Thermal Degradation of Antibiotic Residues: Amphenicols as a Case Study

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ABSTRACT

Veterinary drug residues, though carefully regulated, remain commonly detected in food of animal origin (meat, seafood, dairy products etc.). Worryingly, new pharmaceutically active contaminants are continuously being detected in food. Recently, human pharmaceuticals and other antimicrobial residues occurring as environmental contaminants in aquatic systems were observed to accumulate in various seafood. At the same time, most foods of animal origin are cooked before consumption. In terms of food safety, it is therefore necessary to understand the fate of drug residues in the food supply chain, notably during thermal processing.

Amphenicols (e.g. florfenicol, chloramphenicol) are one class of antibiotics commonly reported in fish/seafood and aquatic system worldwide. In this study, the thermal degradation kinetics of amphenicols were explored using high performance liquid chromatography triple quadrupole tandem mass spectrometry (HPLC-QqQ-MS/MS). Results indicated that the chloramphenicol and florfenicol followed the first-order degradation kinetic, and the degradation rate constant k increased with temperature increase. Then, the identity of thermal degradation products was investigated through two strategies. First, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was applied to investigate the volatile degradation products of three antibiotics and three additional pharmaceuticals. Chloramphenicol, lincomycin, gemfibrozil and diphenhydramine were shown to degrade into a range of previously unidentified volatile compounds. No volatile degradation products were identified for florfenicol. Overall, Py-GC-MS was a fast and effective method to explore the thermal degradation of pharmaceuticals. Finally,

HPLC coupled with high resolution tandem mass spectrometry (in our case HPLC-QTOF-MS/MS) coupled with bioinformatics was applied to identify the thermal degradation products of chloramphenicol in water and to confirm the degradation products in cooked mussels. This approach allowed to find previously unidentified degradation by-products. This work further highlights the need to take into account the impact food processing for the food safety risk assessment of amphenicol antibiotics.

RÉSUMÉ

Des résidus de médicaments vétérinaires, bien qu'ils soient strictement régulés, sont régulièrement détectés dans les aliments d'origine animale (viande, poisson et fruits de mer, produits laitiers…). De manière inquiétante, de nouveaux résidus de médicaments sont continuellement détectés dans les aliments. Récemment, on a observé que divers médicaments et produits antimicrobiens, contaminant les milieux aquatiques, pouvaient s'accumuler dans les poissons et fruits de mer. En termes de sécurité sanitaire des aliments, il est nécessaire de comprendre le devenir des résidus de médicaments dans la chaine de production agroalimentaire, notamment au cours des traitements thermiques.

Les phénicols (chloramphénicol, florfénicol) constituent une famille d'antibiotiques détectés internationalement dans le poisson, fruits de mer et systèmes aquatiques. Dans la présente étude, la cinétique de dégradation thermique des phénicols a été étudiée en utilisant la chromatographie liquide haute performance couplée à la spectrométrie de masse triple quadrupôle (HPLC-QqQ-MS/MS). Les résultats ont montré que les cinétiques de dégradation du chloramphénicol et du florfénicol suivent un modèle de premier ordre, et la constante de dégradation thermique, k, croît avec la température. Par la suite, deux stratégies ont été mises en œuvre pour l'identification des produits de dégradation thermique. Tout d'abord, la pyrolyse-chromatographie en phase gazeuse-spectrométrie de masse (Py-GC-MS) a été utilisée pour l'étude des produits de dégradation volatils de trois antibiotiques et trois autres composés pharmaceutiques. Ainsi, cette approche a montré que le chloramphénicol, la lincomycine, le

gemfibrozil et la diphénhydramine se dégradent en une série de composes volatils. Aucun compose volatil n'a été détecté au cours de la dégradation du florfénicol. Py-GC-MS s'est montre être une technique efficace et rapide pour l'étude de la dégradation thermique des résidus de médicaments. Enfin, la HPLC couplée à la spectrométrie de masse haute résolution (HPLC-QTOF-MS/MS dans notre cas), combinée à la bioinformatique a été utilisée pour identifier les produits de dégradation thermique du chloramphénicol dans l'eau, et confirmer leur présence au cours de la cuisson d'une autre matrice, de la moule. Cette approche a permis à nouveau de détecter des nouveaux produits de dégradation. Ce travail de recherche met en exergue la nécessité de prendre en compte l'impact de la transformation alimentaire pour l'évaluation des risques liés à la présence de résidus de phénicols dans les aliments.

