested that the TBPs formed were more toxic than its parent compound. Chlorination of methotrexate (MTX) was also found to lead to the formations of several TBPs (Yin et al., 2017b). MTX is one of the most common cytotoxic drugs, which has been widely used in the therapy of solid tumors and leukemias since the 1940s (Farber et al., 1948). During chlorination of MTX, 4-amino-3-chlorinated-N 10-methylpteroylglutamic (monochloro-MTX) and 4-amino-3,5-dichloro-N 10-methylpteroylglutamic (dichloro-MTX) were detected (Figure 2.3). The effects of MTX and monochloro-MTX on the cell cycle progression of the zebrafish liver cell line were evaluated by Yin et al. (2017b). The results showed that these TBPs inhibited the proliferation of zebrafish liver cells. Bedner and MacCrehan (2006) reported that during chlorination of acetaminophen several TBPs formed, such as 1,4-benzoquinone and N-acetyl-p-benzoquinone imine (NAPQI) and two ring chlorination products, chloro-4-acetamidophenol and dichloro-4-acetamidophenon (Figure 2.4). The toxicity study of 1,4-Benzoquinone and NAPQI showed that the Lethal Dosage (LD50) values of 1,4-Benzoquinone and NAPQI were 8.5 mg/kg and 20 mg/ kg, respectively. These values indicate that 1,4-Benzoquinone and NAPQI are much more toxic than acetaminophen (500 mg/kg). Hence, identification of TBPs are important, and further study needs to be done so that environment can be protected from these undesired byproducts.

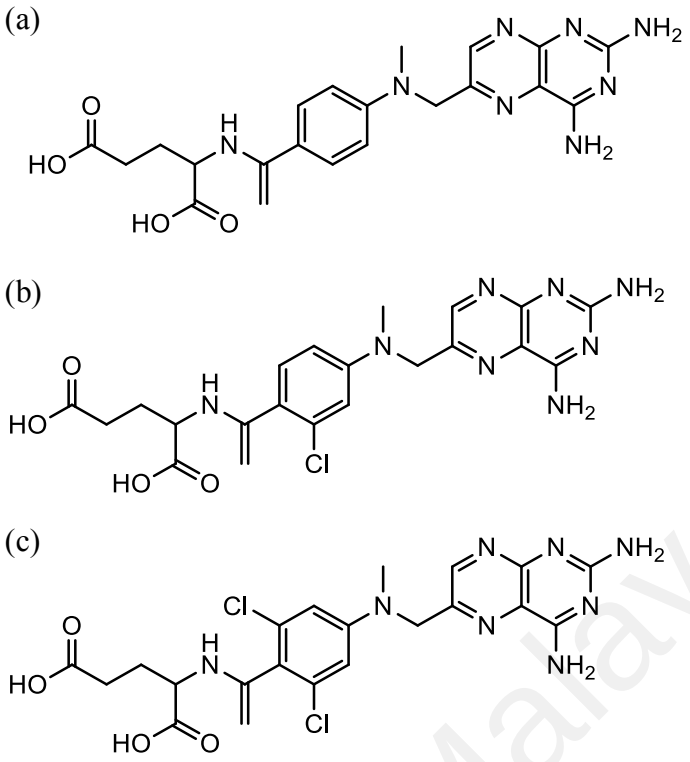



**Figure 2.3:** The chemical structure of (a) MTX, (b) monochloro-MTX and (c) dichloro-MTX (Yin et al., 2017b).

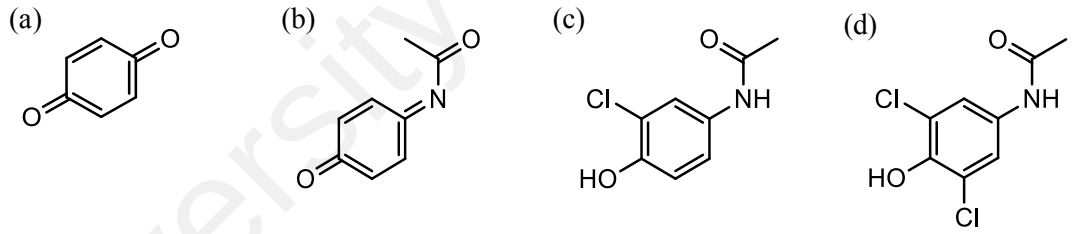



**Figure 2.4:** The chemical structure of (a) 1,4-benzoquinone (b) NAPQI, (c) chloro-4-acetamidophenol and (d) dichloro-4-acetamidophenon (Bedner and MacCrehan, 2006).

## 2.4 Advanced oxidation process (AOP) in water treatment

AOP has been defined as ambient temperature processes which involve the generation of highly reactive radicals, especially the •OH in sufficient quantity for efficient water purification (Glaze et al., 1987). AOP are highly efficient methods that accelerate the oxidation process and degrade a wide range of organic and inorganic substances that presence in water (Joseph et al., 2009). Primo et al. (2008) reported the application of AOP in the treatment of landfill leachate in northern Spain. This study showed that AOP

removed the recalcitrant organic matter and color of landfill leachates. A case study in China also showed that berberine containing wastewater was successfully treated with AOP (Cui et al., 2015). Figure 2.5 shows the classification of AOP.

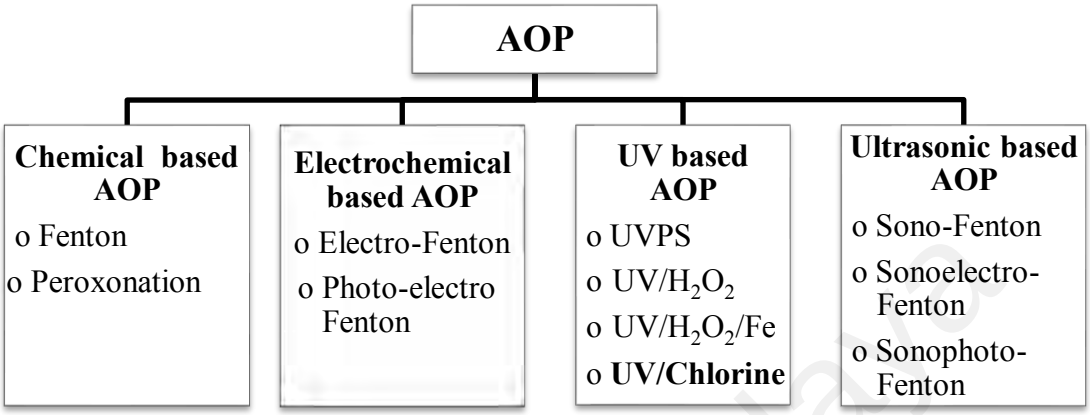



**Figure 2.5:** Classification of AOP.

Fenton oxidation is one of the oldest and the commonly used methods in the chemical oxidation process in water treatment (Flox et al., 2006). This method used Fenton's reagent, which is a mixture of soluble Fe(II) salt and $H_2O_2$ to generate •OH for destroying micropollutants (Andreozzi et al., 1999; Gogate and Pandit, 2004). Eq. 2.3 shows a catalytic reaction based on electron transfer between Fe(II) and $H_2O_2$ during Fenton process to produce •OH. •OH is a strong oxidant with the non-selective property, and it can oxidize a broad spectrum of compounds. Hence, this method is often found to be efficient in the destruction of toxic wastes and non-biodegradable compounds (Chen and Pignatello, 1997). Unfortunately, during Fenton reaction, other reaction such as hydroperoxyl radicals (•$O_2H$) formation (Eq. 2.4) and decomposition of $H_2O_2$ reaction (Eq. 2.5) also occurred. These two reactions become a major drawback for Fenton process because part of $H_2O_2$ consumed •OH and reduce the amount of •OH for oxidation process (Eq. 2.4). Also, a significant amount of oxidant is often required during Fenton process because $H_2O_2$ decomposed continuously to molecular oxygen and water as shown in Eq. 2.5. However, several studies have significantly improved the oxidation efficiency of

Fenton reaction by combining it with electrochemical reaction (Brillas et al., 2010; Oturan et al., 2009), sonolysis techniques or photolysis reaction.

$$Fe^{2+} + H_2O_2 \rightarrow \bullet OH + Fe^{3+} + HO^- \quad \text{(Eq. 2.3)}$$

$$\bullet OH + H_2O_2 \rightarrow \bullet O_2H + H_2O \quad \text{(Eq. 2.4)}$$

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad \text{(Eq. 2.5)}$$

Electro-Fenton process (EF) was the first method proposed for electrochemical-based AOP (Oturan and Aaron, 2014). This indirect electro-oxidation method has more advantages as compared to conventional Fenton reaction. The significant benefits of EF are higher degradation rate of organic pollutants because of the continuous formation of $Fe^{2+}$, on-site production of $H_2O_2$ to avoid risk during chemical transportation, storage and handling (Oturan et al., 1999; Oturan, 2000). However, electro-Fenton processes have some problems concerning $H_2O_2$ production. The production of $H_2O_2$ is slow because oxygen has low solubility in water and the current efficiency under reduced pH ($pH < 3$) is low (Ting et al., 2008).

Ultrasonic based AOP consists of the combinations of Fenton with several types of sonolysis technique, such as the sono-Fenton technique, sonoelectro-Fenton and sonophoto-Fenton (Sathishkumar et al., 2016). According to Afonso-Olivares et al. (2016), these processes are safe, clean and versatile. Besides, the ability of ultrasound waves to correctly transmitted through opaque water systems as compared to ultraviolet light makes sonochemical AOP as one of promising water treatment in recent years. But, these techniques are extremely susceptible and vulnerable to the operational parameter and need to be controlled by someone with good knowledge and understanding of the physical and chemical phenomena involved (Ince et al., 2001).

The UV based AOP technologies are simple, clean, relatively inexpensive method for chemical activation in AOP (Oturan and Aaron, 2014). UV irradiation has been widely

used for disinfection process. Consequently, UV irradiation has been coupled with powerful oxidants such as $H_2O_2$, (Lu et al., 2018; Shi et al., 2018) and various catalyst such as $Fe^{3+}$ and $TiO_2$ (Alvarez-Corena et al., 2016), to generate •OH for AOP. Several studies have shown the effectiveness of UV/ $H_2O_2$ in the removal of micropollutants such as dyes and pharmaceuticals (Autin et al., 2013; Jung et al., 2012; Lutterbeck et al., 2015). As reported by de Souza Santos et al. (2015), the percentage removal of 670 ppm of norfloxacin during UV/ $H_2O_2$ process was 97% with the presence of 450 ppm of $H_2O_2$. Another study by Jung et al. (2012) showed the complete removal of antibacterial activity when 0.1 mM of amoxicillin was exposed with UV in the presence of 10 mM of $H_2O_2$ for 20 min. However, a drawback of this AOP method is the weak molar absorption coefficient of $H_2O_2$ in the UV region, and, consequently, it is necessary to use a high concentration of $H_2O_2$ for efficient oxidation of organic pollutants (Galindo et al., 2000). Also, when the concentration of $H_2O_2$ above the optimum value, $H_2O_2$ tends to accumulate in water and act as a radical scavenger (Glaze et al., 1987) and hence, it suppressed the removal of organic compounds during water treatment (Balcıoğlu and Ötker, 2003). Therefore, replacing $H_2O_2$ is necessary.

By replacing $H_2O_2$ with FAC, UV/chlorination has been reported as one of the efficient processes in removal of micro-pollutant (Wu et al., 2017) such as hormone and pharmaceuticals in water (Sichel et al., 2011; Nam et al., 2015; Xiang et al., 2016). As reported by Zhu et al. (2018), UV/chlorination showed the higher removal of 10 µM of phenacetin as compared to other UV based AOP, such as UV/$H_2O_2$ and UV/persulfate (UV/PS). The removal of phenacetin through UV/chlorination process is 95.7%. Meanwhile, during UV/$H_2O_2$ and UV/PS process, the percentage of removal of phenacetin is only 27.5 and 21.0%, respectively. A study by Xiang et al. (2016), showed that the rate constant for the degradation of ibuprofen during UV/chlorination was $3.1 \times 10^{-3}$ $s^{-1}$ and during UV/$H_2O_2$ process, the rate constant was found to be 3.3 times slower.

This result shows the degradation of ibuprofen during UV/chlorination is faster than UV/$H_2O_2$ process. According to Dong et al. (2017), the rate of degradation of chloramphenicol was also higher during UV/chlorination as compared to UV/$H_2O_2$ process. They concluded that this is due to the higher UV quantum yields of •OH during UV/chlorination process. Also, the •OH scavenging rate during the degradation of chloramphenicol using UV/chlorination was much lower than UV/$H_2O_2$ (Watts and Linden, 2007). Therefore, the radicals generated can effectively contribute to chloramphenicol degradation.

UV/chlorination is one of the advanced oxidation process, which is a combination of FAC and UV to enhance the degradation of pollutants in water. Recently, studies show the effectiveness of this process in degradation of pollutants, especially in the removal of pharmaceuticals. Several studies have demonstrated the effectiveness of UV/chlorination process compare to UV and chlorination process during degradation of pharmaceuticals. As reported by Lu et al. (2018) during UV/chlorination and UV irradiation process of clofibric acid, the percentage removal was 85.5% and 55.2%, respectively. Meanwhile, during chlorination, only 5.5% removal of clofibric acid was achieved in 5 min. A study by Nam et al. (2015) showed the same observation, chlorination alone just removed 2% of metoprolol in 4 h. Meanwhile, during UV and UV/chlorination process manage to remove 19.0% and 99.9% of metoprolol, respectively. Wang et al. (2016a) reported that only 0.1% and 5.5% of carbamazepine was removed using UV and chlorination process, respectively. However, UV/chlorination showed the highest removal efficiency with 98.0% removal of carbamazepine within 5 min.

### 2.4.1 The Chemistry of UV/chlorination Process

During UV/chlorination process, UV photolysis of FAC produces reactive species such as •OH and •Cl (Wolfe, 1990). •OH is a non-selective radical and it reacts with a variety of contaminants at nearly diffusion-controlled rates, whereas, •Cl is a selective oxidant which is preferable to react with the aromatic ring and electron rich moieties of organic compounds (Mártire et al., 2001; Fang et al., 2014; NIST, 2016). Others reactive species produces during UV/chlorination reaction are $\bullet Cl_2^-$ and •ClO. These two radicals also react selectively with organic matter (NIST, 2016). $\bullet Cl_2^-$ is formed from the reaction between •Cl and chloride ion (Kläning and Wolff, 1985), meanwhile, •ClO is produced when •Cl and •OH react with HOCl or $OCl^-$ as shown in Table 2.3 (Jayson et al., 1973; Matthew and Anastasio, 2006; Zehavi and Rabani, 1972).

**Table 2.3**: Principle reactions during UV/chlorination.

| No. | Reaction | Rate constant ($M^{-1} s^{-1}$) | References |
|---|---|---|---|
| 1 | $HOCl + h\nu \rightarrow \cdot OH + \cdot Cl$ | $k_1 = (1.3 \pm 0.3) \times 10^{-3}$ | Qin et al., (2014) |
| 2 | $OCl^- + h\nu \rightarrow \cdot OH +$ other products | $k_2 = (9.0 \pm 0.2) \times 10^{-9}$ | Qin et al., (2014) |
| 3 | $\cdot Cl + Cl^- \rightarrow \cdot Cl_2^-$ | $k_3 = 6.5 \times 10^{-9}$ | Jayson et al., (1973) |
| 4 | $HOCl + \cdot OH \rightarrow \cdot ClO + H_2O$ | $k_4 = 2.0 \times 10^{-9}$ | Watts and Linden, (2007) |
| 5 | $HOCl + \cdot Cl \rightarrow \cdot ClO + Cl^- + H^+$ | $k_5 = 3.0 \times 10^{-9}$ | Deng et al., (2014) |
| 6 | $OCl^- + \cdot OH \rightarrow \cdot ClO + OH^-$ | $k_6 = 8.8 \times 10^{-9}$ | Deng et al., (2014) |
| 7 | $OCl^- + \cdot Cl \rightarrow \cdot ClO + Cl^-$ | $k_7 = 8.2 \times 10^{-9}$ | Jayson et al., (1973) |

The simultaneous formation of •OH and reactive chlorine species (RCS) •Cl, $\bullet Cl_2^-$ and •ClO) is an added advantage of UV/chlorination as a complementary solution for the degradation of a wide range of micropollutants as compared with conventional AOP. Previous studies have reported that •OH was the predominant radical in the degradation of ibuprofen (Xiang et al., 2016) and chloramphenicol (Qin et al., 2014) using UV/chlorination. The second-order rate constant ($k_{app}$) •OH and for the reaction between

ibuprofen and chloramphenicol were reported as $(7.4 \pm 1.2) \times 10^{-9}$ $M^{-1}$ $s^{-1}$ and $2.0 \times 10^{-9}$ $M^{-1}$ $s^{-1}$, respectively. Meanwhile, RSC was found to play an essential role in the degradation of trimethoprim (Wu et al., 2016) and benzoic acid (Fang et al., 2014). According to Guo et al. (2017), during degradation of micropollutants, •OH and RCS are expected to react differently towards organic compound, and this process is depending on the chemical structures of the organic compounds. Recently, it was revealed that •ClO also played an essential role in the degradation of gemfibrozil and bezafibrate using UV/chlorination process with $k_{app}$ of $(4.2 \pm 0.3) \times 10^{-8}$ $M^{-1}$ $s^{-1}$and $(3.6 \pm 0.1) \times 10^{-1}$ $M^{-1}$ $s^{-1}$, respectively (Kong et al., 2018). According to Zhao et al. (2011), the unreacted FAC during UV/chlorination process provides protection to water distribution system against pathogen. The finding showed the added advantage of UV/chlorination in water treatment as compared to $H_2O_2$ based AOP which often leave the unreacted toxic $H_2O_2$ after water treatment (Ishak et al., 2017).

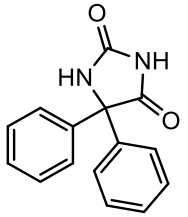
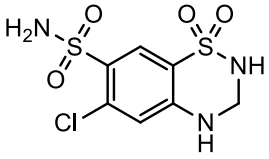
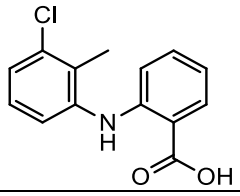
pH and FAC dosage are two essential operating parameters that influence the efficiency of UV/chlorination in degradation of micropollutants (Deng et al., 2014; Fang et al., 2014; Wang et al., 2012). Previous studied showed that the degradation rate of pharmaceuticals was significantly enhanced under acidic conditions (pH < 6.5). Meanwhile, under alkaline conditions, the degradation rate of pharmaceuticals was found to be inhibited (Nam et al., 2015; Wang et al., 2016a; Wang et al., 2016b; Xiang et al., 2016). According to Nowell and Hoigne (1992), HOCl yields more •OH as compared to $OCl^-$. Hence, in an acidic condition, the rate of degradation of pharmaceuticals is much higher than the basic condition due to the higher molar fraction of HOCl. Meanwhile, under alkaline conditions, the radical scavenging rate is also higher due to the presence of $OCl^-$ (Fang et al., 2014). Wang et al. (2012) stated that the rate constant of •OH scavenging by $OCl^-$ is higher than HOCl.

Several studies have proven that the rate of degradation of pharmaceuticals increased with increasing FAC (Guo et al., 2016; Kong et al., 2018; Wang et al., 2016a; Wang et al., 2016b). According to Zhu et al. (2018), when FAC dosage increased from 100 to 300 μM, the rate of degradation of phenacetin was found to increase due to the formation of the higher amount of oxidative radicals, such as •OH and •Cl. However, the rate of degradation was found to increased less rapidly at 400 μM showing the scavenging effects of •OH and •Cl by FAC as shown in Table 2.3 (Reaction 4-7). A study by Dong et al. (2017), the rate of degradation of chloramphenicol was found to increased linearly as the FAC dosage increased from 0.25 to 1.0 mM. However, as the FAC dosage beyond 1.0 mM, FAC was found to lower the rate of degradation of chloramphenicol. Therefore, it is important to study the effects of pH and FAC dosage on the degradation of micropollutants to achieve the highest efficiency of UV/chlorination in pollutants treatment.

## 2.5 Background of Selected Pharmaceuticals

In this study, DPH, HCTZ and TOL were selected for this study. These three pharmaceuticals were frequently detected in the effluent of WWTPs and the aquatic environment worldwide (Bueno et al., 2012; Huerta-Fontela et al., 2011; Kim et al., 2014; Oosterhuis et al., 2013; Vanderford and Snyder, 2006; Zuccato et al., 2005). The presence of these compounds in the environment indicating these selected pharmaceuticals are poorly removed by currently available WWTP. Table 2.4 shows the structure and the chemical properties of the selected pharmaceuticals.

**Table 2.4**: The chemical properties and structures of selected pharmaceuticals.

| Compound | Structure | Chemical formula | $pK_a$ | Water solubility |
|---|---|---|---|---|
| 5,5-Diphenylhydantoin (DPH) |  | $C_{15}H_{12}N_2O_2$ | 8.3 [a] | 20 mg/L [d] |
| Hydrochlorothiazide (HCTZ) |  | $C_7H_8ClN_3O_4S_2$ | 8; 9.5 [b] | 980 mg/L [e] |
| Tolfenamic acid (TOL) |  | $C_{14}H_{12}ClNO_2$ | 4.3 [c] | Not soluble [f] |

[a] Agarwal and Blake, 1968; [b] Parmar et al., 2014; [c] Pentikäinen et al., 1982; [d] Varia et al., 1984; [e] Kadam et al., 2011; [f] Ahmed et al., 2015

### 2.5.1 DPH

DPH is a non-sedative antiepileptic drug, which has been widely used to treat epilepsy, decrease the excitatory neurotransmission and enhance the γ-aminobutyric acid-mediated inhibition (Luchian et al., 2015). DPH is a highly protein-bound drug, which is metabolized by the cytochrome P450 enzyme in the liver and secreted by the kidneys (De Schoenmakere et al., 2005). DPH has been detected in various water samples such as river water and the effluents of wastewater treatment plant with the concentration ranging from 56 to 78.5 ng/L (Dong et al., 2015; Huerta-Fontela et al., 2011). The ecotoxicity of DPH is seldom reported. However, DPH was found to produce reproductive and embryotoxic effects in mice. DPH was also found to cause hypoalbuminemia and renal failure in the human body (Imam et al., 2014; NTP, 1993).

### 2.5.2 HCTZ

HCTZ is one of the thiazides which acts as the antihypertensive drug, and it has been widely prescribed for the management of edema and used in the treatment of hypertension (Bucher et al.,1990; Ranjan et al., 2017). Diuretics are primarily used to modify the volume and the composition of body fluids and commonly used to treat high blood pressure. Thiazide drugs inhibit sodium reabsorption in the early distal convoluted tubule, hence lower the blood pressure reading. According to Razak (2004), HCTZ is not metabolized and excreted unchanged in urine. Hence, HCTZ is frequently detected in surface water and WWTP effluent with concentration ranging from 91.0 to 255.8 ng/L (Estrada-Arriaga et al., 2016; Kim et al., 2014; Oosterhuis et al., 2013). So far, ecotoxicity of HCTZ has not been reported elsewhere.

### 2.5.3 TOL

TOL is one of the nonsteroidal anti-inflammatory drugs (NSAIDs) which is effective in reducing acute migraine attack (Hakkarainen et al., 1979). In general, NSAIDs are used to relieve pain, and to suppress inflammation in the way that similar to steroids, but without steroids side effects. According to Roberts and Morrow (2001), NSAIDs also acts as painkiller which is effective against low-intensity or moderate-intensity pain. Their antipyretic activity reduces the body temperature in febrile states, but their main clinical application is as anti-inflammatory agents in the treatment of musculoskeletal disorders, such as rheumatoid arthritis and osteoarthritis. TOL is fenamate family and has a diphenylamine structure typically found in emerging contaminants (Venkataraman et al., 2014). Recently, TOL was detected in hospital effluent and river water with concentration ranging from 311 to 462 μg/L (Guan et al., 2016). Regarding toxicity, tolfenamic acid is known to cause upper gastrointestinal side effects as compared to other NSAIDs (Hansen and Pedersen, 1986). Zhang et al. (2013) reported that TOL inhibited

tumorigenesis in mice and lead to induce anti-cancer effects which mediated by various cellular mechanisms (Jeong et al., 2013; Lee et al., 2008; Lee et al., 2010).