CONTRIBUTIONS OF AUTHORS

The authors involved in the thesis and their contributions to the various articles are as follows:

Lei TIAN is the M.Sc. candidate who designed and conducted all the experiments in consultation with the supervisors. She performed data collection and analysis. Additionally, she prepared drafts of all the manuscripts for scientific publications.

Dr. Stéphane BAYEN is the thesis supervisor, under whose guidance the research was conducted. He assisted the candidate in designing and conducting the experiments as well as correcting, proofreading, reviewing and processing manuscripts for the publications.

The literature review in Chapter 2 is reviewed and organized by Lei TIAN under the supervision of Dr. Stéphane BAYEN. Salma KHALIL contributed to Chapter 2 and wrote two paragraphs on tetracyclines in section 3.4. These two paragraphs are part of the manuscript submitted for publication and are kept in the present thesis to give an overview of the topic for all antibiotics.

LIST OF PUBLICATIONS AND PRESENTATIONS

Part of this thesis has been prepared as manuscripts for publications in refereed scientific journals:

Lei Tian, Salma Khalil, Stéphane Bayen. Effect of thermal treatments on the degradation of antibiotic residues in food. *Critical Reviews in Food Science and Nutrition.* (Status: *Accepted*)

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Lei Tian, Stéphane Bayen. Thermal degradation kinetics of chloramphenicol and its degradation products investigated by HPLC-QqQ-MS/MS and HPLC-QTOF-MS/MS. (Status: *in preparation*)