# CHAPTER 3: RESEARCH METHODOLOGY

## 3.1 Materials and Stock Solutions

DPH (99%), HCTZ (98%) and TOL (99%) were purchased from Alfa Aesar (England). Sodium dihydrogen phosphate monohydrate, di-sodium hydrogen phosphate dihydrate, formic acid (98-100%), acetic acid (100%) and sulfuric acid (95-97%) were purchased from Merck (Germany). Ascorbic acid (99%) was obtained from Acros Organics (China), and BSTFA and TMCS (99:1) were obtained from Supelco Analytical (USA). NaOCl solution with available chlorine 4.00-4.99% and sodium thiosulfate ($Na_2S_2O_3$) were obtained from Sigma-Aldrich (USA). HPLC grade acetonitrile and methanol were obtained from RCI Lab Solution (Thailand), and ammonium hydroxide solution > 25% in water was purchased from Fluka (USA). Starch was obtained from BDH Chemicals (England). DPH and HCTZ stock solution was prepared by dissolving an appropriate amount of DPH and HCTZ in methanol and ultrapure water (UK) with the ratio 9:1. Meanwhile, TOL stock solution was prepared by dissolving it in acetonitrile. The stock solution of NaOCl was prepared daily by diluting it with ultrapure deionized water. Standardization for the concentration of FAC stock solution was performed using iodometric method (Adam and Gordon, 1995).

## 3.2 Concentration of Free Available Chlorine (FAC)

Iodometric titration method was used for the determination of FAC in the NaOCl solution (Adam and Gordon, 1995). 5 mL of NaOCl solution was diluted to 100 mL using ultrapure deionized water. In a conical flask, 10 mL of diluted NaOCl solution, 20 mL of concentrated potassium iodide, 50 mL of 10% sulfuric acid and 100 mL of deionized water were added. Then, the mixture was titrated with $Na_2S_2O_3$ until the pale yellow solution was obtained. The starch solution was added to the mixture as an indicator, and the mixture was quickly titrated with the $Na_2S_2O_3$ solution until the color turned to colorless. FAC concentration was determined in triplicate.

## 3.3 Kinetic Experiments

Chlorination was carried out according to the previous study (Khalit and Tay, 2016b) with slight modification. Chlorination of selected pharmaceuticals was carried out in a 50 mL jacketed beaker, and the temperature was controlled at 25 ± 1 °C. All chlorination experiments were conducted under pseudo-first order kinetics condition where the concentration of FAC was at least 50 times higher than the concentration of selected pharmaceuticals. The pH of the solution was controlled at 5 to 8 by using phosphate buffer (50 mM). Chlorination was initiated by adding an aliquot of NaOCl solution to the selected pharmaceuticals solution. At the defined time interval, 1 mL of the reaction mixture was rapidly transferred into an HPLC vial that containing the ascorbic acid solution.

For UV/chlorination, UV lamp with the wavelength of 254 nm (Tank Master™ Ultraviolet Liquid Storage Sanitizer, Atlantic Ultraviolet) was inserted in the middle of 100 mL jacketed beaker that containing the solution of selected pharmaceuticals to ensure the samples were exposed to UV light and the temperature was maintained at 25 ± 1 °C using a circulating water bath. As chlorination, the reaction was started by adding an aliquot of NaOCl solution and by turning on the UV lamp. Ascorbic acid was used to quench the reaction. The concentration of selected pharmaceuticals was monitored using HPLC. At least two replications of each experiment were carried out, and more replications were executed in cases where the variation between each measurement of concentration exceeded 5%.

## 3.4 Chlorination and UV/chlorination of Selected Pharmaceuticals in Lake Water and Tap Water

To simulate the real water treatment conditions, experiments were performed using lake water and tap water obtained around Kuala Lumpur (Malaysia). For chlorination process,

each water sample was spiked with selected pharmaceuticals at 1 μM concentration and transferred into 20 mL vial. Then, FAC solution ranging from 20 to 60 μM were added into the vials. The vials were shaken vigorously after the addition of FAC solution for 24 h. For UV/chlorination process, each water sample was spiked with 20 to 60 μM of selected pharmaceuticals and exposed to UV light for 6 min. All sample was measured by HPLC, and the experiments were carried out at room temperature (25 - 30 °C).

### 3.5 Determination of Transformation by-products (TBPs)

For the identification of the TBPs of selected pharmaceuticals, 99 μM of DPH, 90 μM of HCTZ and 20 μM of TOL solutions were treated with chlorination, UV irradiation and UV/chlorination at pH 6, 7, and 8. The molar ratios of FAC to selected pharmaceuticals were kept at 0.5:1, 1:1, and 2:1. In chlorination process, the samples were shaken for 24 h. For UV irradiation and UV/chlorination process, the sample was exposed to UV light for 10 min. The TBPs of DPH were extracted using dichloromethane via liquid-liquid extraction method. The extracts were silylated using BSTFA and TMSCl (99:1) mixture for 4 h at 70 °C. Silylated extracts were dried using nitrogen stream and re-dissolved in 100 μL of dichloromethane. A 1.0 μL aliquot of the solution was injected to GC-MS.

The TBPs of HCTZ was extracted using Solid phase extraction (SPE) (Tay et al., 2009). SPE cartridge (LiChrolut SCX, 40-63 μm, 200 mg for 3 mL and PP-tubes) was condition with 6 mL of methanol and equilibrated with acidified water (pH 2). After conditioning and equilibrating, the acidified samples (pH 2) were loaded into SPE cartridge at the consistent flow rate of ~1-2 drops/second. Then, the SPE cartridge was rinsed with deionized water. The TBPs and its parent compound were eluted using 5% ammonium hydroxide in methanol before analyses using LC-QqQ-MS analysis. TBPs of TOL were analyzed directly using LC-MS without pre-concentration.

### 3.6 Determination of the ecotoxicity of selected pharmaceuticals after chlorination and UV/chlorination treatments

In this study, *E. Coli* suspension was prepared using Epower™ microorganism pellet (USA). Each pellet contains 4.3 x $10^3$ colony forming units (CFU). For the preparation, microorganism pellet was transferred using forceps to the phosphate buffer solution with pH 7.2 in a vial. The vial was incubated at 34 – 38 ºC for 30 min to assure complete hydration. Then, the vial was vortexed to obtain a homogeneous suspension. 1 mL of the *E. Coli* suspension was then added to a Total Bacteria broth tube. The tube was incubated at 35 ºC for 12 h. This *E. Coli* suspension was used for ecotoxicity determination.

Toxicity of selected pharmaceuticals solution before and after treating with chlorination and UV/chlorination was evaluated using commercially available ToxTrak™ test kit (Hach, 2014). This toxicity test is based on the colorimetric method which measured the rate of blue color resazurin, a redox-active dye, reduced to pink color resorufin by the *E. Coli* respiration (Gmurek et al., 2015). The presence of toxic substances reduces the rate of resazurin reduction. The change in absorbance was measured using GENESYS 10S UV-Vis spectrophotometer (USA) at the wavelength of 603 nm as suggested by Hach (2014).

For the colorimetric test, one ToxTrak Reagent Powder Pillow was added to an empty vial. 5.0 mL of deionized water and 5 mL of sample was added. Then, two drops of Accelerator Solution were added followed by 5 mL of the inoculum. The vials were inverted several times to homogenize the mixture. The absorbance of control and sample were recorded. After the absorbance of control decreased by 0.60 (± 0.10) nm (~45 min), the absorbance of the sample was recorded. The values of absorbance were used to calculate the percent inhibition of samples by using Eq. 3.1.

$$\%\text{Inhibition} = \left[1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100\right] \qquad \text{(Eq. 3.1)}$$

## 3.7 Instrumental Analysis

### 3.7.1 High Performance Liquid Chromatography (HPLC)

HPLC was used to monitor the concentration of selected pharmaceuticals in kinetics study. HPLC (Shimadzu, Japan) system used in this study 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 (10 mm×4.6 mm; Merck, Germany) and a reversed-phase Supelco Ascentis C18 column (150 mm×4.6 mm; Sigma-Aldrich, USA) was used for separation. The flow rate of HPLC was 1 mL/min. The solvent program and detection wavelength of HPLC for each selected pharmaceutical are shown in Table 3.1.

**Table 3.1:** HPLC solvent program and detection wavelength for selected pharmaceuticals.

| Compound | Solvent Program | Detection wavelength (nm) | Column |
|---|---|---|---|
| DPH | Isocratic with 20A:80B | 202 | Chromolith RP-18 |
| HCTZ | Isocratic with 25A:75B | 271 | Supelco Ascentis C18 |
| TOL | Isocratic with 78A:22B | 282 | Supelco Ascentis C18 |

*A = acetonitrile; B = 0.1% formic acid

### 3.7.2 Triple Quadrupole liquid chromatography mass spectrometer (LC-QqQ-MS)

The Agilent 6490 LC-QqQ-MS equipped with electrospray ionization (ESI) was used to analyze the TBPs of HCTZ and TOL. A reversed-phase InertSustain C18 (3 μM, 2.1 × 150 mm; GL Science, Japan) was used for separation. The column temperature was set at 35 $^{o}$C, and the flow rate at 0.2 mL/min. For MS, the nitrogen gas temperature was 200 $^{o}$C and 14 L/min. The nebulizer pressure was 45 psi with sheath gas temperature of 300 $^{o}$C

and the flow rate of 11 L/min. The capillary voltage was set at 3.0 kV and chamber current at 0.73 µA. The solvent programs and polarity of LC-QqQ-MS are shown in Table 3.2.

**Table 3.2**: LC-QqQ-MS solvent programs and polarity.

| Compound | Solvent Program | Polarity | Column |
|---|---|---|---|
| HCTZ | Isocratic with 25C:75D | Negative | InertSustain C18 |
| TOL | Isocratic with 78A:22B | Positive | InertSustain C18 |

*A = acetonitrile; B = 0.1% formic acid; C = methanol:water (80 : 20, v/v) ; D = 0.1% acetic acid

### 3.7.3 Gas Chromatography Mass Spectrometry (GC-MS)

Shimadzu gas chromatograph-mass spectrometry (GCMSQP 2010 Plus) equipped with an Rtx-5MS capillary column (30 m × 0.25 mm I.D., 0.25-µm film thickness, Restek) was used to analyze the TBPs of DPH. Helium (purity 99.99%) with an average velocity of 40 cm/s was used as the carrier gas. The GC oven temperature program was as follows: isothermal at 60 °C for 2 min, 60–150 °C at 30 °C/min, then 150–310 °C at 4 °C/min, and hold at 310 °C for 10 min. The injection port and transfer line were maintained at 300 and 310 °C, respectively. The mass spectrometry data were acquired in the electron impact mode (70 eV) over the mass range of 50–600 Da. The ion source temperature was 220 °C.

# CHAPTER 4: RESULT AND DISCUSSION

## 4.1 Kinetics of the chlorination of selected pharmaceuticals

The rate of reaction of the individual organic compound such as pharmaceuticals with FAC in water is a second-order reaction (Deborde and Von Gunten, 2008), first-order in FAC and first order in the organic compound as shown in Eq. 4.1:

$$-\frac{d[\text{phar}]}{dt} = k_{\text{app}}[\text{phar}]_t[\text{FAC}]_t \qquad \text{(Eq. 4.1)}$$

where $[\text{phar}]_t$ is the concentrations of selected pharmaceuticals, $[\text{FAC}]_t$ refers to the concentration of FAC and $t$ is the reaction time. In this study, chlorination was performed using pseudo-first order reaction condition where the concentration of FAC was ($[\text{FAC}]_0$) kept at 50 times higher than the of selected pharmaceuticals. Under this condition, the concentration of FAC was found to remain unchanged throughout the study. Therefore, Eq. 4.1 was simplified as Eq. 4.2:

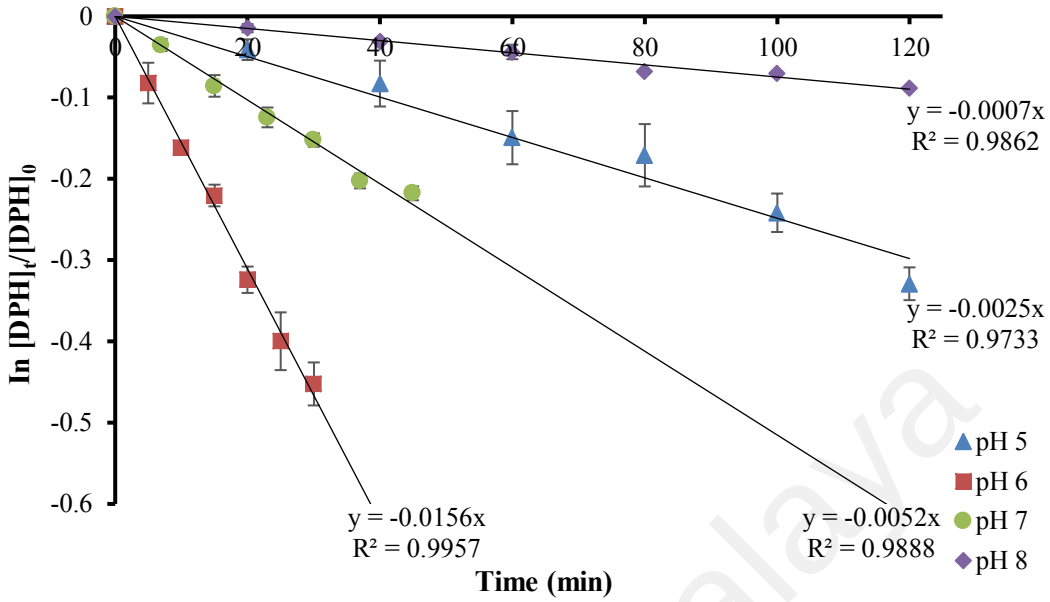
$$-\frac{\text{d}[\text{phar}]_t}{\text{dt}} = k_{\text{obs}}[\text{phar}]_t \qquad \text{(Eq. 4.2)}$$

The pseudo-first-order rate constant ($k_{\text{obs}}$) represents $k_{\text{app}} \times [\text{FAC}]_0$. By integrating Eq. 4.2, Eq. 4.3 was obtained.

$$\text{In}\frac{[\text{phar}]_t}{[\text{phar}]_0} = -k_{\text{obs}}t \qquad \text{(Eq. 4.3)}$$

where $[\text{phar}]_0$ represents the initial concentration of selected pharmaceuticals. Based on Eq. 4.3, the $k_{\text{obs}}$ values can be determined by plotting $\text{In}\frac{[\text{phar}]_t}{[\text{phar}]_0}$ against $t$. Figure 4.1 shows a linear plot for $\text{In}\frac{[\text{phar}]_t}{[\text{phar}]_0}$ versus $t$ determined at different pHs and the coefficient of determination ($R^2$) values for all selected pharmaceuticals were ranged from 0.9733 to 0.9972. This result further confirmed the degradation of selected pharmaceuticals proceeded via pseudo-first- order condition.

(a) DPH




(b) HCTZ

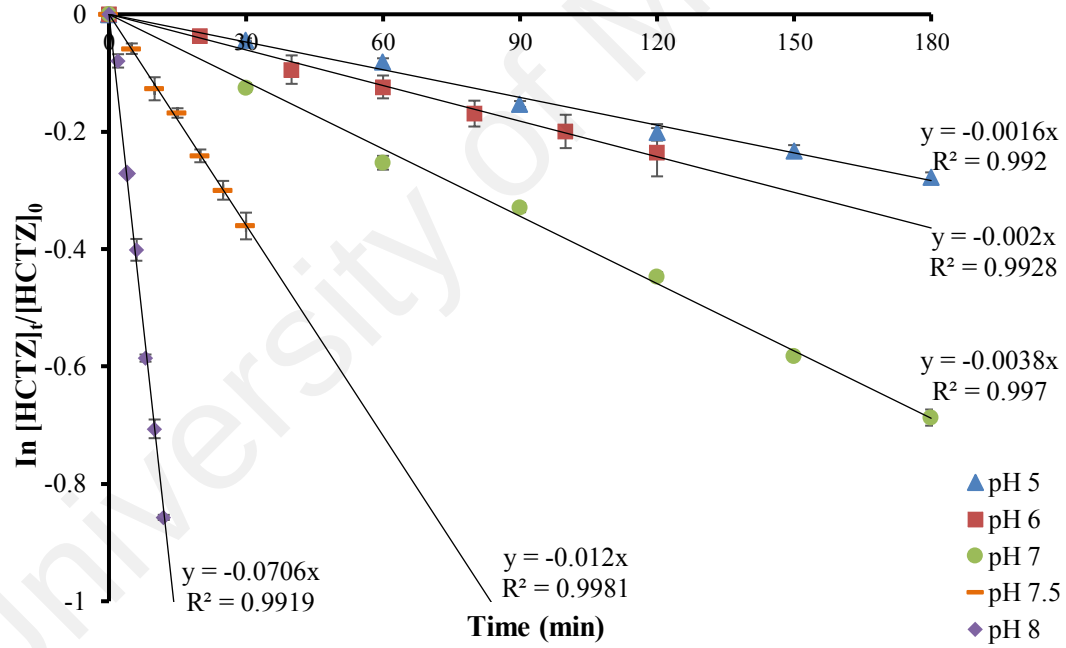



**Figure 4.1:** Pseudo-first-order kinetics plot for the degradation of selected pharmaceuticals via chlorination at pH 5 to 8. ([T = 25±0.1 °C, $[DPH]_0$ = 19.8 µM, $[FAC]_0$= 990 µM] $[HCTZ]_0$ = 20 µM, $[FAC]_0$= 1000 µM] $[TOL]_0$ = 20 µM, $[FAC]_0$= 1000 µM]).

(c) TOL

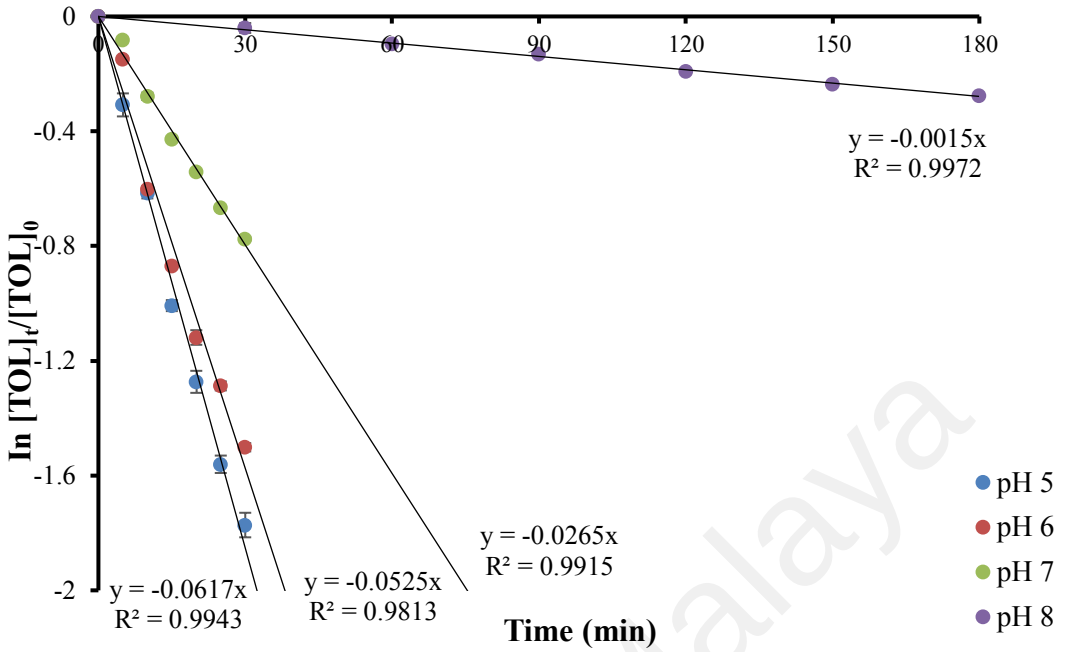



**Figure 4.1**, continued

### 4.1.1 Variation of $k_{app}$ against pH

HOCl with the pKa of 7.54 dissociates in water to form $OCl^-$ (Chamberlain and Adams, 2006; Acero et al., 2010). According to Roberts et al. (1998), solution pH can greatly affect the dissociation of hypochlorous acid (HOCl) and the rate of reactions between FAC with organic matter. Hence, the effect of pH on $k_{app}$ for the reaction between FAC and selected pharmaceuticals were investigated in detail. The molar fraction of HOCl (αHOCl) and $OCl^-$(αOCl) was calculated by using the Henderson–Hasselbalch as shown in equation Eq. 4.4. Figure 4.2 shows the variation of the molar fraction of FAC in water with pH. As shown in Figure 4.2, the values of αHOCl are decreased with increasing pH. This shows that the degree of dissociation of HOCl in water is a pH-dependent process (Cai et al., 2013). In this study, the pH ranging from 5 to 8 was selected to study the influence of pH on the $k_{app}$ (Figure 4.2). This selected pH range also represents the pH condition for most of the environmental water samples (Al-Badaii et al., 2013; Suratman et al., 2015). Also, the αHOCl and αOCl could vary from 1 to 0.24 and 0 to 0.76, respectively.

$$pH = pK_a - \log_{10}\frac{[HOCl]}{[OCl^-]} \qquad \text{(Eq. 4.4)}$$

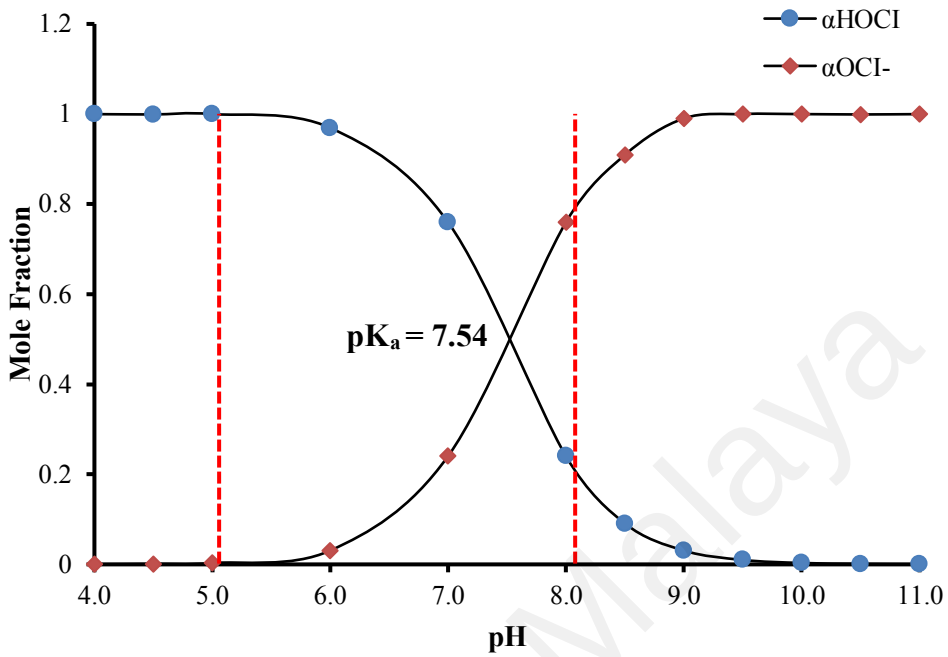



**Figure 4.2:** The distribution diagram of FAC in water at different pH values.

Figure 4.3 shows the variation of $k_{app}$ of DPH with pH. The values of $k_{app}$ were obtained by dividing $k_{obs}$ with $[FAC]_0$. Based on Figure 4.3, the $k_{app}$ was found to increase from 2.52 to 15.74 $M^{-1}s^{-1}$ when the pH was increased from 5 to 6. However, the $k_{app}$ was found to decrease to 5.24 and 0.81 $M^{-1}s^{-1}$ when the pH was further increased to 7 and 8, respectively. These results showed that the $k_{app}$ values are pH dependent. As shown in Figure 4.3, the $k_{app}$ was found to decrease with decreasing HOCl molar fraction when the pH was increased from 6 to 8. Due to low reactivity of $OCl^-$ toward organic compounds, the reaction between $OCl^-$ and organic compounds often neglected (Sharma, 2008). Therefore, the obtained results indicated that the rate of degradation of DPH at pH 6 to 8 was dominated by the reaction between DPH and HOCl.