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CONTENTS

LIST OF ABBREVIATIONS

4eTC=4-epitetracycline

ATC=anhydrotetracycline

BL=Blank

BPA=Bisphenol A

CHL=Chloramphenicol

CTC=Chlortetracycline

DAD=Diode array detector

DOC=Doxycycline

DP=Degradation percentage

Ea=activation energy

EFSA=European Food Safety Authority

ESI=Electrospray ionization

FDA= Food and Drug Administration

GC=Gas chromatography

HPLC=High performance liquid chromatography

HRAM=High resolution accurate mass

JECFA=The Joint FAO/WHO Expert Committee on Food Additives

LC=Liquid chromatography

Ln A=collision frequency

LOD=Limit of detection

MIC=Minimal inhibitory concentration

MRM=Multiple reaction monitoring

MRLs=Maximum residue levels

MRPLs=Minimum required performance limits

MS/MS=Tandem mass spectrometry

MS=Mass spectrometry

OTC=Oxytetracycline

PAD=Photodiode array detector

Py-GC-MS=Pyrolysis GC-MS

QC=Quality control

QqQ=Triple quadrupole

QTOF=Quadrupole time-of-flight

SPE=Solid-phase extraction

SP=Spiking

TC=Tetracycline

TIC=Total ion chromatogram

TOF=Time-of-flight

UHPLC=Ultra high performance liquid chromatography

UV-Vis=Ultraviolet visible

CHAPTER 1. INTRODUCTION

Antibiotics are a kind of antimicrobial which are used for medical treatment for both humans and animals, as well as for growth promotion in animal production. Major classes of antibiotics used in animal farming include tetracyclines, macrolides, sulfonamides, penicillins and amphenicols (Wang and Ma, 2008; Liu et al., 2014; FDA, 2014; EFSA, 2014). Veterinary drug administration to animals, typically through animal feed, leads to residues of these antibiotics remaining in the animal tissue that is to be consumed. Furthermore, these residues may also be left in the environment such as water and land, after which they could be absorbed by other animals and human beings (Sarmah et al., 2006). As a result, antibiotic residues are regularly detected in many food categories including meat, eggs and seafood products (Donkor et al., 2011; Done and Halden, 2014). Another reason is the abuse of antibiotics in animal agriculture, which also worsen the drug resistance in human pathogens (Goldman, 2004; Landers et al., 2012). It has been reported that the antibiotic resistance is associated with food animals as the mechanism of antibiotic resistance gene transfer is contributed by the animals and not only linked to antibiotic use itself (Mathew et al., 2007). Some antibiotic residues can trigger adverse effects on human health including allergic reactions in hypersensitive individuals or affect the gut microbiota in humans (Levine, 1960; Franco et al., 1990). Besides potential direct effect on human health, antimicrobial use was also identified as a cause of antimicrobial resistance in the food production system, an emerging public health issue of global concern (McDermott et al., 2002; Grundmann et al., 2006).

Chloramphenicol and florfenicol belong to the amphenicols, a family of antibiotics commonly used in bovine and aquaculture for many years (EFSA, 2014). Although chloramphenicol is not authorized to use in food-producing animals in many places in recent years (Shakila et al. 2006; EFSA, 2014), it is frequently detected in the environment and food (Na et al., 2013; Tittlemier et al., 2007; Sheridan et al., 2008). According to the survey of EFSA (2014), the majority of chloramphenicol is detected in fishery products of South-East Asian origin. In most of the cases, the residue level of chloramphenicol is lower than 10 μg/kg in fish, however, the exceptions could exceed 300 μg/kg (EFSA, 2014). Florfenicol is permitted for use in food-producing animals especially in fish (Gaunt et al., 2013), and was also reported as an environment contaminant in water (Na et al., 2013) and fish (0.11- 172.6 μg/kg) (Barani & Fallah, 2015).

Antibiotic residues such as chloramphenicol are recognized to be genotoxic and can induce cancer to humans (JECFA, 2004). Since some trace residues may be present in food of animal, food safety risk assessment procedures require to understand their fate and degradation during food processing (e.g. during cooking or storage). The degradation of antibiotics is generally assessed by measuring the change in either the antimicrobial activity or the concentration of parent antibiotic residue in the food or the food extracts using analytical techniques based on chromatography. Earlier studies used microbiological assays to assess the degradation of antibiotic residues, based on the difference in microbial activity before and after treatment (O'Brien et al., 1981; Shakila et al., 2006; Franje et al., 2010). Since the degradation products of antibiotics may be bioactive, biological tests may not always reflect a real reduction of the

concentration of the parent antibiotic (Traub and Leonhard, 1995). As a result, the utilization of robust techniques based on chromatography, especially HPLC, has become more popular for studying the thermal degradation of antibiotics. Based on these tools, it was estimated that some antibiotics can degrade almost completely during cooking (See Chapter 2). Many parameters such as temperature, time and the food matrix can affect the thermal degradation of antibiotics. Eventually, a first-order model has been applied and proven to be the degradation kinetics of sulfonamides, tetracyclines and quinolones. To date, the degradation of amphenicols was studied, but the degradation kinetic of amphenicols at high temperature (100 ℃) has not been reported.

Although many studies have reported the degradation of antibiotics in the literature, only a few have actually identified the degradation products (Fritz and Zuo, 2007; Hsieh et al., 2011; Junza et al., 2014; Franje et al., 2010). This knowledge gap hinders the understanding of the fate of antibiotic residues in the food supply chain. More worryingly, some of few identified degradation products may remain bioactive and toxic. For example, enrofloxacin and difloxacin degraded to ciprofloxacin and sarafloxacin, two common antibiotics, respectively (Junza et al., 2014). One degradation product of penicillin, the penicillin acid, may induce allergic reaction in sensitive populations (Sullivan et al., 1981). Florfenicol can degrade into thiamphenicol (Franje et al., 2010; Mitchell et al., 2015), which is also registered as an amphenicol antibiotic. Therefore, the potential adverse health effects of the degradation of antibiotics needs to be furthered investigated. The fate of amphenicols in the food matrix is not completely elucidated.

The identification of the degradation products mentioned earlier was based on chromatography

coupled with low resolution mass spectrometry (GC-MS or LC-MS). Other techniques, such as pyrolysis-GC (Py-GC) coupled with low resolution MS have been reported in the literature for the study of the thermal degradation of pure chemicals (Lehotay & Hajšlová, 2002), but to date, Py-GC has not been applied to study the degradation of antibiotics. Py-GC has the advantage to study the volatile degradation products which are out of the capacity of GC-MS or LC-MS. However, because of the low resolution, the identification of degradation products with these tools is usually limited to the study of simple matrices, such as ultrapure water or pure chemicals. Low resolution MS is however not able to unambiguously identify the structure of all the degradation products in more complex matrices, such as food samples. In the last decade, the hyphenation of chromatography to tandem mass spectrometry (MS/MS) and high resolution mass spectrometry (e.g. Orbitrap or hybrid quadrupole-time-of-flight (Q-TOF) mass spectrometers) developed rapidly. The high-resolving power of these techniques can provide additional structural information for samples with high complexity such as food (Ferrer & Thurman, 2003). The measurement of accurate molecular masses can quickly help in determining the formula for unknown compounds, which has been a common strategy in recent years (Nägele & Moritz, 2005). Because most of the antibiotic residues, their metabolites and their degradation products are relatively less volatile, polar and water soluble (García-Galán et al., 2008), liquid chromatography coupled to high-resolution accurate mass tandem mass spectrometry (LC-HRAM-MS/MS) emerged as a method for the study of complex matrices. LC-HRAM-MS/MS (TOF and LTQ-Orbitrap) was recently applied by Junza et al. (2014) for the

study of quinolones in milk. To date, no one has reported the use of LC-HRAM-MS/MS for the study of amphenicols.

The overall objective of the present thesis was to study the thermal degradation of two amphenicols (chloramphenicol and florfenicol) in model solutions. Then the degradation products of chloramphenicol were confirmed in real tissue matrices. Mussel tissues were selected because amphenicol residues may be encountered in these mollusks, as they were reported to be used as the indicator of environment due to their very high bioaccumulation and a low biotransformation potential to contaminants (Smolders et al., 2003). Furthermore, there is high population of mollusks in marine environment, and it is a relatively easy to raise in the laboratory. More specifically, the objectives of this study were to: (i) identify the thermal degradation products of amphenicol antibiotics and other pharmaceuticals using Py-GC-MS (AIM 1), (ii) study the thermal degradation kinetics of chloramphenicol and florfenicol using HPLC-DAD and HPLC-MS/MS and understand the parameters that affect the thermal degradation of amphenicols (AIM 2), and apply LC-HRAM-MS/MS to identify the degradation products of chloramphenicol in model solutions and in actual mussel tissue matrices during cooking (AIM 3).

A literature review describing the effect of thermal treatment on antibiotics in food matrices is presented in Chapter 2. The application of Py-GC-MS as a fast and effective tool to study thermal degradation products of antibiotics is demonstrated in Chapter 3. In Chapter 4, the thermal kinetics of amphenicols in water and the effect of temperature on the degradation rates are reported. The results of the identification of degradation products of chloramphenicol in

water and in mussel tissues are presented in Chapter 5. Finally, in Chapter 6, conclusions of this study and perspectives for future studies are proposed.

CHAPTER 2. LITERATURE REVIEW

2.1. Introduction

Antibiotics are used for medical treatment for both humans and animals, as well as for growth promotion in animal production. Major classes of antibiotics used in animal farming include tetracyclines, macrolides, sulfonamides and penicillins and amphenicols (Wang and Ma, 2008; Liu et al., 2014; FDA, 2014). Veterinary drug administration to animals, typically through animal feed, leads to residues of these antibiotics remaining in the animal tissue that is to be consumed. Furthermore, these residues may also be left in the environment such as water and land, after which they could be absorbed by other animals and human beings (Sarmah et al., 2006). As a result, antibiotic residues are regularly detected in many food categories including meat, eggs and seafood products (Donkor et al., 2011; Done and Halden, 2014). Some antibiotic residues can trigger adverse effects on human health including allergic reactions in hypersensitive individuals or the change of R+ enteric organisms' amount in human body (Levine, 1960; Franco et al., 1990). Besides potential direct effect on human health, the widespread use of antibiotic residues in food may also cause antibiotic resistance, an emerging public health issue of global concern (McDermott et al., 2002; Grundmann et al., 2006). For this reason, regulations are in place to set the Maximum Residue Limits (MRLs) of antibiotic residues permitted in food products. However，MRLs apply to the quantity of residues in the raw food commodity, without considering the changes that occur during processing. As most foods of animal origin are typically consumed after cooking or processing, knowing the effect of