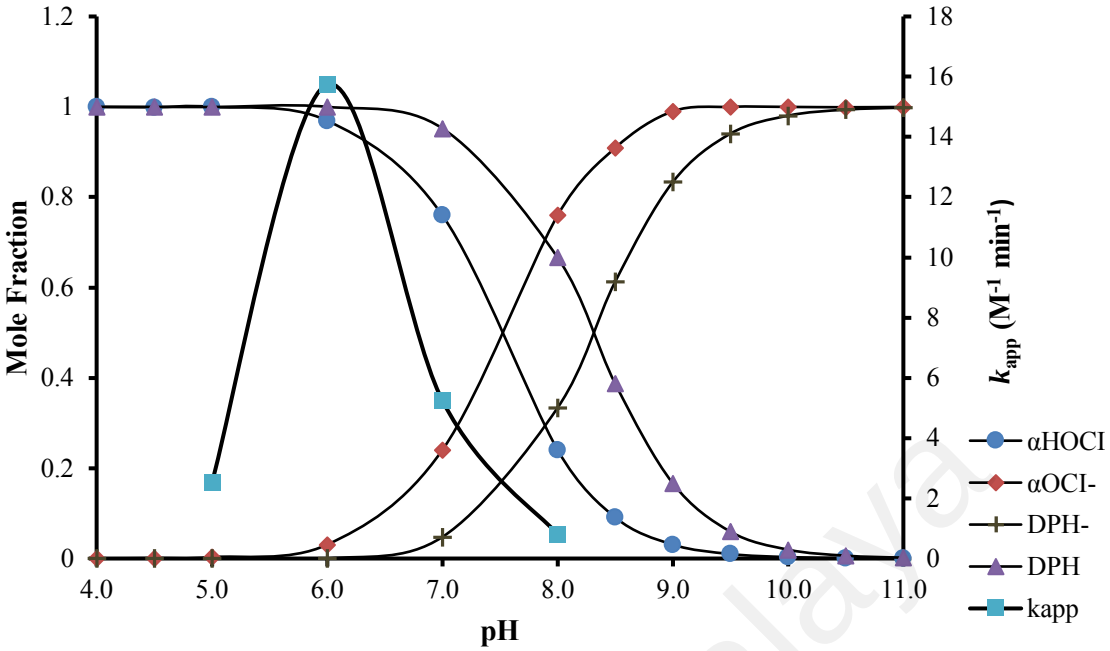



**Figure 4.3:** Second-order rate constant for chlorination of DPH from pH 5 to 8. [T = 25 ± 0.1°C, $[DPH]_0$ = 19.8 µM, $[HOCl]_O$ = 990 µM].

For DPH, the NH groups are acidic due to the presence of electron withdrawing carbonyl groups. Therefore, with the pKa of 8.3 (Agarwal and Blake, 1968), DPH could dissociate and formed anions ($DPH^-$) (Figure 4.4). The increase in the magnitude of $k_{app}$ from pH 5 to 6 (Figure 4.3) suggested that the anion species of DPH are more reactive toward HOCl as compared to the neutral form of DPH. This is due 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 with the negative charge (Figure 4.5). The presence of negative charge could increase the reactivity of DPH via the nucleophilic attack on HOCl and consequently increase the rate of DPH decomposition during chlorination (Khalit, 2016). A similar observation was also reported in the chlorination of sulfamethoxazole (Dodd et al., 2005) and amoxicillin (Acero et al., 2010).

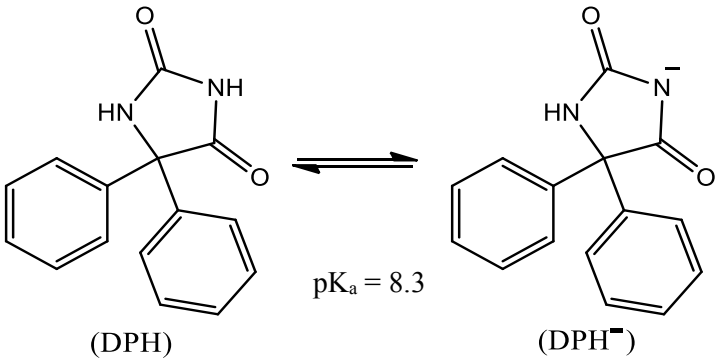



**Figure 4.4:** The neutral and deprotonated form of DPH.

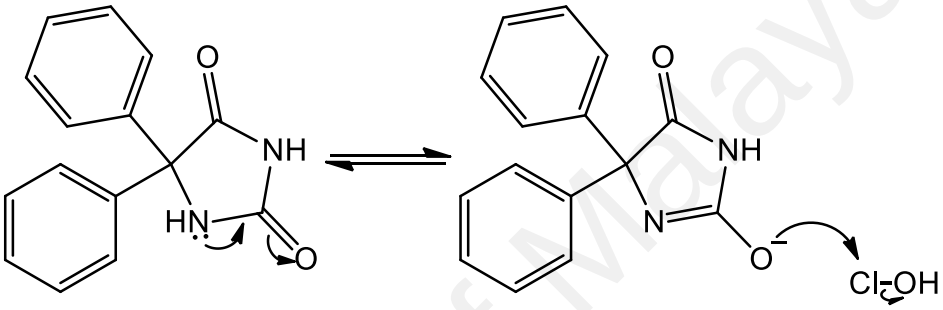



**Figure 4.5:** Delocalization of lone pair for DPH.

As shown in Figure 4.6, when pH increases, the $k_{app}$ of HCTZ was found to increase from 1.6 to 70.6 $M^{-1}$ $min^{-1}$. The degradation of HCTZ was not influenced by decreasing αHOCl. HCTZ can appear as neutral or deprotonated ions as shown in Figure 4.6. When the pH increased from 5 to 8, the molar fraction of anionic HCTZ was found to increase (Figure 4.6). The increase of $k_{app}$ with increasing pH showed that the deprotonated form of HCTZ is more reactive toward chlorination as compared to its neutral form. This result showed that the ionization of sulfonamide group has a significant impact on the reactivity of HCTZ in chlorination (Figure 4.6) (Soufan et al., 2012).

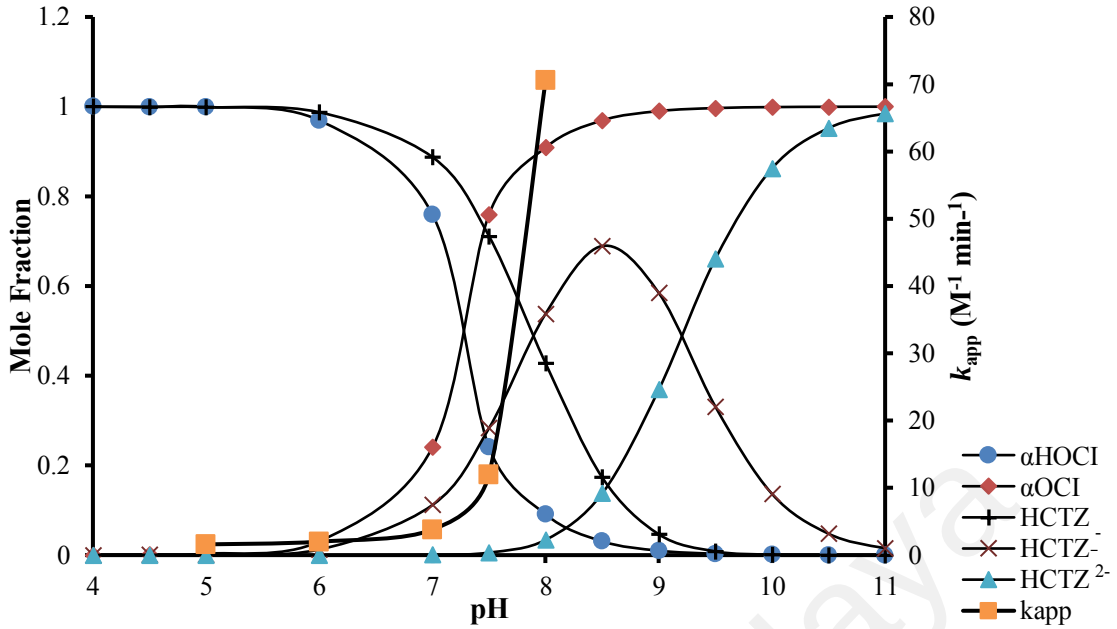



**Figure 4.6:** Second-order rate constant for chlorination of HCTZ from pH 5 to 8 (T = 25 ± 0.1 °C, $[HCTZ]_0$ = 20 µM, $[FAC]_0$ = 1000 µM).

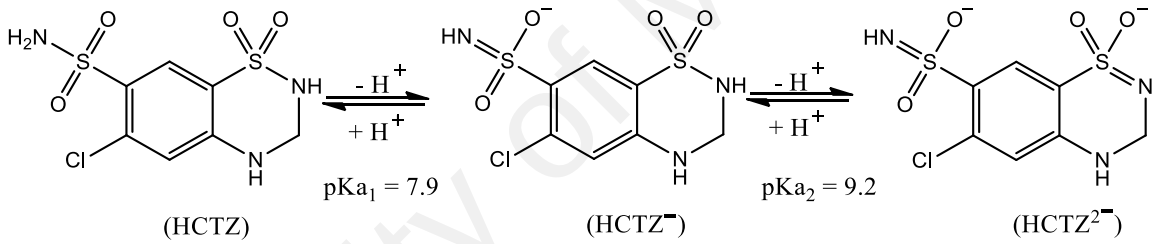



**Figure 4.7:** The ease of degradation of HCTZ and its anionic and dianionic species.

Based on Figure 4.8, the $k_{app}$ of the reaction between TOL and FAC was found to decrease with increasing pH from 5 to 8. The rate of reaction between TOL with FAC decreased sharply with decreasing αHOCl. This result showed that the degradation of TOL was dominated by the reaction with HOCl. The results also showed that the dissociation of TOL (Figure 4.9) does not give significant effect on the $k_{app}$ of the reaction between TOL and FAC. Furthermore, $TOL^-$ hardly reacts with $OCl^-$ (Sharma, 2008) hence $k_{app}$ decreased significantly under basic condition. Therefore, it is proposed that the degradation of TOL largely depends on the concentration of reactive HOCl.

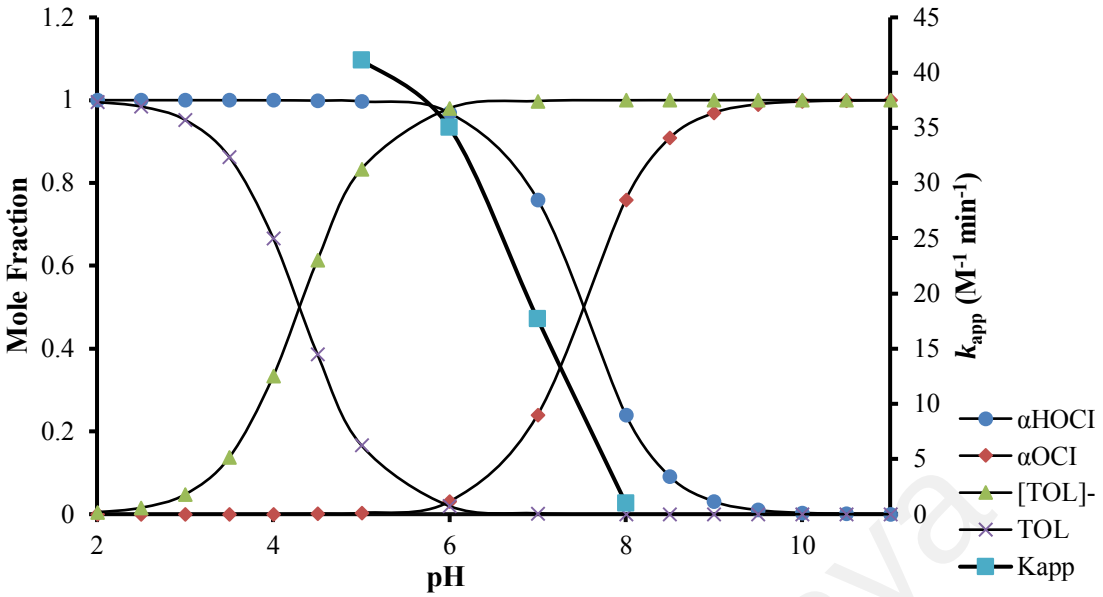
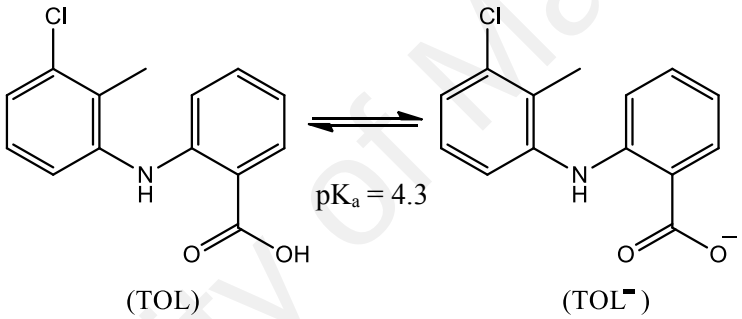



**Figure 4.8:** Second-order rate constant for chlorination of TOL from pH 5 to 8 (T = 25 ± 0.1 °C, $[TOL]_0$ = 20 µM, $[FAC]_0$ = 1000 µM).




(TOL) (TOL$^-$)

**Figure 4.9:** The neutral and deprotonated form of TOL.

### 4.1.2 Comparison of second-order rate constant ($k_{app}$) between selected pharmaceuticals

Figure 4.10 shows the variation of $k_{app}$ of selected pharmaceuticals with pH. In this study, the reactivity of pharmaceuticals in chlorination process was found to be influenced by its functional group. According to Deborde and Von Gunten (2008), for pharmaceuticals with complex chemical structures, the main reaction sites can be predicted by considering the FAC reactivity at the various functional group.

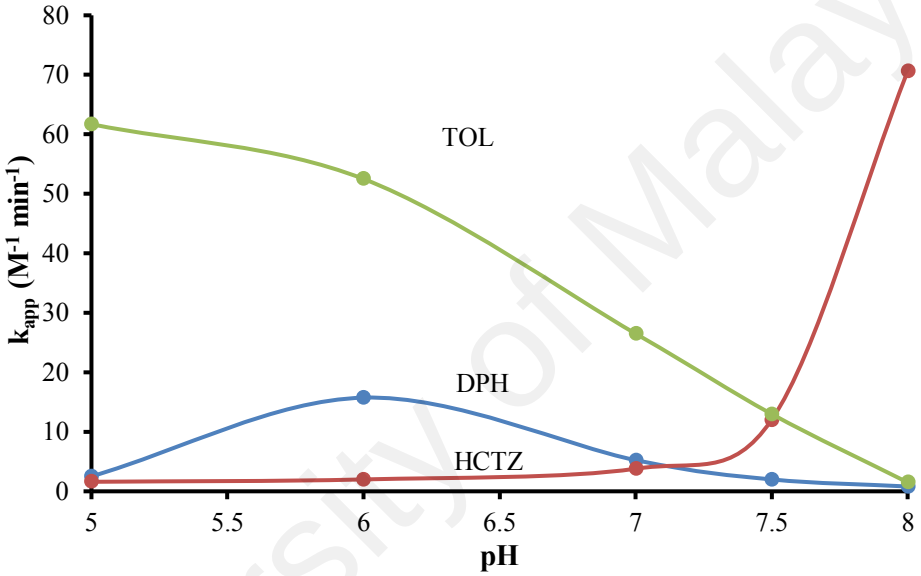



**Figure 4.10:** The variation of second-order constant of DPH, HCTZ and TOL with pH.

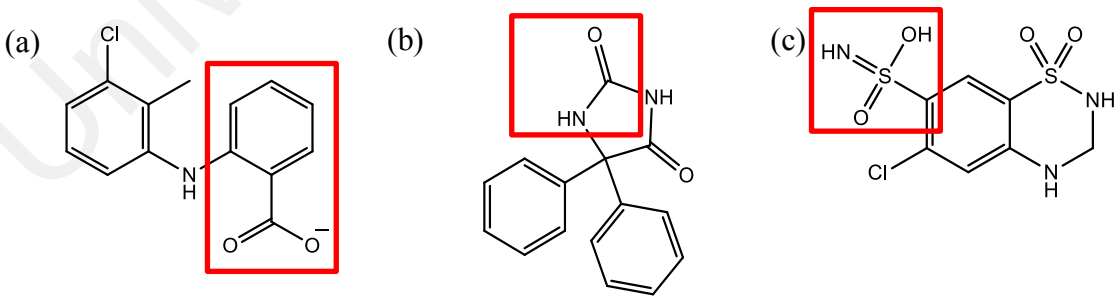



**Figure 4.11:** The structure of (a) $TOL^-$, (b) DPH and (c) HCTZ.

Based on Figure 4.9 the $k_{app}$ of TOL was the highest followed by DPH and HCTZ at the pH ≤ 7. At pH ≤ 7, the major forms of selected pharmaceuticals were $TOL^-$, DPH (neutral) and HCTZ (neutral) as shown in Figure 4.11. TOL shows the highest $k_{app}$ due to

the presence of carboxylate group, which increase the electron density of the aromatic ring of TOL (Wu et al., 2017). The electron rich aromatic ring reacts more favorably with HOCl via addition reaction (refer to section 4.4.3). As a result, chlorinated TOL was detected as TBPs (refer to section 4.4.3).

For DPH, main reaction site for chlorination is imidazole-2,4-dione moiety with amide as the functional group (refer to Figure 4.22). The low reactivity of DPH during chlorination as compared to TOL might due to the low reactivity of amide as compared to the aromatic ring. The $k_{app}$ of HCTZ was the lowest as compared to TOL and DPH. This might be due to the presence of chlorine atom attached to the aromatic ring of HCTZ, which acts as electron withdrawing group that reduced the electron density of the aromatic ring. Consequently, it reduces the reactivity of the aromatic ring of HCTZ towards HOCl. When pH > 8, $k_{app}$ of TOL and DPH was decreased due to the decreased of αHOCl. However, for HCTZ the $k_{app}$ was increased significantly. This was due to the deprotonation of HCTZ to produce anion HCTZ. Compound with anionic sulfonamide groups was found to be more reactive toward chlorination as compared to its neutral counterpart (Dodd and Huang, 2004).

## 4.2 Degradation of selected pharmaceuticals during UV/chlorination

Figure 4.12 shows the degradation of selected pharmaceuticals by chlorination, UV irradiation, and UV/chlorination processes (with and without *tert*-Butanol). Chlorination of DPH showed the minimal degradation with only 9.0% of DPH was removed in 6 min. DPH was found to undergo photolysis by UV irradiation with 36.6% of DPH removal. By using UV/chlorination, the removal of DPH was further enhanced to 79.8%. The degradation of HCTZ during chlorination process was low with only 6.4% removal and increase to 16.9% during UV irradiation of HCTZ. But, during UV/chlorination, the degradation of HCTZ was increased significantly to 62.6%. The removal of TOL during

chlorination and UV irradiation are 36.2% and 12.8%, respectively. The degradation of TOL was further enhanced to 75.7% during UV/chlorination. From this observation, it shows that the chlorination process was not effective with only 6.1 to 36.2% removal of selected pharmaceuticals. UV irradiation of selected pharmaceuticals also showed low removal efficiency with 12.8 to 36.6% only. By using UV/chlorination, the removal of selected pharmaceuticals was enhanced 62.6% to 79.8%. This result indicated that the presence of UV irradiation during chlorination has significantly improved the efficiency of pharmaceuticals removal due to the formation of •OH and •Cl from the UV photolysis of FAC (Qin et al., 2014; Wang et al., 2016a). To prove the presence of radical species, *tert*-butanol, a radical scavenger was added during UV/chlorination. As shown in Figure 4.12 (a), the percent removal of DPH was reduced to 58.2% with the presence of 5 mM of *tert*-butanol. The percent removal of HCTZ and TOL were also degraded with a presence of 10 mM of *tert*-butanol. This result further proved the contribution of radical species in the degradation of selected pharmaceuticals during UV/chlorination.

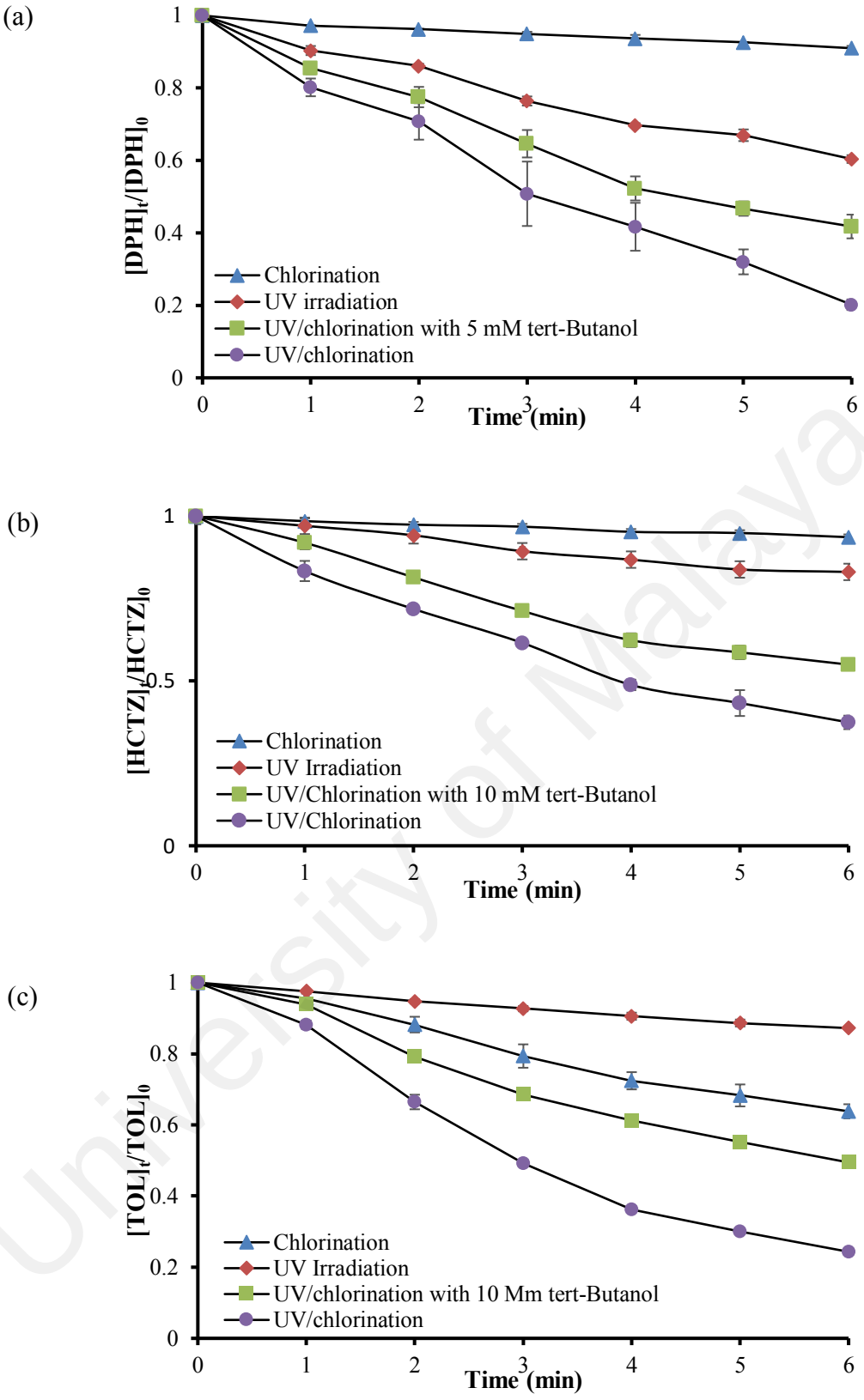



**Figure 4.12:** Degradation of selected pharmaceuticals as a function of time during chlorination, UV irradiation and UV/chlorination process (with and without *tert*-Butanol). (pH = 7, T = 25±0.1 $^{o}$C, [ (a) $[DPH]_0$ = 100 µM], [HOCl] = 750 µM (b) $[HCTZ]_0$ = 50 µM, [HOCl] = 50 µM, (c) $[TOL]_0$ = 20 µM [HOCl] = 100 µM]).

### 4.2.1 Effects of pH and chlorine dosage of selected pharmaceuticals during UV/chlorination

FAC dosage and pH of the solution are two important parameters that influence the efficiency of UV/chlorination in the removal of organic pollutants (Qin et al., 2014; Wang et al., 2016b). The effect of chlorine dosage on the selected pharmaceuticals removal was evaluated at the different initial concentration of FAC ranging from 0 to 750 μM. The removal of DPH was found to increase from 72.8 to 82.0% when the concentration of FAC was increased from 250 to 750 μM (Figure 4.13a). As compared with UV irradiation alone (at 0 μM of FAC), the pseudo-first-order rate constant ($k_{obs}$) was found to increase by almost double with the presence of 250 μM of FAC (Figure 4.13b). Figure 4.14a shows the removal of HCTZ reached 62.5% with the presence of 50 μM of FAC. The percentage removal of HCTZ was further improved to 92.7% with 150 μM of FAC. Meanwhile, the degradation of TOL during UV/chlorination with 150 μM of FAC was only18.9%. But, when FAC dosage was increased to 500 μM the degradation of TOL increased to 67.3% (Figure 4.15a). These results indicated that the amount of radicals generated from the photolysis of FAC exerted much influence on the degradation of selected pharmaceuticals. The increased of FAC dosage enhanced the production of radical species, which accelerated the selected pharmaceuticals degradation (Guo et al., 2016). The similar effect of FAC dosage on the removal of phenacetin (Zhu et al., 2018), bezafibrate (Shi et al., 2018) and gemfibrozil (Kong et al., 2018) during UV/chlorination was also reported by previous studies. A recent study has shown that $k_{obs}$ reached plateaus at high $[FAC]_0$ due to full photo absorbance by FAC is achieved (Wang et al., 2016a). Under this condition, the production of radical species is not increased even with higher $[FAC]_0$. Wang et al. (2016a) had demonstrated that the $k_{obs}$ could be further enhanced when the full photon absorption was achieved by increasing the UV irradiance. However,

changing the UV irradiance during water treatment process is an unlikely process since it associated with higher cost operational cost.

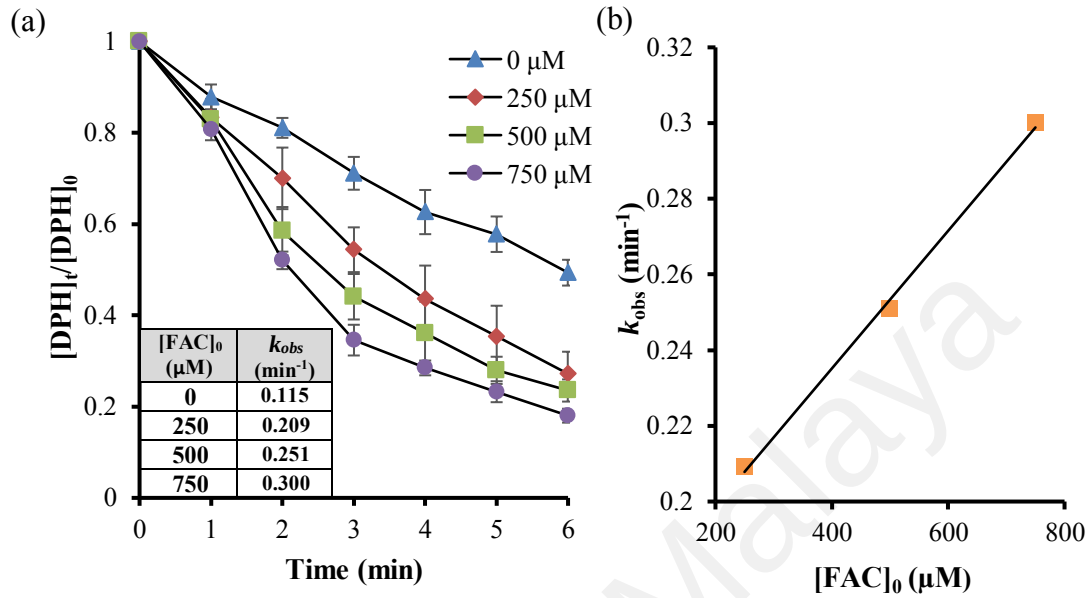



**Figure 4.13:** (a) The degradation of DPH as a function of time with different $[FAC]_0$ and (b) The variation of $k_{obs}$ with $[FAC]_0$. [pH = 7, T = 25±0.1°C, $[DPH]_0$ = 50 µM].

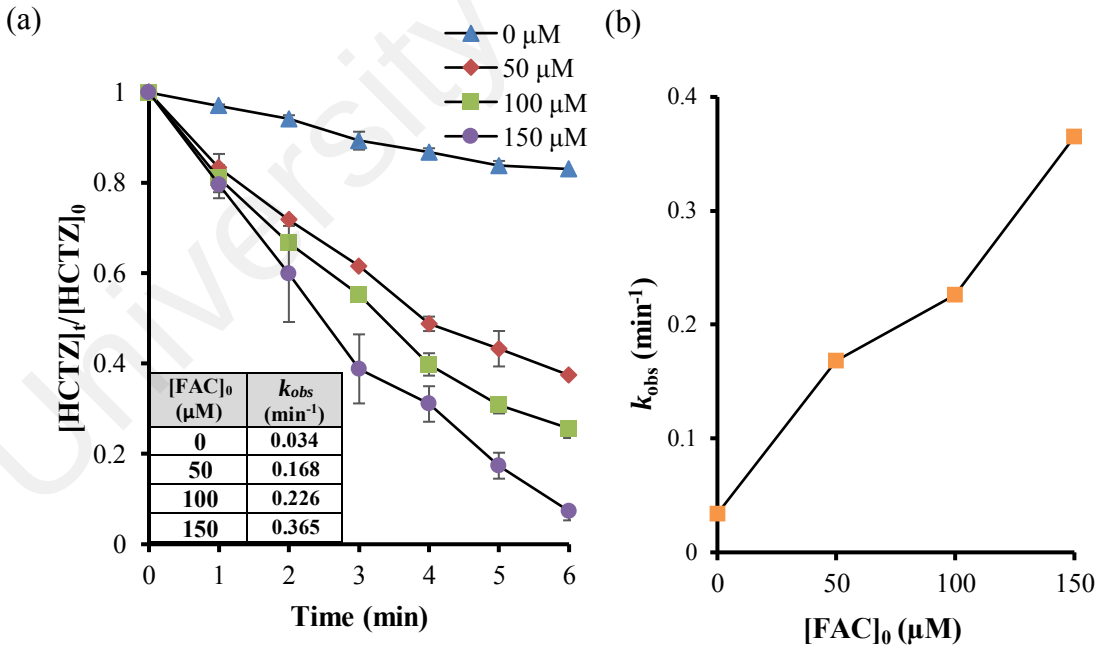



**Figure 4.14:** (a) The degradation of HCTZ as a function of time with different $[FAC]_0$ and (b) The variation of $k_{obs}$ with $[FAC]_0$. [pH = 7, T = 25±0.1°C, $[HCTZ]_0$ = 50 µM].

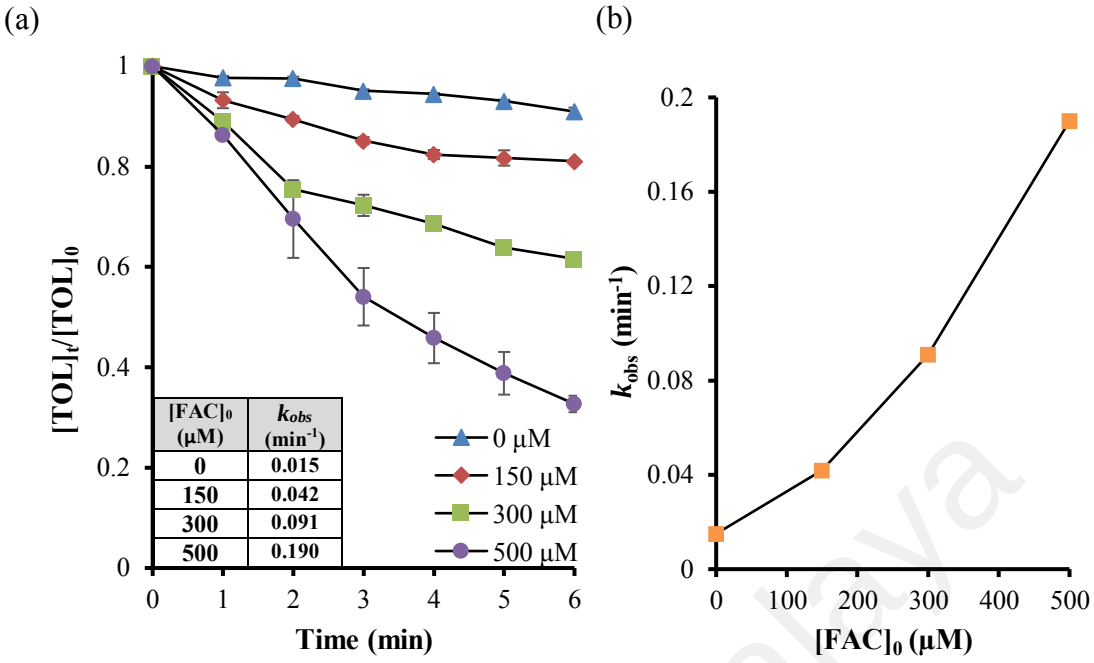



**Figure 4.15:** (a) The degradation of TOL as a function of time with different $[FAC]_0$ and (b) The variation of $k_{obs}$ with $[FAC]_0$. [pH = 7, T = 25±0.1°C, $[TOL]_0$ = 50 µM].

The effect of pH on the removal of selected pharmaceuticals was evaluated at pH ranging from 5 to 8. As shown in Figure 4.16 - 4.18, the removal of selected pharmaceuticals by UV/chlorination were more efficient under the acidic condition as compared to neutral and basic conditions. After 3 min of reaction, the percentage removal of DPH at pH 5 was 67.5%. This value was found to decrease to 46.3% when the pH was increased to 8. HCTZ and TOL also show the same results, degradation rate was also decreased with increasing pH after 4 min of reaction. The range of percent removal of HCTZ and TOL at pH 5 - 8 were reduced by 9.0% and 54.6%, respectively.

The degradation rate of selected is higher at acidic conditions due to the higher molar fraction of HOCl. Quantum yields of HOCl at 254 nm light and ambient temperature has been found to be much higher than $OCl^-$ (Watts and Linden, 2007). Therefore, the higher molar fraction of HOCl at acidic condition could enhance the formation of reactive radicals and consequently, increased the rate of selected pharmaceuticals degradation (Kong et al., 2016). The decreased of $k_{obs}$ with increasing

pH also can be explained by the scavenging effect of $OCl^-$ on the radical species. $OCl^-$ is the predominant species at high pH and it was found to react with •OH and •Cl at the rate of $8.8 \times 10^9$ and $8.2 \times 10^9$ $M^{-1}s^{-1}$ (Fang et al., 2014).

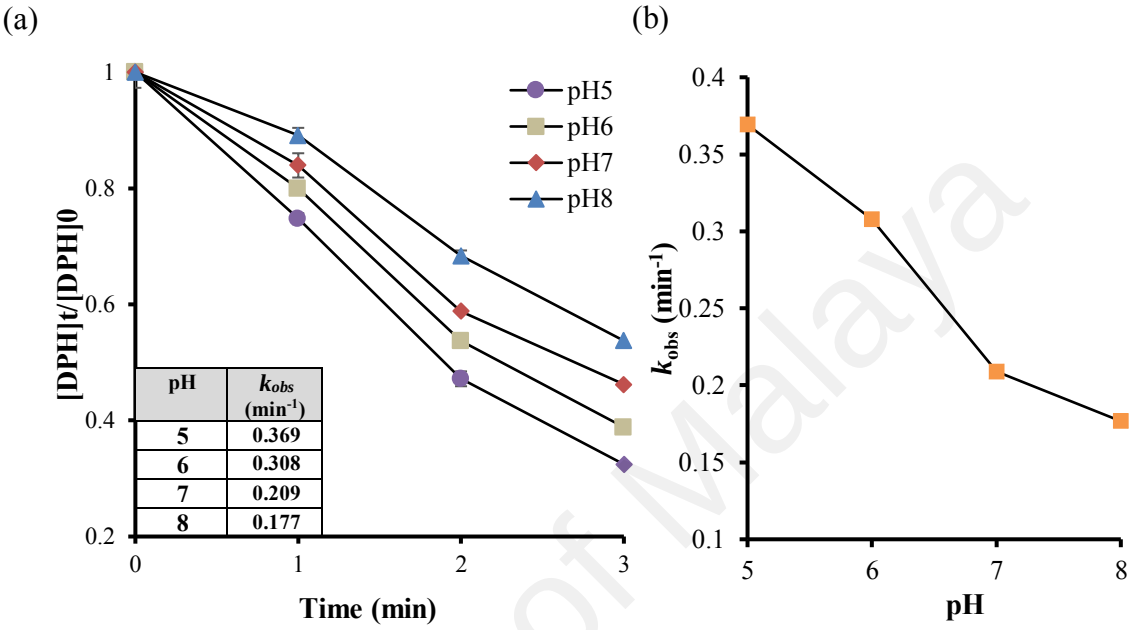



**Figure 4.16:** (a) The degradation of DPH as a function of time with different pH and (b) The variation of $k_{obs}$ with pH. [pH = 7, T = 25±0.1°C, $[DPH]_0$ = 50 µM, $[FAC]_0$ = 250].

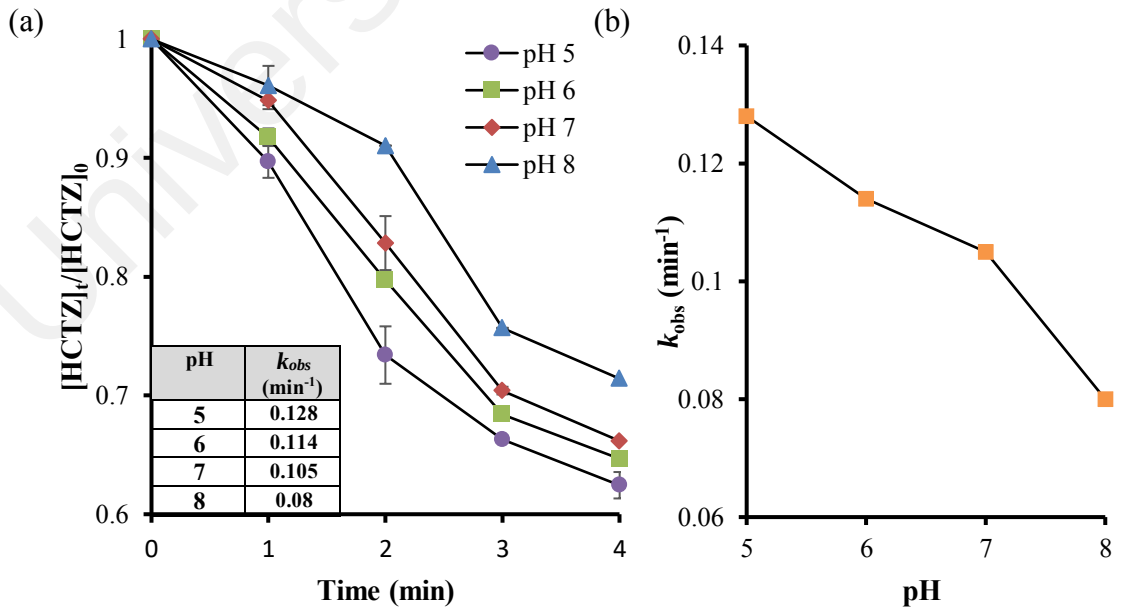



**Figure 4.17:** (a) The degradation of HCTZ as a function of time with different pH and (b) The variation of $k_{obs}$ with pH. $[FAC]_0$ = 50 µM, T = 25±0.1°C, $[HCTZ]_0$ = 50 µM].

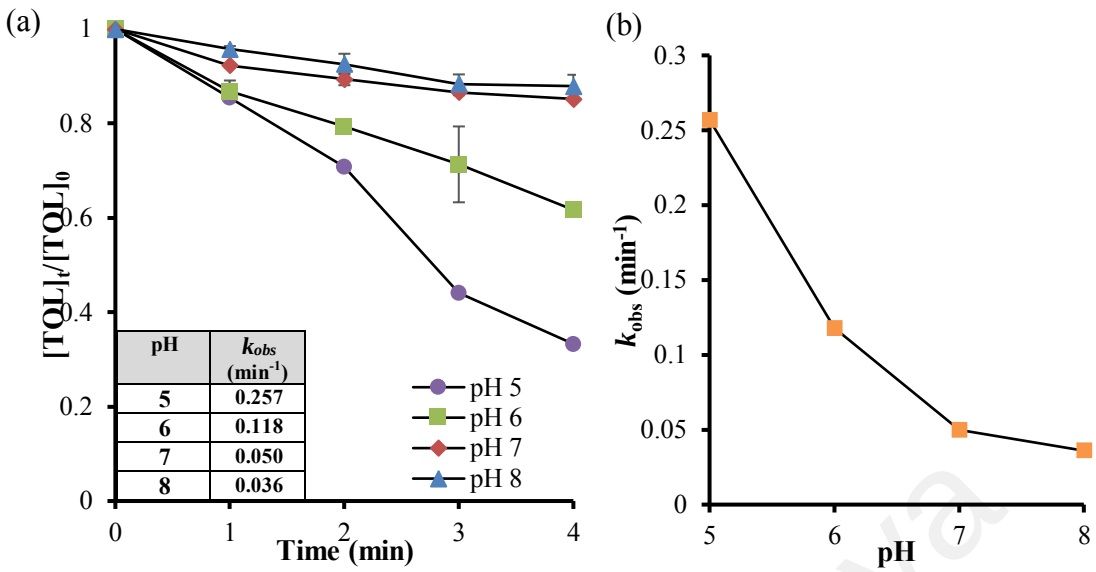
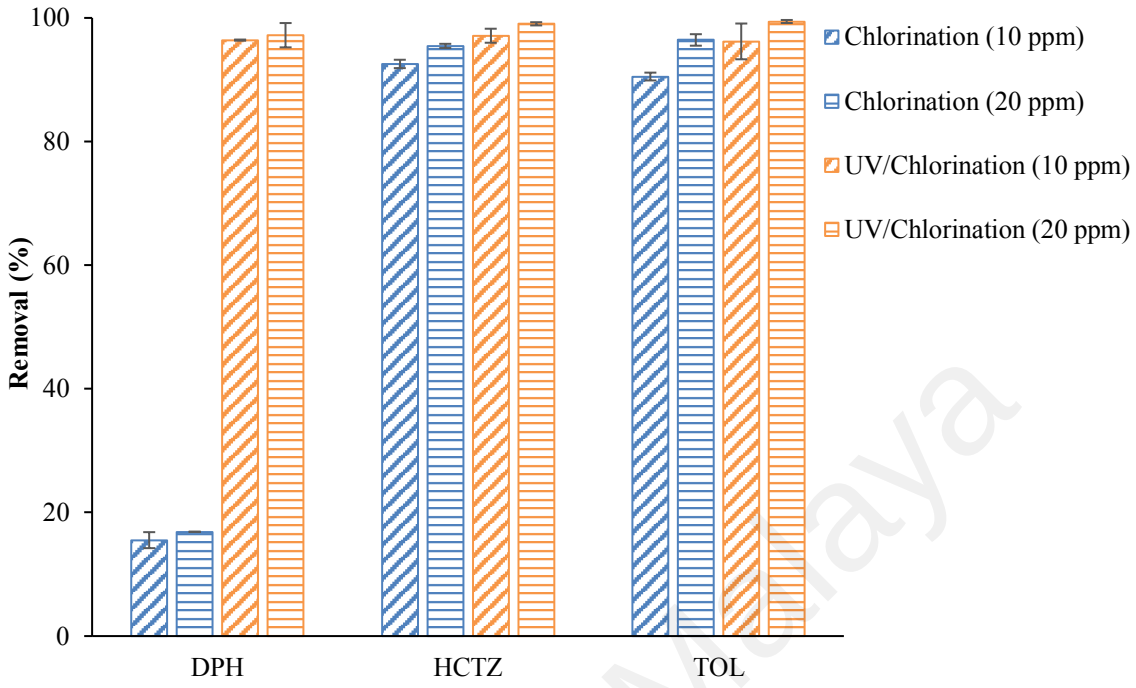



**Figure 4.18:** (a) The degradation of TOL as a function of time with different pH and (b) The variation of $k_{obs}$ with pH. [pH = 7, T = 25±0.1°C, $[TOL]_0$ = 20 µM, $[FAC]_0$ = 120].

## 4.3 Removal of selected pharmaceuticals in different water matrices during chlorination and UV/chlorination

Figure 4.19 shows the percentage removal of selected pharmaceuticals during chlorination and UV/chlorination in lake and tap water. Based on Figure 4.19, the removal of DPH was the lowest with only 12.97 to 19.10% of removal as compared to HCTZ and TOL during chlorination in the real water samples. Meanwhile, the removal of HCTZ and TOL in real water samples were in the range of 86.82 to 99.05% and 84.65 to 97.18%, respectively. A study by Huerta-Fontela et al. (2011) also showed that DPH is resistant against the degradation in chlorination process of surface water, even though chlorination was found to be efficient in degrading other pharmaceuticals that bearing electron-donating moieties such as phenol and aniline group (Lee and von Gunten, 2010).

(a) Lake water




(b) Tap water

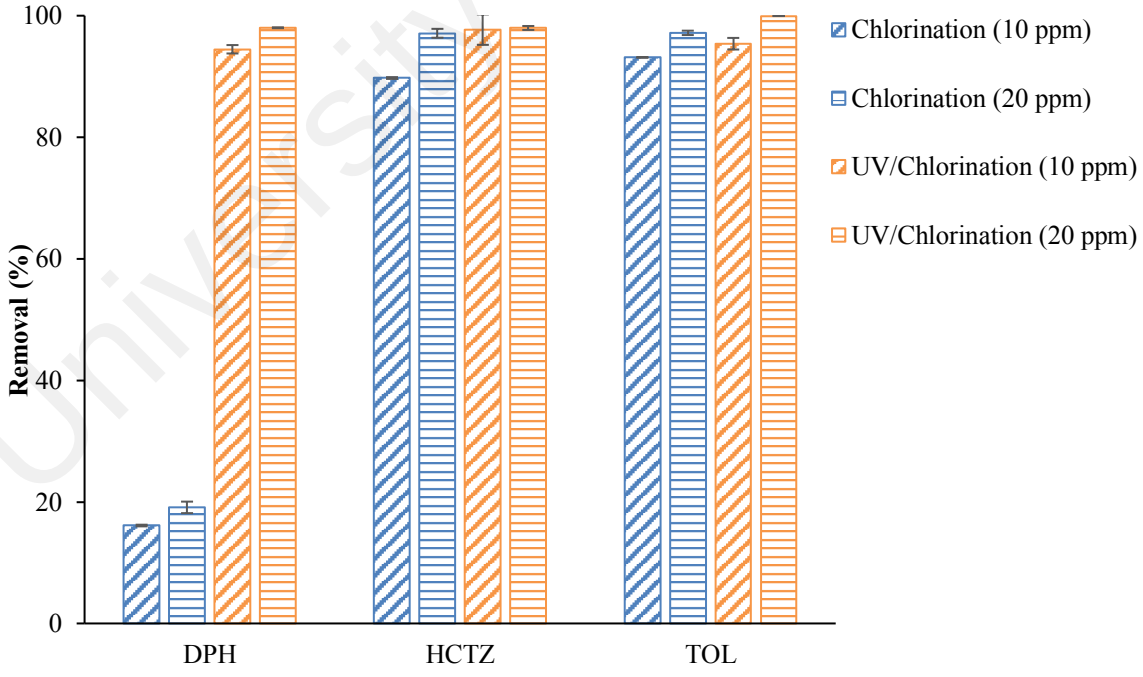



**Figure 4.19:** Percentage removal of selected pharamcaeuticals during chlorination and UV/chlorination in (a) lake water and (b) tap water. [selected pharmaceuticals] = 1ppm.

The removal of selected pharmaceuticals was higher during UV/chlorination as compared to chlorination process alone. As shown in Figure 4.19, the removal of DPH during chlorination was only in the range of 12.97 to 19.10%, but during UV/chlorination, the removal of DPH was found to increase to 94.48 to 98.02%. The removal of HCTZ during chlorination in lake water and tap water samples when 10 ppm of FAC was used was 95.55% and 89.75%, but during UV/chlorination, the removal of HCTZ was increased to 97.12% and 97.71%. TOL also shows higher removal during UV/chlorination as compared to chlorination. The removal of TOL during chlorination was 90.52% and 93.14% and increased to 96.20% and 95.39% during UV/chlorination. These results show that the UV/chlorination process could enhance the removal of pharmaceuticals in real water samples especially for the pharmaceuticals that resistant against the degradation by chlorination. Hence, the UV/chlorination can be a potential treatment method to treat the pharmaceuticals in real water samples.