different thermal treatments on the residues is essential when assessing human exposure, determining MRLs, and evaluating toxicity. Shahani et al. (1956) first reported the effect of heating on penicillin G in water and milk. Since then, various studies have investigated the thermal stability of antibiotics under different heating conditions such as domestic cooking, commercial pasteurization and canning. The aim of the present paper is to review this literature to get a better understanding of the degradation of antibiotic residues in food and the implications for food safety. In particular, information on the degradation kinetics of antibiotics was compiled to assess the relationships between the degradation of antibiotics and processing time and temperature. Studies on the influence of other parameters (e.g. initial concentration of residue in the raw food, the pH and the presence of additives) are also discussed. Finally, this paper reviews the current knowledge on the mechanisms of structural degradation and the identity of all the degradation products.

2.2. Material and methods

2.2.1 Selected literature

The scientific literature (1956-2015) was screened for information about the degradation of antibiotic residues in food using combination of keywords such as "*antibiotics*" (or the name of the chemical), "*cooking*", "*thermal degradation*" and "*food"*. Available literature published in Chinese was also screened. A total of 105 papers were evaluated out of which 84 papers are presented in this review.

2.2.2 Degradation percentage (DP) and degradation rate constant (k)

Quantitative information about the disappearance (degradation) of the antibiotic residue is mostly available in the literature through parameters such as degradation percentages (DP) or degradation rate constants (k).

For the biological test, the degradation was measured by the change of antimicrobial activity such as the minimal inhibitory concentration (MIC) and the inhibition zone diameter (detailed in section 3.1).

For the analysis based on chromatography, degradation percentages are calculated according to Equation (1), where C_0 and C_{final} are the concentrations of the chemicals before and after heating respectively. These concentrations are measured using various tools as discussed in section 3.1, and should account for the change in food weight during cooking (e.g. water loss). While the degradation percentage is relatively simple, it does not integrate any kinetic or mechanistic considerations. As a result, direct comparison of degradation percentages across studies is often inappropriate as experimental conditions (time, temperature, etc) are generally different.

$$Degradation\ percentage\ (\%) = \left(1 - \frac{C_{final}\ (corrected\ by\ weight\ change)}{C_0}\right) \times 100 \quad \text{(Equation 1)}$$

Alternatively, comparison of the various degradation experiments can be derived using the degradation rate constant k. To date, degradation rates in food have been calculated only in a few studies (Fuliaş et al., 2010; Roca et al., 2010; 2011; 2013).

Following the hypothesis that the degradation of antibiotic compounds follows a first-order model, k was first applied in studying antibiotic residues by using the following equation (Fuliaş

et al., 2010) based on the equation developed by Martin (1993):

$$\frac{\partial[C]}{\partial t} = -k * [C] \qquad \text{(Equation 2)}$$

where the t is the heating time and [C], the concentration of each compound in the sample at a specific point in time t. The integration of Eq. (2) leads to:

$$ln[C] = ln[C_0] - k \times t \qquad \text{(Equation 3)}$$

where C_0 is the initial concentration of the antibiotic. Eq. (2) can be rewritten as:

$$k = \frac{ln[C_0] - ln[C]}{t} \qquad \text{(Equation 4)}$$

The k value can then be computed for each antibiotic from experimental data. The examination of k values allows for a clearer comparison of the stability of antibiotics in food across studies for a specific set of conditions (e.g. for a particular temperature or a specific food matrix), independently of time. The suitability of the first-order model is discussed in section 3.3.1. To date, there is no sufficient kinetic data available for macrolides, aminoglycosides, amphenicols and lincomycin, and in this paper we hypothesized that they also obey the first-order kinetic model for the purpose of comparison across studies.

Some studies expressed the degradation kinetics using the D value, which is the amount of time required for one log reduction in the concentration of residues. D values are related to k values according to:

$$k = \frac{\ln(10)}{D} \qquad \text{(Equation 5)}$$

2.2.3 Statistical analysis

k values were calculated using Microsoft Excel. For statistical analysis, t-test is employed and conducted by JMP statistical software (SAS Institute, North Carolina). $P<0.05$ is treated as significant.

2.3. Results and Discussion

2.3.1 Experimental assessment of antibiotic degradation

2.3.1.1. Experimental design of degradation studies

Generally, liquid samples (except those in water or salt water) were pretreated through deproteinization or extraction by solvent before analysis, while solid samples were always extracted by solvents and then analyzed by chromatography or MIC test to detect the concentration change of antibiotic residue. In solid samples that were treated by baking, microwave heating or frying, the water loss was taken into account by most of the authors for the correction of final results. For boiling samples, the boiling water was also analyzed. Sometimes, the experiment cannot be controlled well due to the specificity of grilling (e.g. the loss of juice during grilling was uncollectible) (Cooper et al., 2011).

In addition to the limitations of detection and weight loss, the procedures of experiments also present limitations in terms of achieving accurate results. Reported cooking temperatures for solid matrices do not, in most cases, reflect the actual temperature antibiotics are exposed to. This is because in most cases, the temperature in cooked solid matrices is not homogenous in

comparison to liquid matrices. Furthermore, antibiotic residues may be unevenly distributed in treated animals (Moats, 1999). Even in the same body part or organ, the residue concentrations vary at different points (O'Brien et al., 1981). To achieve the homogeneous residue distribution, some studies used ground tissues before heat treatment (Moats, 1999), but this does not accurately represent real domestic cooking or commercial food processing. Thus, data for solid matrices are rejected from the comparison of k values.

2.3.1.2. Quantification of antibiotic degradation

The degradation of antibiotics is generally assessed by measuring the change in either the antimicrobial activity or the concentration of parent antibiotic residue in the food or the food extracts using analytical techniques based on chromatography.

Earlier studies used microbiological assays to determine the reduction in veterinary drug residues. These assays determine the difference in microbial activity before and after treatment, from which a degradation percentage in the biologically active compound was calculated. Tests such as the minimal inhibitory concentration test (MIC) (Traub and Leonhard, 1995) and the inhibition zone diameter (Javadi, 2011) have been applied to detect the changes of antimicrobial activity. Shit et al. (2008) reported the use of Delvotest® (DSM, the Netherlands), a commercial test kit for antibiotic residue in food, to detect the presence of furazolidone in cooked chicken tissue based on the bioactivity. However, the Delvotest® can only test the presence/absence of active antibiotics but not the actual concentration of antibiotic residues. As the degradation

products of antibiotics may be bioactive, biological tests may not always reflect the real concentration of the parent compound (Traub and Leonhard, 1995; McCracken and Kennedy, 1997). The utilization of techniques based on chromatography or a combination of microbial methods with chromatography allowed for more accurate results (Franje et al., 2010; Hsieh et al., 2011).

As the use of chromatographic analysis became more widespread, liquid chromatography (HPLC and more recently UHPLC) coupled to a range of detectors started to become the method of choice for the quantification of antibiotic concentrations in food. LC-based analysis first requires the extraction of the target analyte from the food matrix. Liquid food matrices such as milk or food juice can be extracted using solid-phase extraction (SPE) (Sun et al., 2010). Solid food matrices are usually extracted with solvent and/or buffer mixtures optimized to obtain good extraction recoveries (Ridgway, Lalljie and Smith, 2007). For example, the standard extraction solvent utilized in tetracycline analysis is a McIlvaine-EDTA buffer system (Anderson, Rupp et al. 2005), although other solvents such as methanol, citrate buffer, and trichloroacetic acid are sometimes used. Eventually, target antibiotics in the extract are quantified using HPLC coupled to detectors such as UV-Vis detectors (including diode array detectors), fluorescence detectors or mass spectrometry (Joshi, 2002). Many studies reported the use of HPLC coupled with UV-Vis or fluorescence detection. While these instruments allow for the quantification of the parent antibiotic in food extracts, they seldom allow for the unambiguous identification of the degradation products.