### 4.4 Identification of TBPs for selected pharmaceuticals

TBPs of selected pharmaceuticals were determined using GC-MS and LC-QqQ-MS. Chlorination and UV/chlorination of DPH found to produce non-polar TBPs. Some of these TBPs are difficult to ionize. Therefore, GC-MS was used to detect TBPs of DPH after liquid-liquid extraction. TBPs of HCTZ and TOL were not detected using GC-MS. These results were due to the high solubility of TBPs of HCTZ and TOL in water. These TBPs are less likely to be extracted using liquid-liquid extraction. Hence, structure elucidation of TBPs of HCTZ and TOL was performed using LC-QqQ-MS. The analysis was performed by comparing mass spectrometric data of the initial pharmaceuticals solution as a control sample with the data collected from the treated pharmaceuticals solution. The structure of all TBPs was suggested based on its fragmentation pattern in MS and MS/MS spectrum. Since most of these compounds are not reported before.

### 4.4.1 TBPs of DPH

GC-MS analysis of the extract from the chlorinated and UV/chlorination treated DPH solutions indicate the generation of a variety of TBPs) (Table 4.1). In this study, TBPs were extracted from the water after the reaction, and the extracts were then converted to trimethylsilyl-derivatives before GC-MS analysis. The detected TBPs were identified based on its fragmentation pattern in the mass spectra (Figure 4.21). The peak for the silylated DPH appears at a retention time of 26.9 min (Figure 4.20). The mass spectrum of silylated DPH (Figure 4.21a) shows the molecular ion peak ($M^{\bullet+}$) at *m/z* 396. Other significant peaks in the mass spectrum, which are attributed to the fragments lost from $M^{\bullet+}$ of DPH, are *m/z* 381, 341, 281, and 176. The fragment at *m/z* 381 was formed through the elimination of methyl group. Ion at *m/z* 341 is the product ion of *m/z* 381. It was formed through the elimination of a methyl and a carbonyl group from *m/z* 381 ion. Further fragmentation of the fragment at *m/z* 341 through the elimination of two phenyl groups formed fragment at *m/z* 176. The fragment at *m/z* 281 is another product ion of the fragment at *m/z* 381. This ion was formed through the elimination of trimethylsilyl group and two methyl groups from the fragment ion at *m/z* 381.

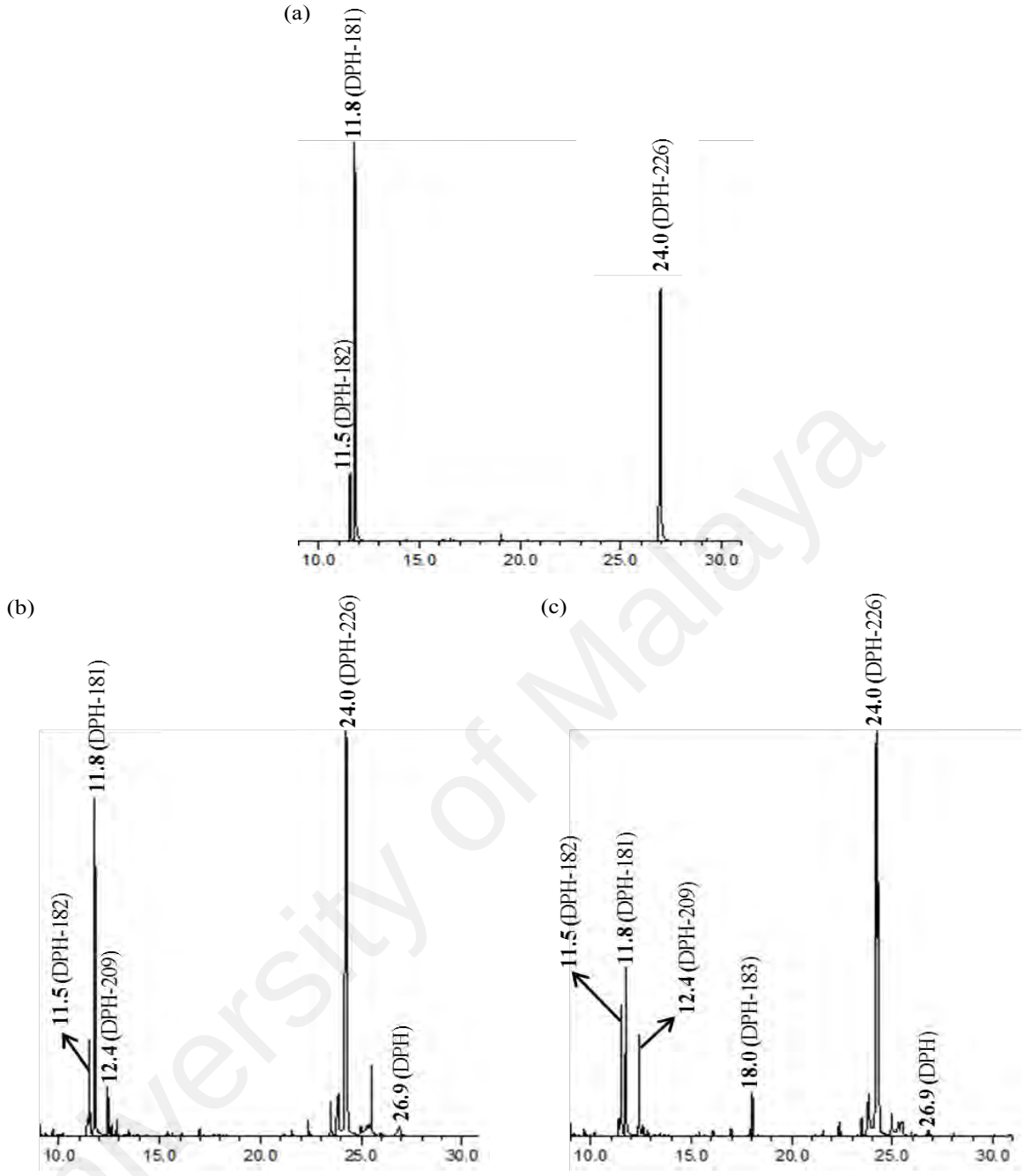
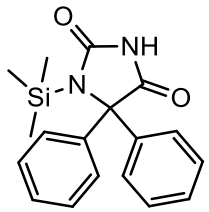
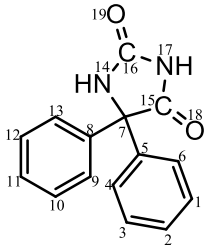
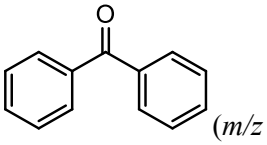
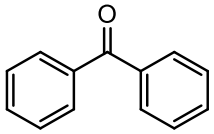
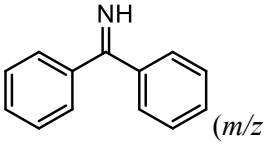
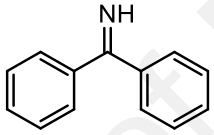
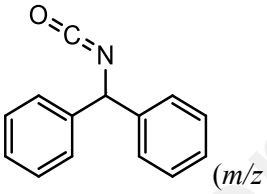
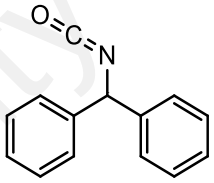
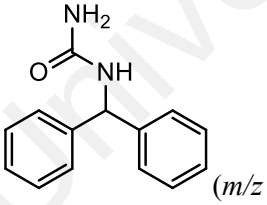
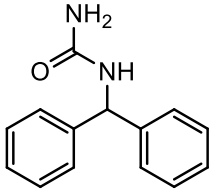
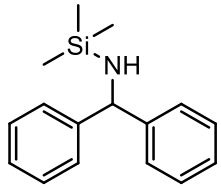
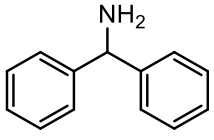
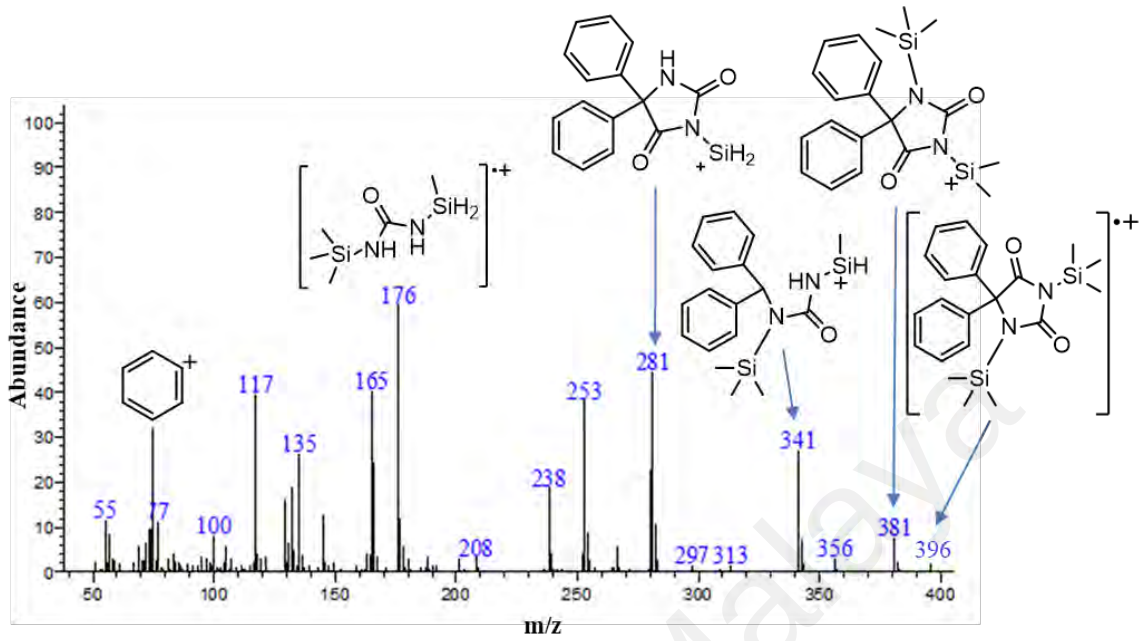



**Figure 4.20:** GC-MS chromatography; (a) Chlorination, (b) UV/chlorination and (c) UV irradiation.

**Table 4.1** Proposed transformation by-products of DPH during chlorination, UV irradiation and UV/chlorination.

| Compounds identified in GC-MS ($M^{\bullet+}$) | RT (min) | Structure of proposed TPBs [Label] | Chlorination | UV/ Chlorination | UV irradiation alone |
|---|---|---|---|---|---|
|  (*m/z* 396) | 26.9 |  [DPH] | ✓ | ✓ | ✓ |
|  (*m/z* 182) | 11.5 |  [DPH-182] | ✓ | ✓ | ✓ |
|  (*m/z* 181) | 11.8 |  [DPH-181] | ✓ | ✓ | ✓ |
|  (*m/z* 209) | 12.4 |  [DPH-209] | | ✓ | ✓ |
|  (*m/z* 226) | 24.0 |  [DPH-226] | | ✓ | ✓ |
|  (*m/z* 255) | 18.0 |  [DPH-183] | | | ✓ |

(a) DPH




(b) DPH-182

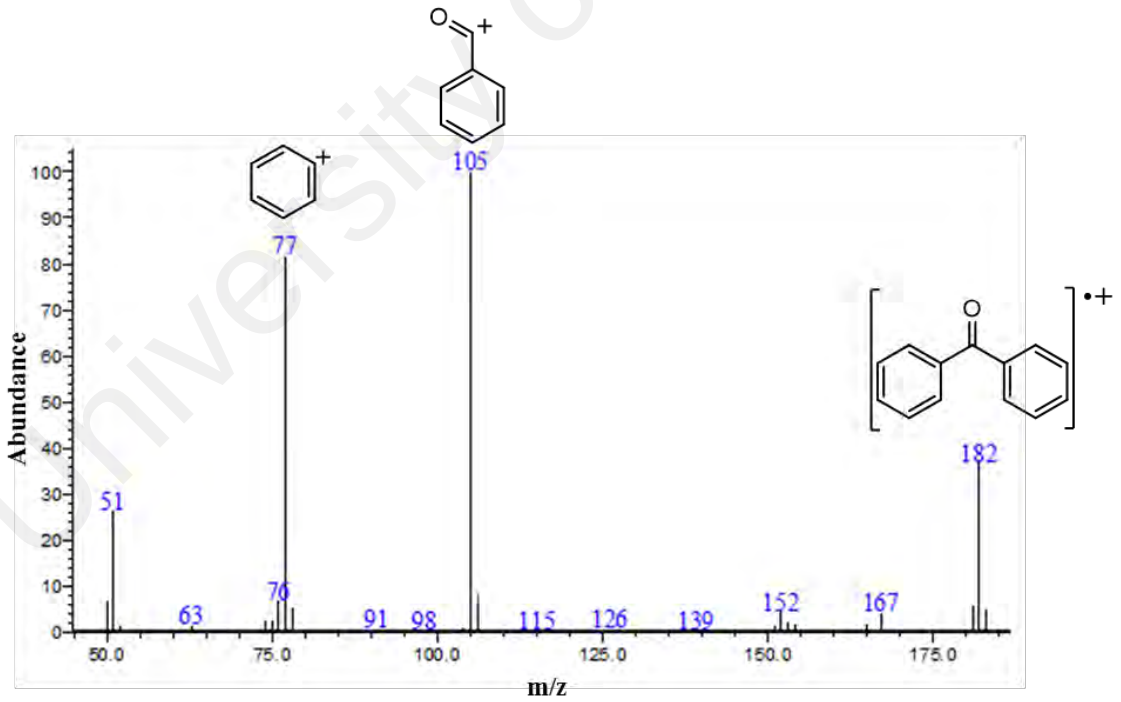
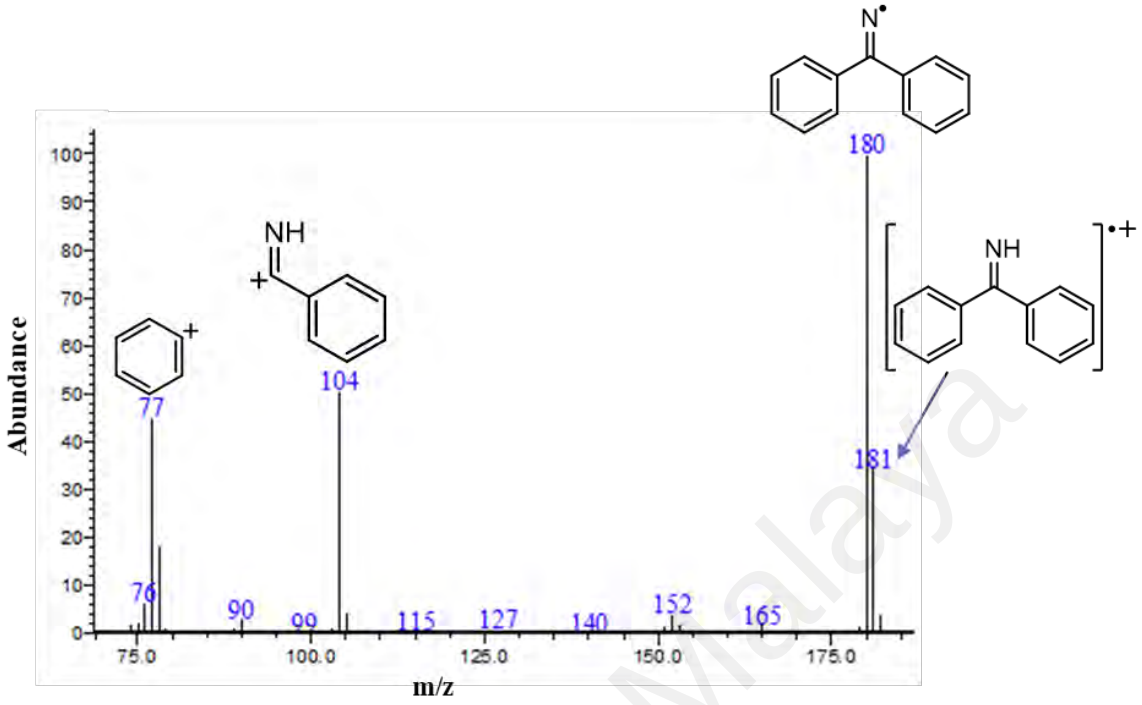



**Figure 4.21:** MS/MS spectrum of DPH and its by-products.

(c) DPH-181




(d) DPH-209

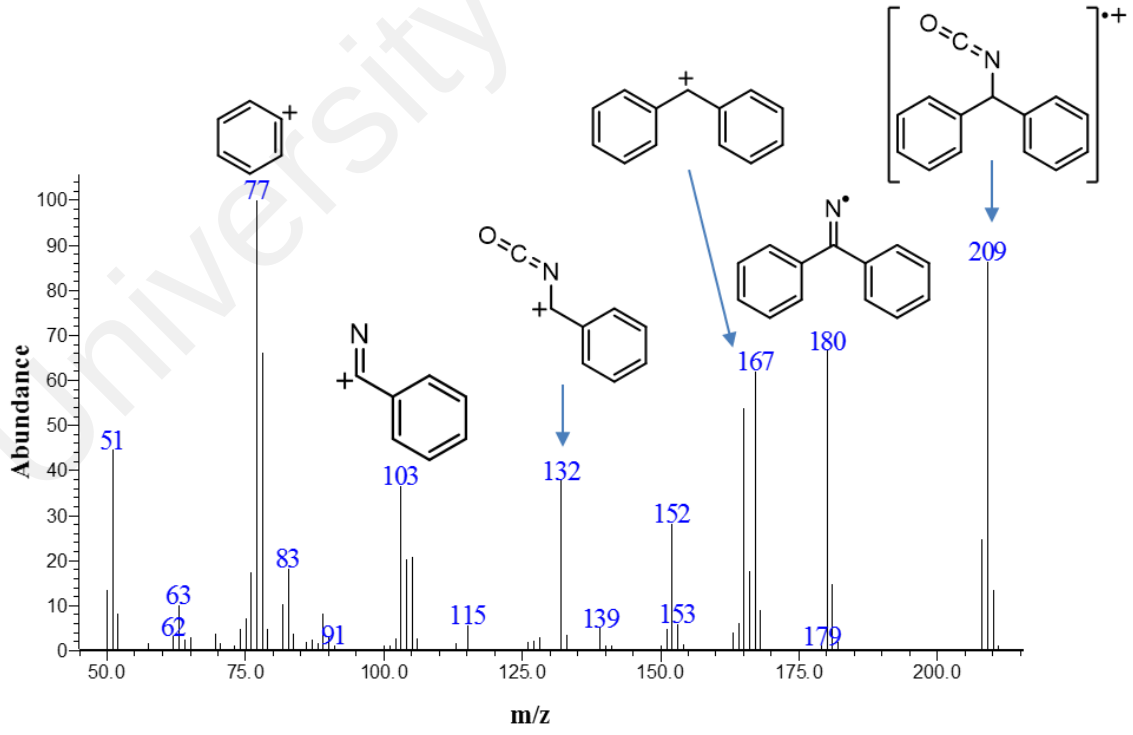
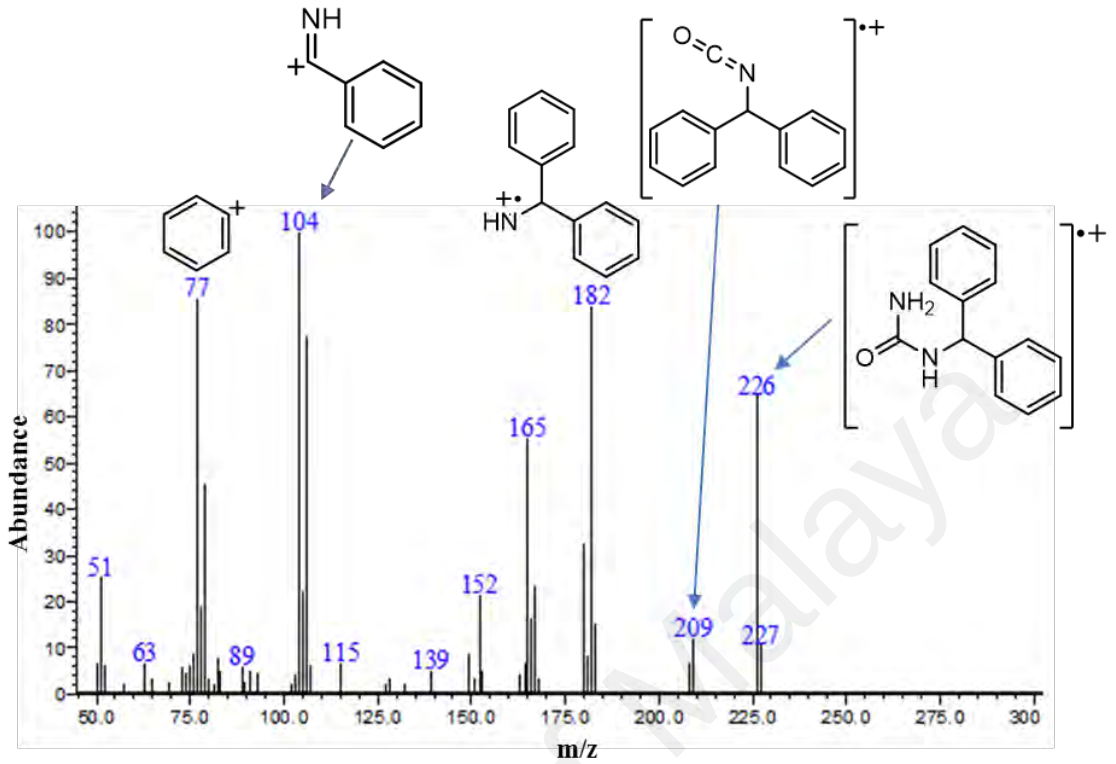



**Figure 4.21,** continued

(e) DPH-226




(f) DPH-183

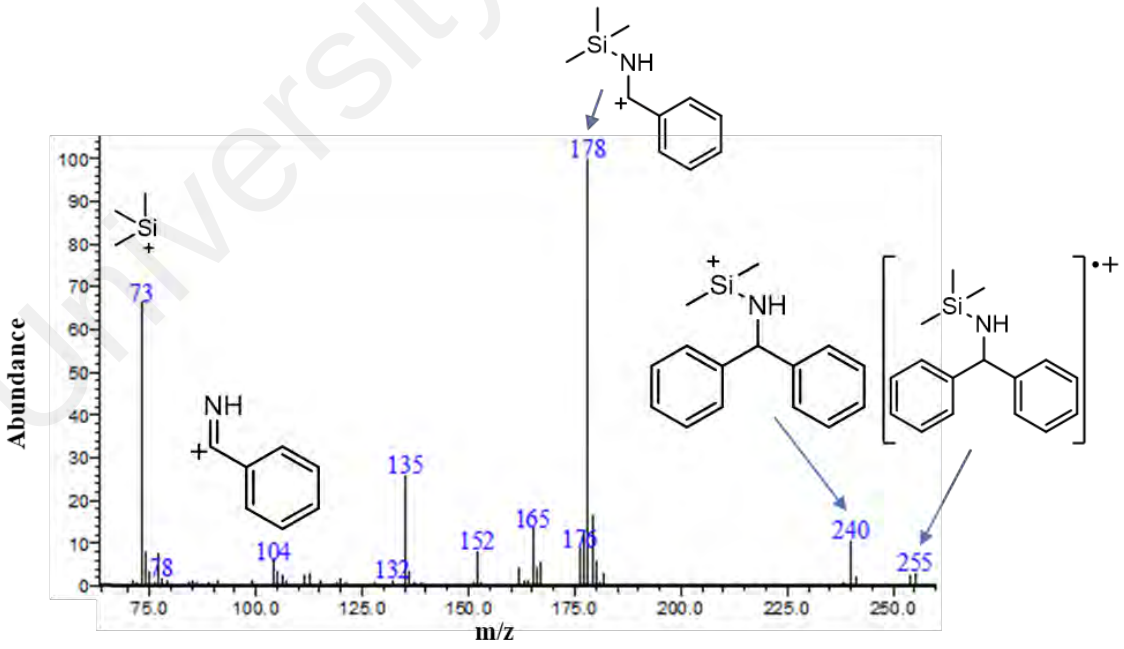



**Figure 4.21,** continued

Chlorination of DPH was found to produce two TBPs namely dibenzophenone (DPH-182) and diphenylmethanimine (DPH-181). The structure of DPH-182 is proposed exclusively based on fragmentation pattern of its mass spectrum (Figure 4.21b). The DPH-182 with the $M^{\bullet+}$ at *m/z* 182 was detected at 11.5 min (Figure 4.20). The main characteristic peaks of DPH-182 are *m/z* 105 and *m/z* 77 which represents $C_6H_5CO^+$ and $C_6H_5^+$, respectively. The retention time for DPH-181 with $M^{\bullet+}$ of *m/z* 181 was found to occur at 11.8 min. DPH-181 shows the main characteristic peaks at *m/z* 104 and *m/z* 77 are which are attributed to $C_6H_5N^+$ and $C_6H_5^+$, respectively. The proposed mechanism for the formation of DPH-181 and DPH-182 are presented in Figure 4.22. For the formation of DPH-181, the reaction is proposed to start from the attack of HOCl at $N_{17}$ of DPH to form chloramine intermediate (***DPH-I***) (Figure 4.22a). According to Hawkins and Davies (1998), the chloramine intermediate is unstable, and it can decompose to form nitrogen-centered radical (***DPH-II***). Radical ***DPH-II*** is proposed to undergo rapid alternation reaction and formed radical ***DPH-III***. Intermolecular rearrangement of radical ***DPH-III*** through the breakdown of $C_{16}$-$N_{14}$ bond forms radical ***DPH-IV***. Further rearrangement of radical ***DPH-IV*** through the breakdown of the $C_{15}$-$C_7$ bond formed DPH-181. The formation of DPH-182 is proposed to start from the reaction between DPH and HOCl at $N_{14}$ of DPH to form chloramine intermediate (***DPH-V***) (Figure 4.22b). Chloramine intermediate is unstable, and it is proposed to decompose to form amine radical (***DPH-VI***) (Hawkins and Davies, 1998). Rearrangement of intermediate ***DPH-VI*** through the breakdown of $C_{15}$-$C_7$ bond forms radical ***DPH-VII***. Radical ***DPH-VII*** could react with dissolved oxygen to form peroxy radical ***DPH-VIII*** (von Sonntag, 2006). Further rearrangement of ***DPH-VIII*** through the elimination of •OH forms DPH-182.

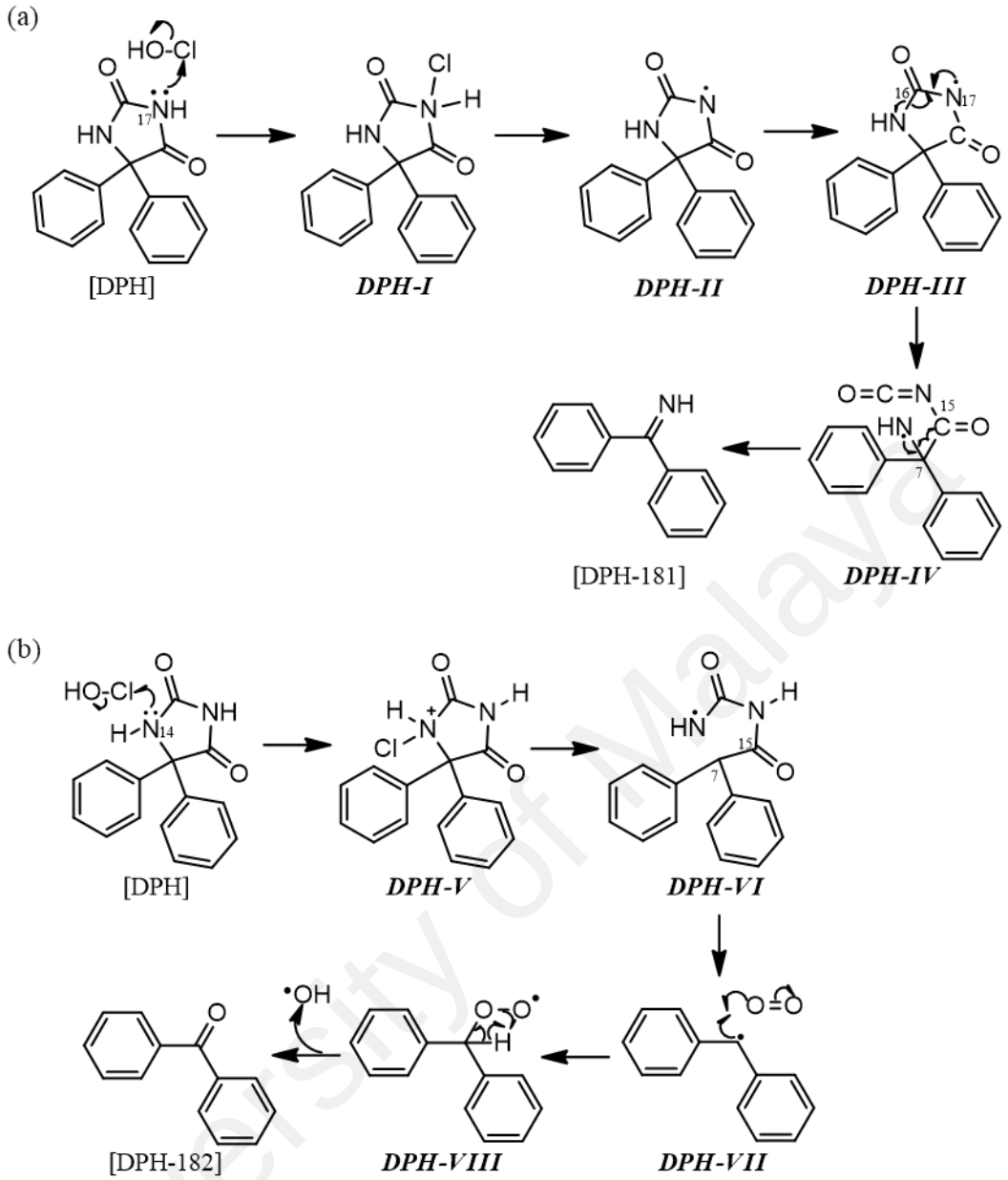



**Figure 4.22:** Mechanism of the formation of (a) DPH-181 and (b) DPH-182 through chlorination of DPH.

During UV/chlorination process, additional two TBPs were detected as compared to chlorination. These two TBPs are DPH-209 and DPH-226 (Table 4.1). The peak of DPH-209 was found to appear at 12.4 min (Figure 4.20). This compound with the $M^{\bullet+}$ of *m/z* 209 shows the significant fragments at *m/z* 180, *m/z* 167 and *m/z* 132, which were generated from the loss of –CO, -NCO and $-C_6H_5$, respectively. The peak for DPH-226 was found to occur at 24.0 min. Based on mass spectrum of DPH-226 (Figure 4.21e), the breakdown of molecular ion peaks at *m/z* 226 through the elimination of $CONH_2$ group

formed a fragment ion at *m/z* 182. The fragment at *m/z* 209 represents the radical cation of (isocyanatomethylene)dibenzene. As DPH-181, DPH-226 also shows the peaks at *m/z* 104, and *m/z* 77. DPH-209 and DPH-226 were only observed when DPH is treated with UV/chlorination process. Under UV/chlorination process, •OH is one of the reactive oxidants (Qin et al., 2014) that degrading the DPH. In order to confirm that DPH-209 and DPH-226 were formed through the •OH pathway, DPH solution was exposed to UV irradiation alone. This condition allowed the reaction between •OH with DPH, and the result showed that DPH-182, DPH-181, DPH-209, and DPH-226 were detected. This result further confirmed that DPH-209 and DPH-226 were formed through the radical reaction pathway. Also, DPH-181 and DPH-182 can be produced through radical reaction. The proposed mechanisms for the formation of DPH-209 and DPH-226 through the radical pathway are presented in Figure 4.23. The formation of DPH-209 is proposed to start with hydrogen abstraction at $N_{14}$ of DPH by •OH to forms radical ***DPH-IX*** (Vel Leitner et al., 2002). Intramolecular rearrangement of radical ***DPH-IX*** through the breakdown of the $C_{16}$-$N_{14}$ bond forms radical ***DPH-X***. Further rearrangement of radical ***DPH-X*** through the elimination of isocyanate radical forms DPH-209. The formation of DPH-226 is proposed to start with hydrogen abstraction at $N_{17}$ of DPH by •OH to form radical ***DPH-XI***. Radical ***DPH-XI*** could react with DPH and forms radical ***DPH-XII***. Further rearrangement of radical ***DPH-XII*** through the elimination of CO group formed radical ***DPH-XII***. Radical ***DPH-XIII*** could then react with DPH to form DPH-226.

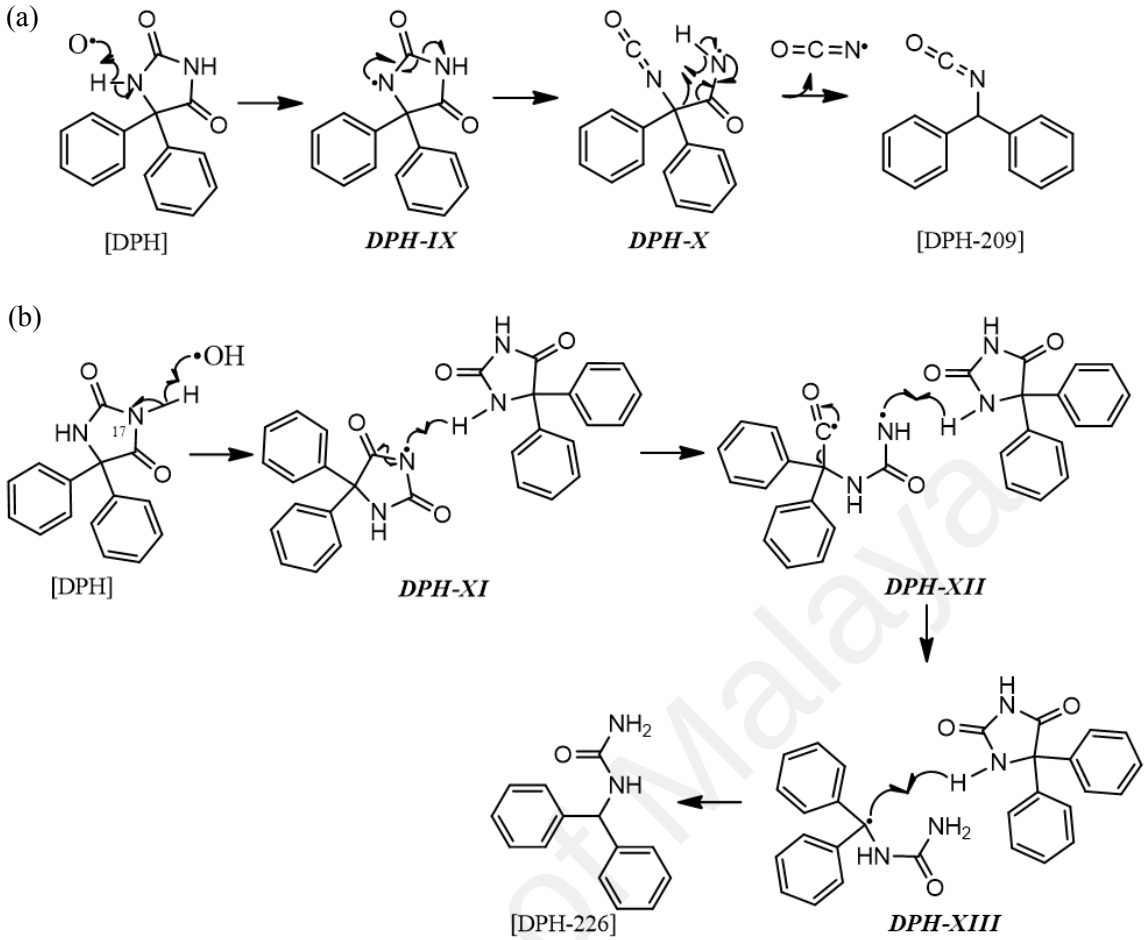


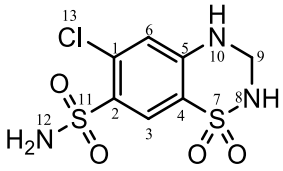
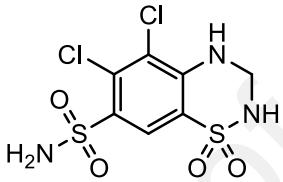
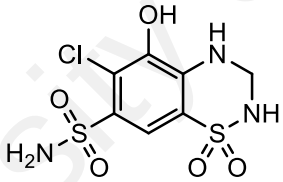
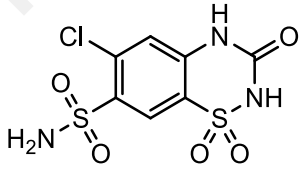

**Figure 4:23:** Mechanism of the formation of (a) DPH-209 and (b) DPH-226 through UV/chlorination of DPH.

### 4.4.2 TBPs of HCTZ

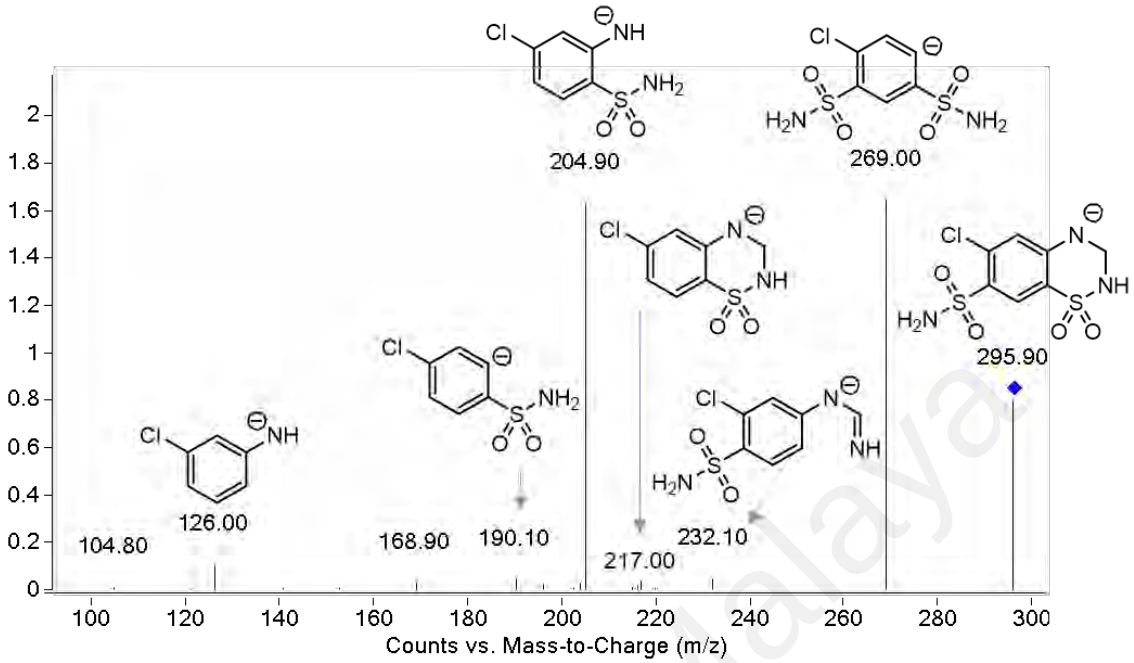
LC-QqQ-MS with ESI negative mode was used to analyze the TBPs from the chlorinated and UV/chlorination-treated HCTZ solutions. ESI negative mode was selected for analysis since HCTZ, and its TBPs formed dominant negative ions. In this study, three stable TBPs were detected for chlorination and UV/chlorination (Table 4.2). These TBPs were formed through chlorination, hydroxylation, and oxidation. HCTZ with the $[M-H]^-$ ion of *m/z* 295.9 shows six significant fragment ions at *m/z* 269.0, 232.1, 217.0, 204.9, 190.1 and 126.0. Fragments ion at *m/z* 269.0 was formed through the breakdown of the heterocyclic ring of HCTZ with the loss of $CH_2$ group and a nitrogen atom from the $[M-H]^-$ ion (Figure 4.24a). The fragment ions at *m/z* 232.1 and *m/z* 217.0 were formed through the loss of sulfur dioxide group of the heterocyclic ring and the sulfonamide group of the aromatic ring from the $[M-H]^-$ ion, respectively. Further fragmentation of fragment ion at

*m/z* 217.0 produced fragment ion at *m/z* 204.9 by the breakdown of the heterocyclic ring and the loss of $CH_2$ group. Fragment ion at *m/z* 190.1 was formed by the elimination of a NH group from fragment ion at *m/z* 204.9. Further fragmentation of fragment ion at *m/z* 204.9 produced fragment ion at *m/z* 126.0 by the elimination of sulfonamide group.

**Table 4.2:** Proposed transformation by-products of HCTZ during chlorination, UV irradiation and UV/chlorination.

| $[M-H]^-$ | MS/MS fragments | Structure of proposed TPBs [Label] | Chlorination | UV/ Chlorination | UV irradiation alone |
|---|---|---|---|---|---|
| 295.9 | 126.0, 190.1, 204.9, 217.0, 232.1, 269.0 |  [HCZT] | ✓ | ✓ | ✓ |
| 330.0 | 222.1, 250.1, 302.0 |  [HCTZ-330] | ✓ | ✓ | |
| 311.9 | 220.0, 232.0, 275.9 |  [HCTZ-312] | ✓ | ✓ | |
| 309.9 | 182.9, 230.0, 274.0 |  [HCTZ-310] | | ✓ | ✓ |

(a) HCTZ




(b) HCTZ-330

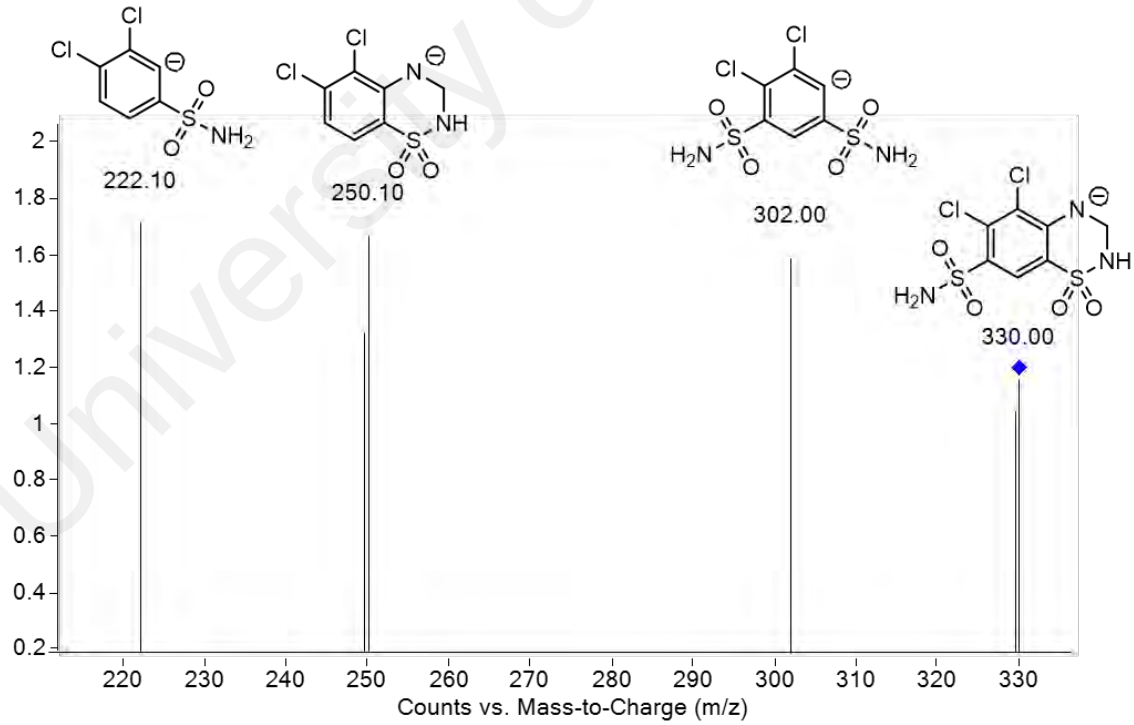
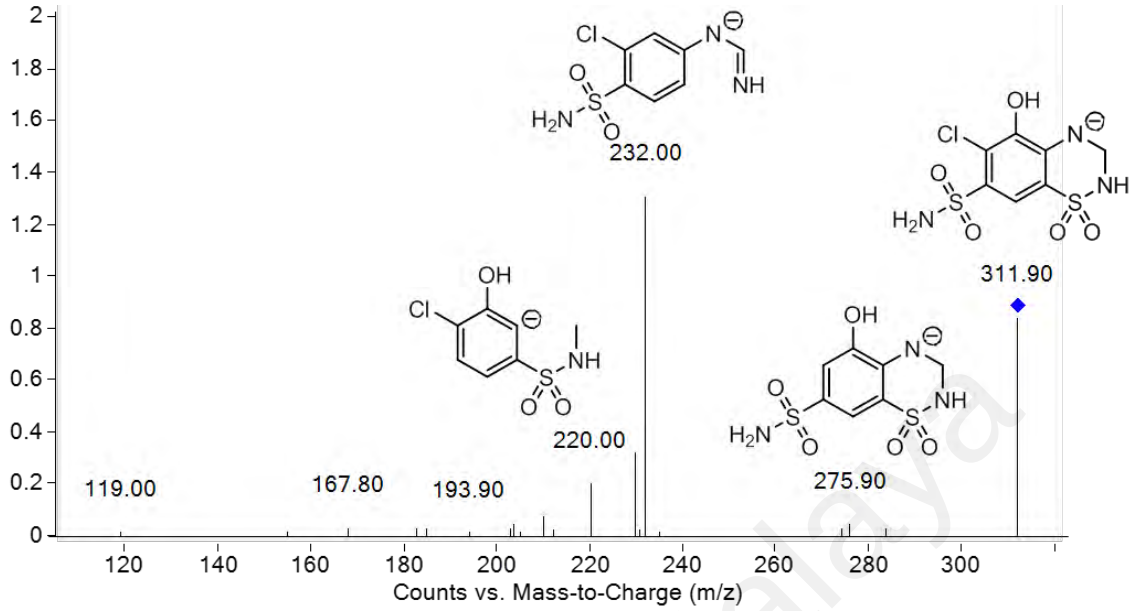



**Figure 4.24** MS/MS spectrum of HCTZ and its by-products.

(c) HCTZ-312




(d) HCTZ-310

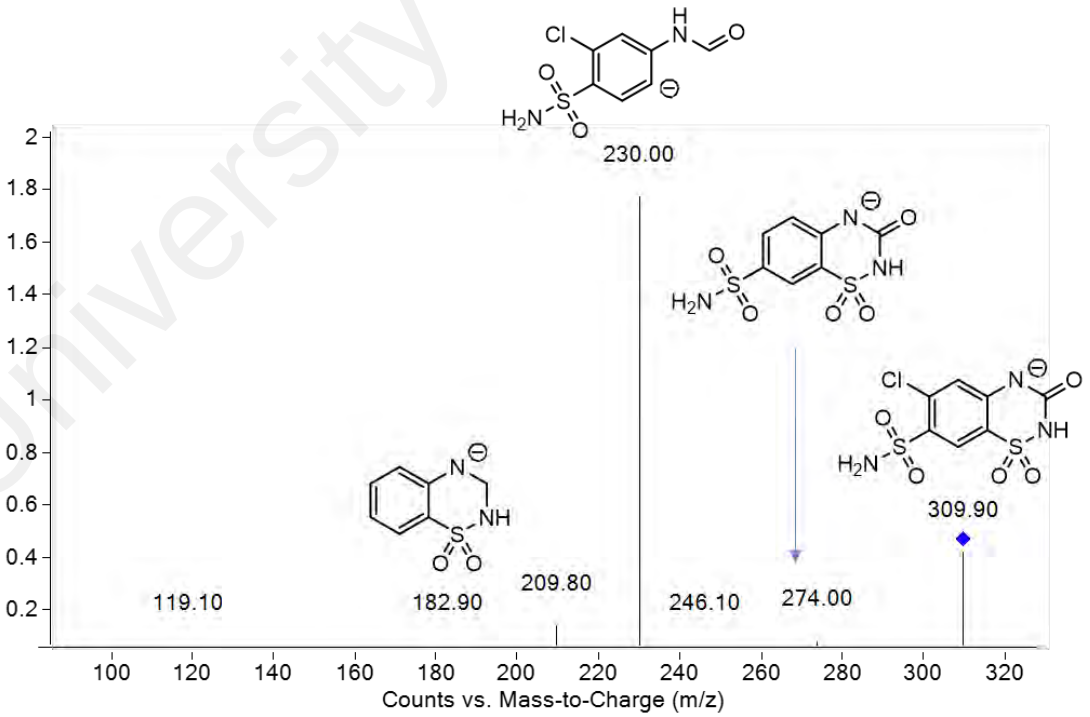



**Figure 4.24,** continued

During chlorination of HCTZ, two TBPs were detected. These TBPs were labelled as HCTZ-330 and HCTZ-312. HCTZ-330 with the $[M-H]^-$ ion of *m/z* 330.0 was the only chlorinated by-product from HCTZ. For this product, the addition of chlorine atom was proposed at the aromatic ring of HCTZ. Since chlorine is the ortho-para director and sulfonamide is the meta director, the addition of chlorine atom was proposed at $C_6$. The significant fragment ions for product ions of HCTZ-330 were detected at *m/z* 302.0, *m/z* 250.1 and *m/z* 222.1 (Figure 4.24b). As HCTZ, fragment ion at *m/z* 302.0 was formed through the breakdown of the heterocyclic ring with the loss of $CH_2$ group and a nitrogen atom from $[M-H]^-$ ion. The elimination of sulfonamide group from the aromatic ring of $[M-H]^-$ ion at $C_2$ formed fragment ion at *m/z* 250.1. Fragment ion at *m/z* 302.0 was also found to form ion at *m/z* 222.1 through the elimination of sulfonamide group at $C_4$ of the aromatic ring. The presence of *m/z* 302, 250.1 and 221.1 confirmed that the chlorine atom was added to the aromatic ring of HCTZ. HCTZ-312 was formed through the hydroxylation of HCTZ. As HCTZ-330, OH group was proposed to be added at the aromatic ring of HCTZ. For HCTZ-312, the most dominant fragment ion in the MS/MS spectrum was *m/z* 232.0. It formed through the breakdown of the heterocyclic ring of HCTZ-312 with the loss of sulfur dioxide and a hydroxyl group of the aromatic ring from $[M-H]^-$. Fragmentation of $[M-H]^-$ ion also produced fragment ion at *m/z* 275.9 by the elimination of chlorine atom. The fragment ion at *m/z* 220 was formed through the breakdown of the heterocyclic ring with the loss of nitrogen atom and a sulfonamide group of the aromatic ring from $[M-H]^-$. The presence of ion at *m/z* 220 confirmed the addition of OH group at the aromatic ring of HCTZ.

Figure 4.25 shows the proposed mechanism for the formation of HCTZ-330 and HCTZ-312. The reaction mechanism for the formation of HCTZ-330 and HCTZ-312 was proposed to start from the attack of an aromatic ring of HCTZ to the positively polarized chlorine atom of HOCl forming a monochlorinated carbocation (***HCTZ-I***).

Rearrangement of ***HCTZ-I*** through the elimination of hydrogen ion forms HCTZ-330. HCTZ-312 was proposed to form from HCTZ-330 via substitution reaction. This reaction was proposed to start with the addition of $OH^-$ to the aromatic ring of HCTZ-330 to from ***HCTZ-II***. Rearrangement of ***HCTZ-II*** through the loss of chloride ion forms HCTZ-312.

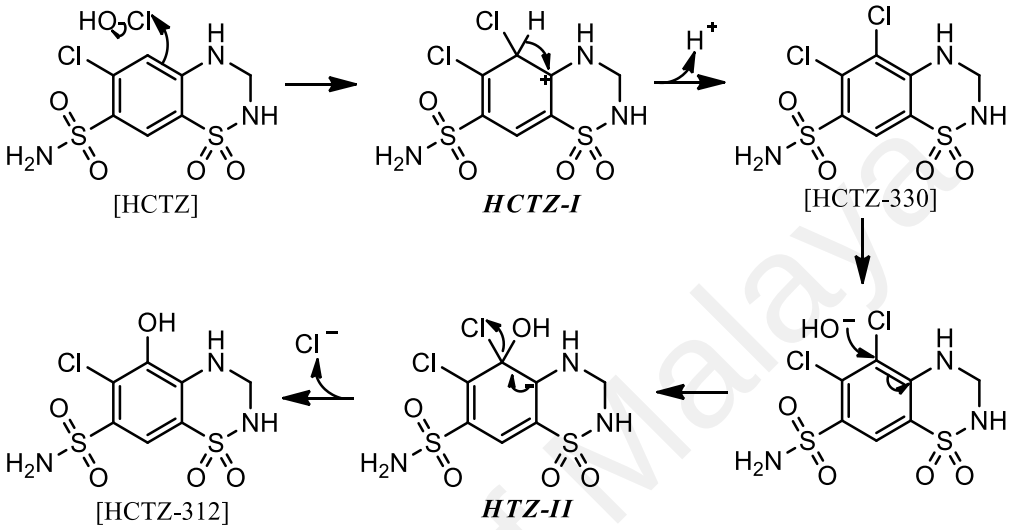



**Figure 4.25:** Mechanism of the formation of HCTZ-312 through chlorination of HCTZ.

During UV/chlorination of HCTZ, three TBPs were detected, namely HCTZ-330, HCTZ-312, and HCTZ310. HCTZ-330 and HCTZ-312 were formed due to the reaction between HCTZ and HOCl as shown in Figure 4.25. HCTZ-310 was an additional TBP formed due to the radical reaction on HCTZ. This reaction was proven when HCTZ-310 was also detected during exposure of HCTZ with UV irradiation. The MS/MS spectrum of HCTZ-310 with $[M-H]^-$ ion of *m/z* 309.9 showed three significant fragment ions at *m/z* 274.0, 230.0 and *m/z* 182.9 (Figure 4.24d). Fragment ion at *m/z* 274.0 were produced from the elimination of the chlorine atom. Then, further fragmentation of fragment ion at *m/z* 274.0 formed fragment ion at *m/z* 182.9 by the elimination of sulfonamide group of the aromatic ring and an oxygen atom of the heterocyclic ring. The presence of *m/z* 182.9 further confirmed the presence of carbonyl group at C9. Fragments ion at *m/z* 230.0 was formed through the breakdown of the heterocyclic ring with the loss of sulfonamide group

and oxygen atom from $[M-H]^-$ ion**.** Figure 4.26 shows the proposed mechanism for the formation of HCTZ-310. The mechanism for the formation of HCTZ-310 is proposed to start with hydrogen abstraction by •OH to forms radical ***HCTZ-III***. Radical ***HCTZ-III*** could react with dissolved oxygen to form peroxy radical ***HCTZ-IV*** (von Sonntag, 2006). Further rearrangement of ***HCTZ-IV*** through the elimination of •OH forms HCZT-309.

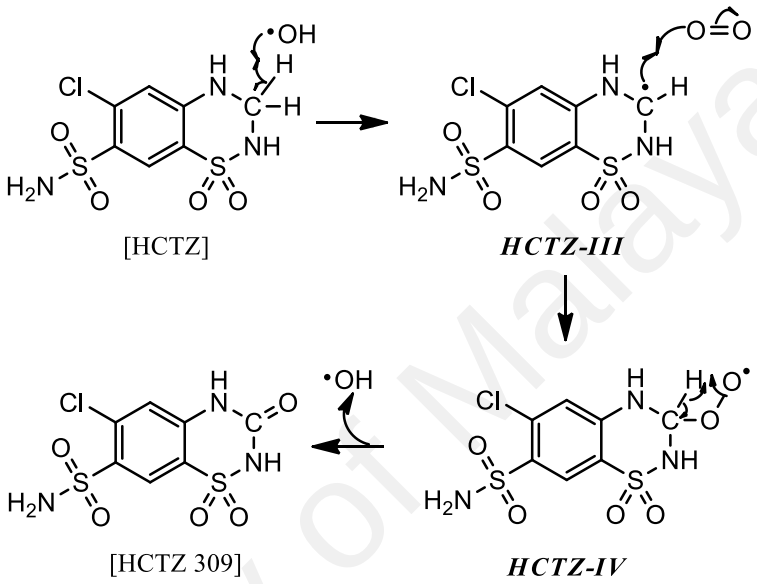
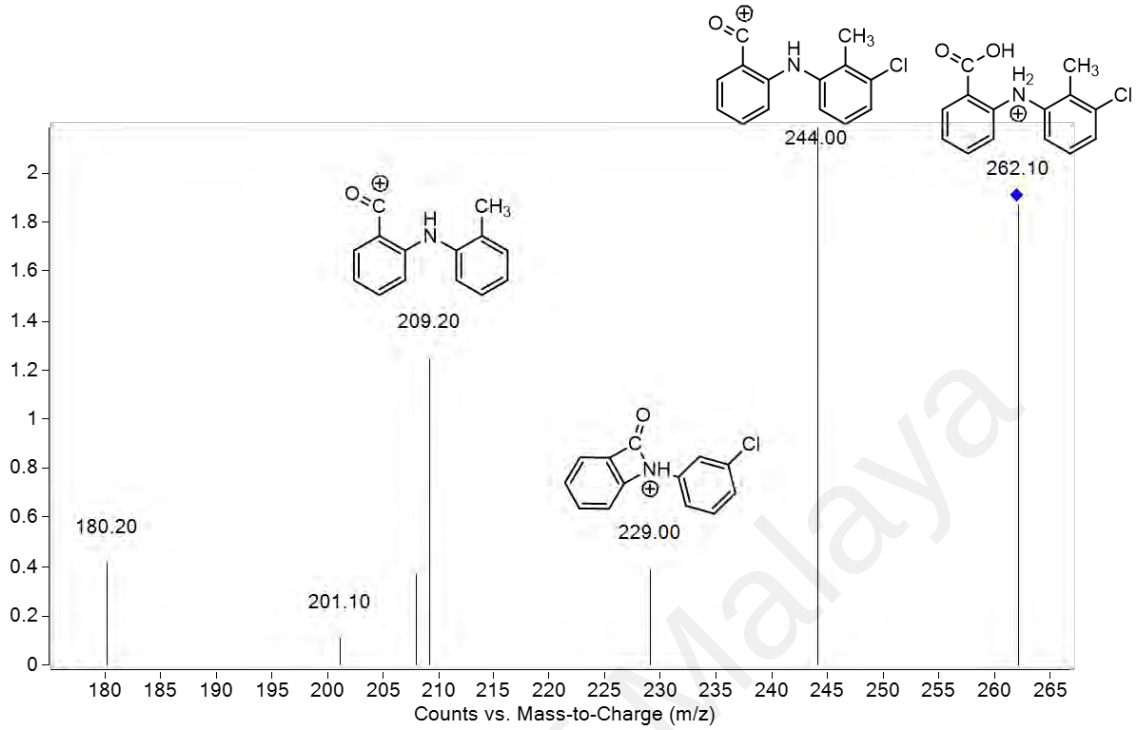



**Figure 4.26:** Mechanism of the formation of HCTZ-309 through UV/chlorination of HCTZ.

### 4.4.3 TBPs of TOL

The mass spectra of TOL with $[M+H]^+$ ion of *m/z* 262.1 showed three significant fragment ions at *m/z* 244.0, 229.0 and 209.2 (Figure 4.27a). The fragment ion at *m/z* 244.0 was produced from the loss of hydroxyl group from the $[M+H]^+$ ion. Further fragmentation of the fragment ion at *m/z* 244.0 through the loss of a methyl group and cyclization formed ion at *m/z* 229.0. Ion at *m/z* 209.2 was formed through the loss of a chlorine atom from ion at *m/z* 244.

(a) TOL




(b) TOL-173

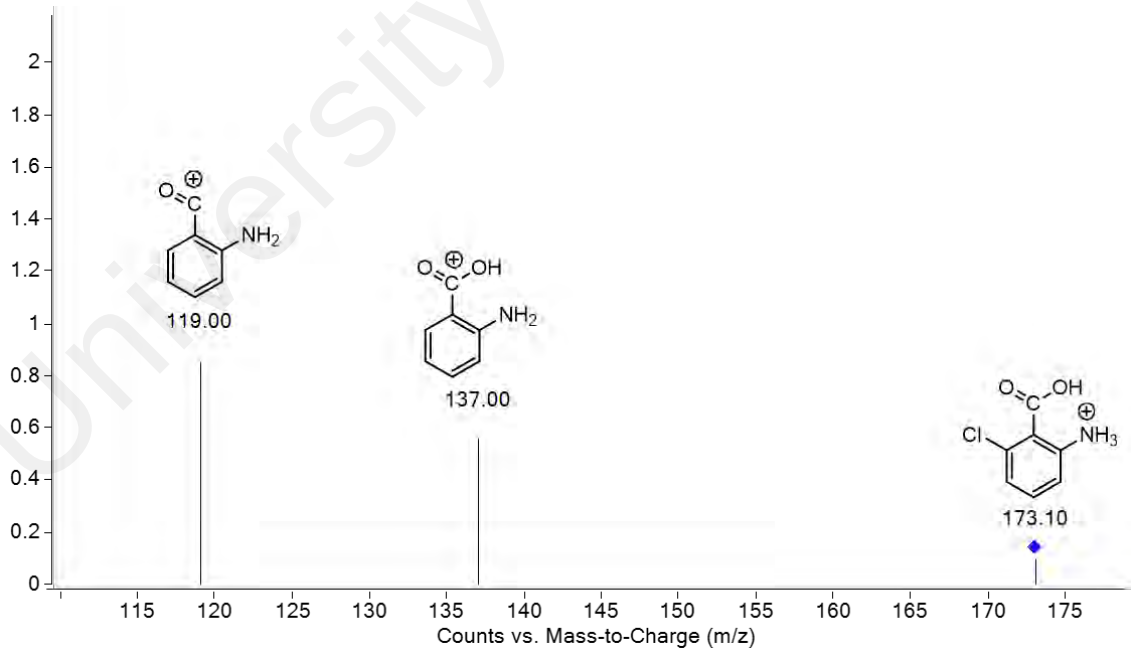



**Figure 4.27:** MS/MS spectrum of TOL and its by-products.

(c) TOL-297

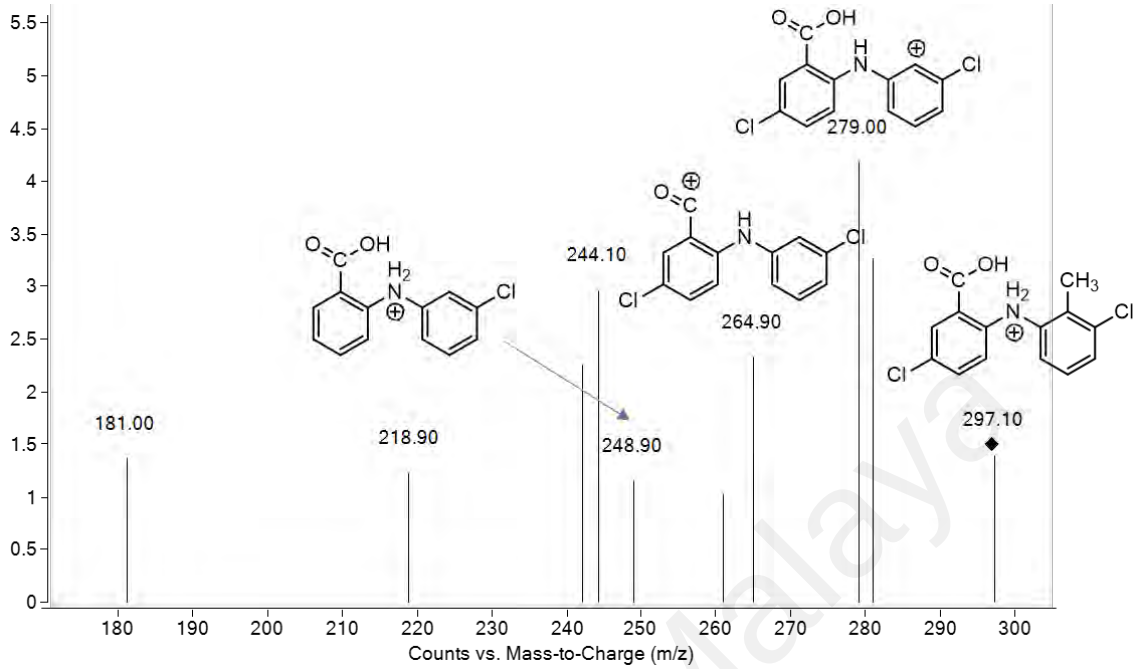



**Figure 4.27,** continued

**Table 4.3:** Proposed transformation by-products of TOL during chlorination, UV irradiation and UV/chlorination.

| $[M-H]^-$ | MS/MS fragments | Structure of proposed TPBs [Label] | Chlorination | UV/ Chlorination | UV irradiation alone |
|---|---|---|---|---|---|
| 262.1 | 209.2, 229.0 244.0 | [TOL] | ✓ | ✓ | ✓ |
| 173.1 | 119.0, 137.0 | [TOL-173] | ✓ | ✓ | ✓ |
| 297.1 | 248.0, 264.9, 279.0 | [TOL-297] | ✓ | ✓ | |

During chlorination and UV/chlorination, two stable TBPs of TOL were detected (Table 4.3). These by-products were TOL-173 (Figure 4.27b) with $[M+H]^+$ ion of *m/z* 173.1 and TOL-297 (Figure 4.27c) with $[M+H]^+$ ion of *m/z* 297.1. For TOL-173, the mass spectrum showed two significant fragment ions at *m/z* 137.0 and *m/z* 119.0. The fragment ion at *m/z* 137.0 was produced through the loss of a chlorine atom from the $[M+H]^+$ ion of TOL-173. Further fragmentation of the fragment ion at *m/z* 137.0 through the loss of hydroxyl group formed the fragment ion at *m/z* 119.0. TOL-297 is a monochlorinated TOL. For this by-product, the addition of chlorine atom was proposed at the aromatic ring A (Table 4.3). This is because aromatic ring A is more electron rich than aromatic ring B, hence aromatic ring A is expected to be more favorable to react with HOCl. Since the carboxylic group is a meta director, hence the addition of chlorine atom was proposed at $C_{11}$. This by-product showed three significance ions at *m/z* 279.0, *m/z* 264.9 and *m/z* 248.9. The fragment ion at *m/z* 279.0 was produced from the loss of methyl group. Further fragmentation of the fragment ion at *m/z* 279.0 through the loss hydroxyl group and chlorine group formed fragment ion at *m/z* 264.9 and *m/z* 248.9, respectively.

The proposed mechanism for the formation of TOL-297 is presented in Figure 4.28. The mechanism for the chlorination is proposed to start with the attack of the aromatic ring A of TOL to the positive chlorine atom of HOCl, forming a monochlorinated carbocation (***TOL-I***). Rearrangement of intermediate ***TOL-I*** through the elimination of one hydrogen atom forms TOL-297. TOL-297 was also detected in UV/chlorination process. This result further showed the involvement of HOCl in the reaction that produced TOL-297 during UV/chlorination. The mechanism for the formation of TOL-173 is proposed to start from the formation of highly reactive intermediate ***TOL-II***. Chloramine ***TOL-II*** is unstable, and it tends to decompose to form ***TOL-III***. Then, ***TOL-III*** is proposed to react with positively charge chlorine atom of HOCl forming a ***TOL-IV***. Rearrangement of intermediate ***TOL-IV*** through the

elimination of hydrogen ion forms TOL-173. TOL-173 was also detected when TOL was treated with UV irradiation. This result showed that TOL-173 also could be produced via the radical pathway. For radical pathway, the formation of TOL-173 is proposed to start with hydrogen abstraction at N of TOL by •OH to form radical ***TOL-V*** (Figure 4.28b). Radical ***TOL-V*** could react with TOL and forms radical ***TOL-VI. TOL-III*** forms after hydrogen abstraction at $N_7$ of radical ***TOL-VI***. Then, the aromatic ring of ***TOL-III*** is proposed to react with positively charge chlorine atom of HOCl to form ***TOL-IV***. Rearrangement of intermediate ***TOL-IV*** through the elimination of hydrogen atom forms TOL-173.

(a)

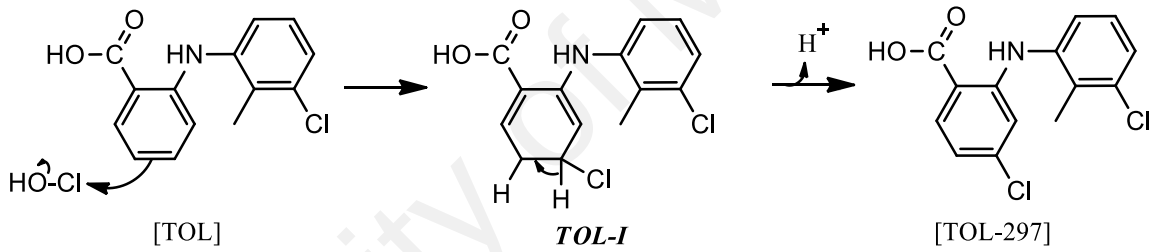



**Figure 4.28:** Mechanism of the formation of (a) TOL-295 and (b) TOL-171 through chlorination and UV/chlorination of TOL.

(b)

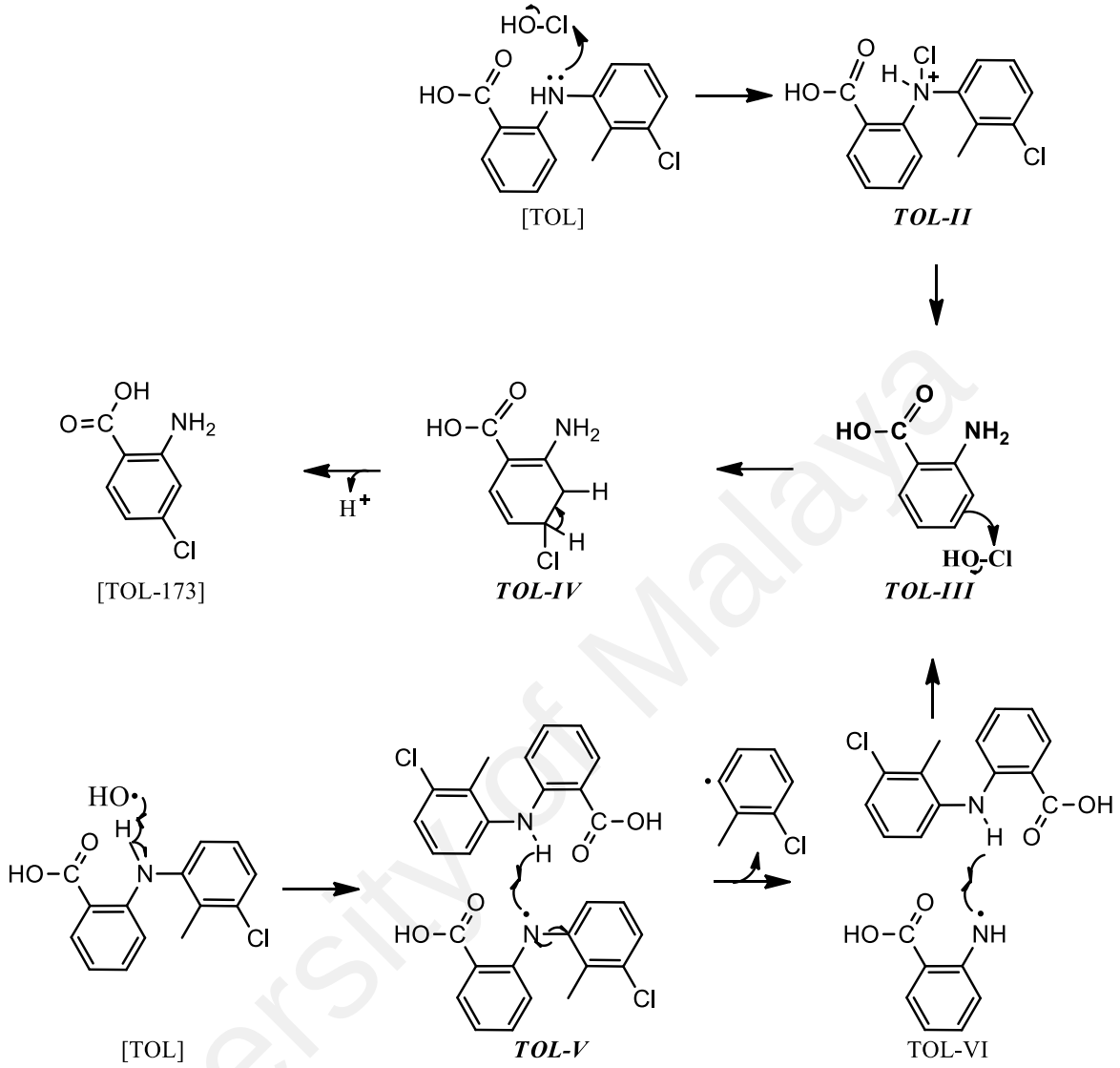



**Figure 4.28,** continued

## 4.5 Ecotoxicity of selected pharmaceuticals after chlorination and UV/chlorination treatments

In this study, the toxicity of selected pharmaceuticals before and after treating with chlorination and UV/chlorination was evaluated using ToxTrak test kit. ToxTrak test has been widely used in water treatment industry to determine the toxicity of drinking water, wastewater and natural waters (Liwarska-Bizukojc et al., 2016). This toxicity determination was based on colorimetric measurement of the percent inhibition of *E. Coli*

activity. In this study, the toxicity of selected pharmaceuticals solution (as control), chlorination and UV/chlorination treated pharmaceuticals solution is presented in the percent inhibition (% Inhibition) of *E. Coli* activity. In addition, the ECOSAR software was also used to determine the toxicity of detected TBPs that generated during chlorination and UV/chlorination process. ECOSAR is a free computer program, which used available toxicity data to predict the acute and chronic toxicity of chemicals by using Structure Activity Relationships (SARs) and Quantitative Structure Activity Relationships (QSARs) (Reuschenbach et al., 2008; USEPA, 2014). Based on the predicted ecotoxicity values, selected pharmaceuticals and its TBPs were classified based on toxicity according to the system established by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) as shown in Table 4.4.

**Table 4.4** Toxicity classification according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

| Toxicity range (mg/L) | Class |
|---|---|
| LC50/EC50/ChV ≤ 1 | Very toxic |
| 1 < LC50/EC50/ChV ≤ 10 | Toxic |
| 10 < LC50/EC50/ChV ≤ 100 | Harmful |
| LC50/EC50/ChV > 100 | Not harmful |

According to Figure 4.29, untreated DPH solution was found to be toxic toward the *E. coli*. For the chlorination, DPH solution was exposed to FAC for 24 h. Chlorination was found to be able to reduce the toxicity of DPH. However, the chlorination treated DPH solution remained toxic. The toxicity of UV/chlorination treated DPH solution was found to be lower than the chlorination treated DPH solution. However, the toxicity of DPH solution was found to remain significant after exposing to 5 min of UV/chlorination treatment. By increasing the reaction time to 10 min, the toxicity of DPH solution was

further reduced to the non-toxic level. This result showed that the toxicity of DPH could be reduced by increasing the reaction time of UV/chlorination process. This result might be due to the removal of toxic DPH and its TBPs when the reaction time was prolonged.

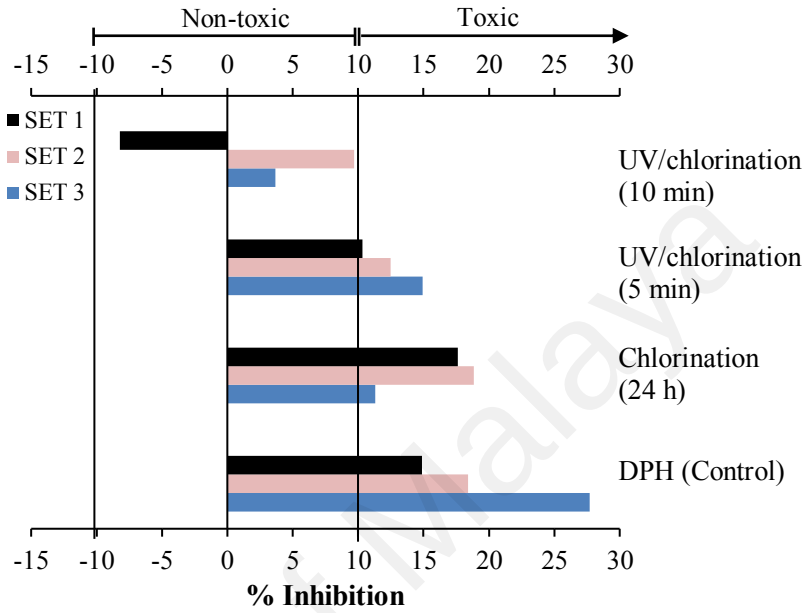



**Figure 4.29:** Percent inhibition of DPH after chlorination, UV irradiation and UV/ chlorination at pH 7.

In Section 4.4.1, chlorination of DPH was found to produce DPH-182 and DPH-181 as TBPs. Meanwhile, UV/chlorination process was found to produce additional three TBPs, namely, DPH-183, DPH-209, and DPH-226. Based on ECOSAR program, the ecotoxicity was presented as $LC_{50}$ values (concentration of tested chemical that is lethal to half of fish and daphnia population after 96 h and 48 h of exposure) and $EC_{50}$ values (concentration of tested chemical that inhibits the growth of 50% of green algae after 96 h of exposure). The ecotoxicity can be classified into acute and chronic toxicity. Acute toxicity refers to the adverse effects of a substance that that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours). Meanwhile, chronic toxicity describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time (more than 0.1 of the expected age of the organism).

According to the acute toxicity (Table 4.5), DPH was found to be non-toxic to fish but it is harmful to both daphnids and algae. For chronic toxicity, DPH was found to be harmful to all tested organism. Most of the detected TBPs were found to be harmful and toxic to all tested organisms. For acute toxicity, DPH-182 and DPH-183 were found to be harmful to all tested organisms. DPH-226 was found to be not harmful to fish. However, it was found to be harmful to both daphnids and algae. DPH-181 was found to be harmful to fish and algae. This compound was found to be toxic to the daphnids. For chronic toxicity, DPH-226 was found to be harmful to all tested organisms. However, DPH-181, DPH-182, and DPH-183 were found to be toxic to all tested organisms. DPH-226 was classified as a very toxic compound to all tested organisms. These results showed that both chlorination and UV/chlorination have the potential to produce TBPs which are more toxic than DPH. Although most of this TBPs were found to be toxic, however, its concentration might too low to produce the toxic effect to *E. Coli*.

**Table 4.5:** Predicted acute toxicity and chronic toxicity of DPH and TBPs proposed for DPH.

| Compound | Acute toxicity (mg/L) | | | Chronic toxicity (mg/L) | | |
|---|---|---|---|---|---|---|
| | Fish ($LC_{50}$) | Daphnids ($LC_{50}$) | Algae ($LC_{50}$) | Fish | Daphnids | Algae |
| DPH | 148.5 | 85.6 | 67.9 | 14.8 | 85.6 | 67.8 |
| DPH-182 | 40.6 | 24.4 | 23.1 | 4.3 | 2.8 | 6.9 |
| DPH-183 | 35.1 | 21.3 | 20.5 | 3.7 | 2.5 | 6.3 |
| DPH-226 | 110.2 | 64.1 | 52.6 | 11.1 | 6.7 | 14.5 |
| DPH-181 | 13.9 | 8.8 | 10.1 | 1.5 | 1.2 | 3.4 |
| DPH-209 | 2.1 | 1.5 | 2.5 | 0.3 | 0.2 | 0.9 |

Not harmful  Harmful  Toxic  Very toxic

Based ToxTrak test, HCTZ was found to be non-toxic. This result is in agreement with the result that reported by Jacob et al. (2016). Jacob et al. (2016) found that HCTZ was non-toxic to *Aliivibrio fischeri*. Meanwhile, HCTZ treated with chlorination for 24 h was found to be toxic. The toxicity of HCTZ solution that treated with UV/chlorination for 5 min was found to be non-toxic. However, the toxicity of HCTZ solution was found

to increase to toxic level after 10 min of treatment. The toxicity of HCTZ solution was toxic during UV/chlorination due to the reaction between UV and HCTZ. As shown in Figure 4.30, the % inhibition of HCTZ solution treated with UV was increased when the reaction time was increased from 10 to 30 min. HCTZ solution was exposed to UV for 5 – 30 min to verify the reaction that contributed to the toxicity of HCTZ during UV/chlorination. The result showed that the toxicity of HCTZ solution that exposed to UV was also increased with reaction time. This result indicated that the TBPs that generated from the radical reaction particularly, the reaction that involved the •OH, were contributed to the toxicity of the HCTZ solution.

In this study, three TBPs were identified for HCTZ. These TBPs are HCTZ-330, HCTZ-312, and HCTZ-310 (Section 4.4.2). The predicted ecotoxicity of HCTZ and its TBPs was tabulated in Table 4.6. According to ECOSAR (Table 4.6), HCTZ and its TBPs were not harmful to fish, daphnia, and algae. Hence, the toxicity of HCTZ solution treated with UV/chlorination is proposed to be contributed by TBPs such as organic acids that generated from the radical reaction which could not be detected by the selected analytical method.

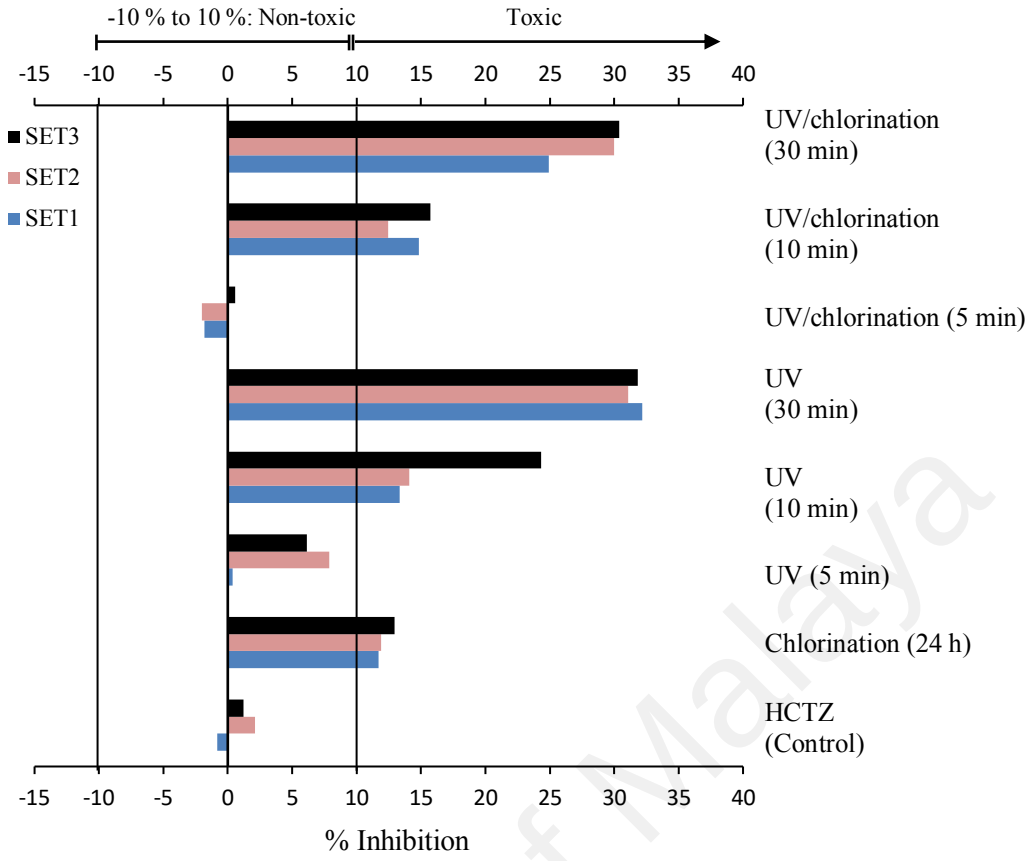



**Figure 4.30** Percent inhibition of HCTZ after chlorination, UV irradiation and UV/ chlorination at pH 7.

**Table 4.6:** Predicted acute toxicity and chronic toxicity of HCTZ and TBPs proposed for HCTZ.

| Compound | Acute toxicity (mg/L) | | | Chronic toxicity (mg/L) | | |
|---|---|---|---|---|---|---|
| | Fish ($LC_{50}$) | Daphnids ($LC_{50}$) | Algae ($LC_{50}$) | Fish | Daphnids | Algae |
| HCTZ | 18684.1 | 8741.7 | 2924.6 | 1453.4 | 497.4 | 497.7 |
| HCTZ -330 | 5497.8 | 2729.0 | 1168.1 | 458.8 | 183.3 | 226.9 |
| HCTZ -312 | 53146.2 | 23786.9 | 6625.0 | 3923.6 | 1196.3 | 1021.3 |
| HCTZ -310 | 6218.4 | 3062.1 | 1265.8 | 531.8 | 200.9 | 241.4 |

Not harmful  Harmful  Toxic  Very toxic

According to Figure 4.31, the untreated TOL and TOL treated with chlorination for 24 h was found to be non-toxic. Meanwhile, TOL solution that treated with UV/chlorination for 5 and 10 min was found to be non-toxic. However, the TOL solution treated with UV/chlorination for 30 min was found to be toxic. Similar to HCTZ, the toxicity of TOL solution was found to increase with reaction time when it was exposed

to UV. This result also indicated that the TBPs that generated from the radical reaction particularly, the reaction that involved the •OH, were contributed to the toxicity of the TOL solution.

TOL-173 and TOL-297 were two main TBPs formed during chlorination and UV/chlorination process of TOL. Based on predicted ecotoxicity by using ECOSAR program (Table 4.7), TOL was found to be toxic and harmful to the tested organisms. TOL-173 was found to be not harmful and harmful to the tested organisms for acute and chronic toxicity, respectively. Meanwhile, TOL-297 was found to be the most toxic TBP for TOL. This compound was far more toxic than TOL.

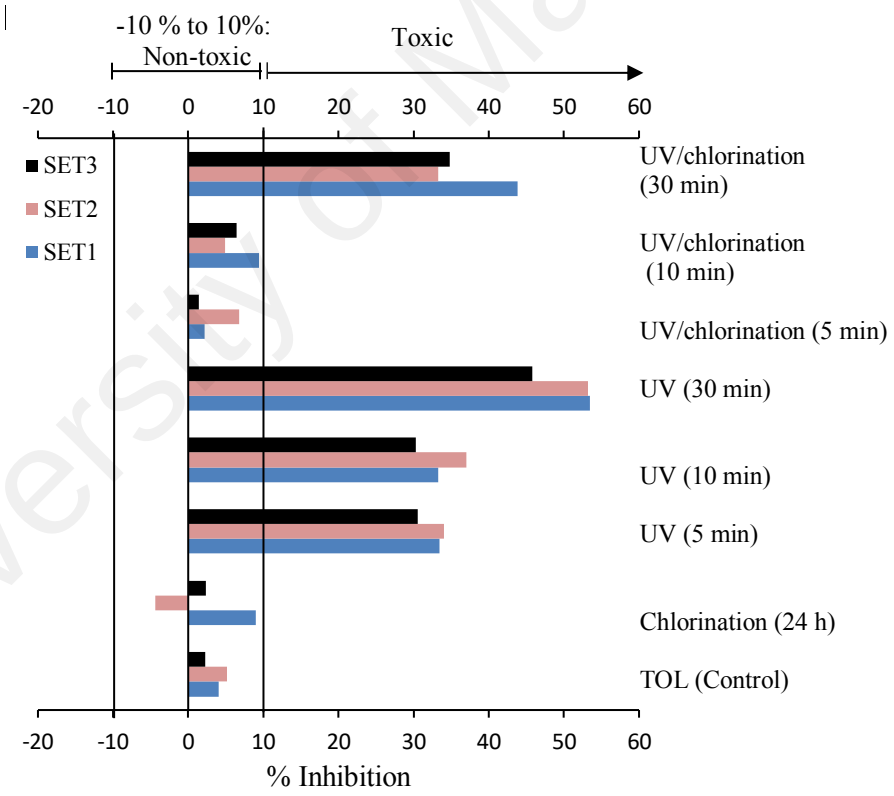



**Figure 4.31** Percent inhibition of TOL after chlorination, UV irradiation and UV/ chlorination at pH 7.

**Table 4.7:** Predicted acute toxicity and chronic toxicity of TOL and TBPs proposed for TOL.

| Compound | Acute toxicity (mg/L) | | | Chronic toxicity (mg/L) | | |
|---|---|---|---|---|---|---|
| | Fish ($LC_{50}$) | Daphnids ($LC_{50}$) | Algae ($LC_{50}$) | Fish | Daphnids | Algae |
| TOL | 2.00 | 1.55 | 4.20 | 0.28 | 0.36 | 2.20 |
| TOL-173 | 665.70 | 352.68 | 197.25 | 59.96 | 28.36 | 44.27 |
| TOL-297 | 0.60 | 0.49 | 1.70 | 0.09 | 0.14 | 1.02 |

Not harmful  Harmful  Toxic  Very toxic

The results of the ecotoxicity studies showed that chlorination of selected pharmaceuticals has the potential to produce toxic solutions, especially for the HCTZ and DPH. However, UV/chlorination which is effective in removing the selected pharmaceuticals was found to produce effluent with higher toxicity than the untreated pharmaceuticals. In general, the result showed that the toxicity of DPH could be reduced by prolonging treatment of UV/chlorination. However, the toxicity of HCTZ and TOL was found to be increased after UV/chlorination treatment. Both HCTZ and TOL are chlorinated compounds. Since some of the TBPs that generated from the UV/chlorination treatment of HCTZ and TOL were non-toxic, it is suggested that the low molecular weight chlorinated organic acids which were not detected during the TBPs identification were suggested as the substances that contribute to the toxicity. According to Warnecke and Gill (2005), the growth of *E. coli* can be inhibited by the chlorinated organic acids such as monochloroacetic acid and dichloroacetic acid. It has been reported that halogenated acetic acids can damage the DNA of *E. Coli* (Giller et al., 1997).

# CHAPTER 5: CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORKS

## 5.1 Conclusions

In this study, the kinetics and mechanisms of the degradation of DPH, HCTZ, and TOL by using chlorination and UV/chlorination were examined. For chlorination reaction, the $k_{app}$ of selected pharmaceuticals were determined using pseudo-first order kinetic model at the pH ranging from 5 to 8. There is three different trends of variation of $k_{app}$ with pH was observed for the selected pharmaceuticals. The variation of $k_{app}$ was mainly influenced by the αHOCl and the degree of dissociation of the selected pharmaceuticals. For DPH, the $k_{app}$ was found to increase from pH 5 to 6 due to the anion species of DPH, which are more reactive toward HOCl as compared to the neutral form of DPH. However, the $k_{app}$ was found to decrease after pH 6. The decreased of $k_{app}$ for DPH with pH was mainly due to the decreased of αHOCl. For HCTZ, $k_{app}$ was found to increase when the pH was increased from 5 to 8. This was due to the presence of more reactive anionic HCTZ. The molar fraction of anionic HCTZ was also found to increase with increasing pH. For TOL, the $k_{app}$ of TOL was found to decrease with increasing pH from 5 to 8. This result was mainly due to the decreased of available HOCl.

For UV/chlorination of selected pharmaceuticals, the effect of operating conditions, which can largely influence the efficiency of treatment method, was evaluated. This experiment was started by comparing the efficiency of UV/chlorination in the removal of DPH, HCTZ and TOL with chlorination of UV irradiation. The results showed that the chlorination process was not effective in removing DPH, HCTZ and TOL with only 6.1 to 36.2% removal. Meanwhile, UV irradiation reaction showed slightly higher removal efficiency of the selected pharmaceutical with 12.8 to 36.6% as compared with chlorination. By using UV/chlorination, the removal of selected pharmaceuticals was enhanced 62.6 to 79.8%. This result indicated that the presence of UV irradiation during chlorination has significantly improved the efficiency of pharmaceuticals removal

due to the formation of radicals. The effects of pH and chlorine dosage on the removal of selected pharmaceuticals during UV/chlorination were evaluated. The effect of chlorine dosage on the selected pharmaceuticals removal was evaluated at the different initial concentration of FAC ranging from 0 to 750 μM. The removal of selected pharmaceuticals was found to increase when the concentration of FAC was increased from 0 to 750 μM. This was due to the increased of FAC dosage that enhanced the production of radical species, which accelerated the selected pharmaceuticals degradation. The effect of pH on the removal of selected pharmaceuticals was evaluated at pH ranging from 5 to 8. The removal of selected pharmaceuticals was found to be more efficient under the acidic condition as compared to neutral and basic conditions. The removal of selected pharmaceuticals is higher at acidic conditions due to the higher molar fraction of HOCl as compared to $OCl^-$ at 254 nm light and ambient temperature. Therefore, the formation of reactive radicals increased and consequently, enhanced the removal of selected pharmaceuticals.

The chlorination and UV/chlorination of selected pharmaceuticals was found to produce various TBPs. The TBPs present during chlorination and UV/chlorination were difference according to the reaction between selected pharmaceuticals with HOCl and •OH. During chlorination, the selected pharmaceuticals were found to react with HOCl via hydroxylation, oxidation and chlorination reactions. Meanwhile, TBPs that identified during UV/chlorination were formed through reaction with both HOCl and •OH. The ecotoxicity of TBPs was predicted individually using ECOSAR software. On the other hand, the ecotoxicity of the chlorination and UV/chlorination treated DPH, HCTZ, and TOL in *E. Coli* was evaluated using ToxTrak test kit. The computational method showed that both chlorination and UV/chlorination of selected pharmaceuticals showed the potential to produce TBPs with the toxicity higher than its parent compounds. However, the toxicity of the TBPs was found to be insignificant in contributing to the toxic effluent

after treatment due to the low concentration or different in the tested organism. Meanwhile, UV/chlorination of TOL and HCTZ solution was found to produce the toxic solution. The toxicity of the solution was suggested to be contributed by low molecular weight TBPs. The potential toxic TBPs were chlorinated organic acids, which were not, detected using the selected analytical method. Chlorinated organic acids have been reported to be toxic to *E. Coli*.

In conclusion, UV/chlorination showed a great potential in treating waters that containing pharmaceuticals. It showed great ability in removing pharmaceuticals, which are resistant against chlorination process. However, this method also showed the potential to produce toxic TBPs and the toxic effluent. In order to allow the application of UV/chlorination in real water treatment, further evaluation, particularly in toxicity of the effluent, needs to be evaluated carefully.

## 5.2 Suggestions for future works

In the future research, the following topics are recommended:

- Assessment of the ecotoxicity of the TBPs that formed during UV/chlorination and chlorination of other pharmaceuticals.
- Assessment of application of UV/chlorination in real water and wastewater treatment process.

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RESEARCH ARTICLE

# Degradation of 5,5-diphenylhydantoin by chlorination and UV/chlorination: kinetics, transformation by-products, and toxicity assessment

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**Abstract** This study investigated the reaction kinetics and mechanism of the degradation of 5,5-diphenylhydantoin (DPH) during conventional chlorination and UV/chlorination. DPH is one of the antiepileptic drugs, which has frequently been detected in the aquatic environment. For chlorination, the second-order rate constant for the reaction between DPH and free active chlorine (FAC) was determined at pH 5 to 8. At pH 6 to 8, the efficiency of chlorination in the removal of DPH was found to be dominated by the reaction involving hypochlorous acid (HOCl). The result also showed that anionic species of DPH was more reactive toward FAC as compared with neutral DPH. For UV/chlorination, the effect of FAC dosage and pH on the degradation of DPH was evaluated. UV/chlorination is a more effective method for removing DPH as compared with conventional chlorination and UV irradiation. The DPH degradation rate was found to increase with increasing FAC concentration. On the other hand, the degradation of DPH was found to be more favorable under the acidic condition. Based on the identified transformation by-products, DPH was found to be degraded through the reaction at imidazolidine-2,4-dione moiety of DPH for both chlorination and UV/chlorination. Toxicity study on the chlorination and UV/chlorination-treated DPH solutions suggested that UV/chlorination is a more efficient method for reducing the toxicity of DPH.

**Keywords** Pharmaceuticals · Water treatment · Advanced oxidation processes · Chemical oxidation · Degradation · PPCPs

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## Introduction

The presence of pharmaceuticals in the aquatic environment has gained increasing attention in recent years. As emerging contaminants, some pharmaceuticals in the environment have been found to produce toxic effects to the living organism (Molinari et al. 2017; Culleres 2015). These toxic effects are such as producing antibiotic resistant bacteria, genotoxicity, and endocrine disruption (Pruden et al. 2006; Halling-Sorensen et al. 1998; Kümmerer 2001; Sumpter 1998). Most of the consumed pharmaceuticals by human are not completely metabolized, and it is excreted into the wastewater which is later treated by wastewater treatment plants. The presence of pharmaceuticals has posed a great challenge to conventional wastewater treatment plants to remove the pharmaceuticals from wastewater as the currently available conventional wastewater treatments plants are not designed for this purpose (Fatta-Kassinos et al. 2011). As a result, numerous studies have shown that pharmaceuticals are present in the effluent of conventional wastewater treatment plants (Gabet-Giraud et al. 2014).

Chlorination is one of the commonly used disinfection methods in water treatment. It also has been used to treat the taste, color, and odor of water (Xiang et al. 2016). Chlorination has been widely used in water treatment plants of many countries due to its low cost (Nam et al. 2015). During water treatment, pharmaceuticals, which escaped from the physicochemical and biological treatments, are often