In the recent years, liquid chromatography coupled with mass spectrometry, notably tandem mass spectrometry, became the gold standard for antibiotic residue analysis, allowing in some cases for the identification of degradation products (Bogialli and Di Corcia, 2009). Thus, McCracken and Kennedy (1997) identified the metabolite of furazolidone residue (AOZ) in pig meat by using HPLC-thermospray mass spectrometry. Grunwald and Petz (2003) explored the degradation of four penicillins in milk by using LC-UV and LC connected to a Thermo Finnigan TSQ 7000 triple quadrupole mass spectrometer. Such an approach helped determine that penicillin G degrades into penillic acid and that cloxacillin degrades into penillic, penilloic and penicilloic acids. It was the first paper that gave a brief explanation of the degradation products of antibiotic residue during milk processing. Later, Junza et al. (2014) identified the thermal transformation products of quinolones in cow's milk by utilizing LC-LTQ-Orbitrap-MS/MS and LC-TOF-MS/MS. These tools helped in identifying the chemical structure, molecular mass and reaction type (decarboxylation, reductive defluorination, etc.) of both the thermal degradation products and the intermediary transformation products (only exist during the heating procedure) (Junza et al., 2014).

2.3.2 Thermal kinetics of antibiotics

Studies investigating the effects of thermal treatment on antibiotic residues most commonly present their findings in terms of the degradation percentage of the residues following the treatment. From the available studies, it can be concluded that, in general, thermal treatment

leads to the degradation of antibiotic residues and consequently a reduction of the residue concentration or bioactivity in the food product. That being said, the values reported in the literature vary widely depending on the type of treatment used, the matrix, the pH, and the temperature. Some researchers applied the degradation rate constant k to study the thermal kinetic of antibiotic, which achieved a good regression coefficient. Later, the kinetics of some antibiotics were proved by calculating the Ea (minimum energy required to start the chemical reaction), lnA (collision frequency) and ΔG^0 (the standard molar Gibbs free energy of activation) by some authors (detailed information was shown in section 3.2.3, section 3.2.6, section 3.2.7 and section 3.3.1).

The range of degradation percentage reported in the literature for antibiotics is summarized in Table 2.1. The complete detailed information is reported in Table S1 of the Supplementary Information. Sections 3.2.1 to 3.2.8 below discuss the results for each specific family of compounds.

2.3.2.1 β-Lactams antibiotics

β-Lactam antibiotics (such as penicillin and cephalosporins) are effective veterinary drugs widely used in animal production, which may result in β-lactam antibiotic residues in food, particularly in milk (Yamaki et al., 2004).

According to reported literature, the degradation percentages of β-lactams residues in food during cooking range from 0.1% to 100% (Table 2.1). The first tests on β-lactams using MIC

tests indicated that most of these antibiotics are unstable during heat treatment (Traub and Leonhard, 1995). The degradation of β-lactams antibiotics was later shown to follow the first-order kinetic model (Fuliaş et al., 2010; Roca et al., 2011), and the degradation percentage was reported to be temperature-dependent (Roca et al., 2011). Grunwald and Petz (2003) noted that, in the case of penicillin in milk, the concentrations influenced the thermal stability, and relatively higher degradation percentages were recorded for lower initial concentrations.

Table 2.1. Summary of thermal degradation range reported in literatures

Antibiotic Family	Compound(s)	Processing Methods	Matrix	DP Range	Reference
β-lactams	Amoxycillin/Ampicillin/Penicillin G/Oxacillin/Dicloxacillin/Cloxacillin	Boiling	Water	8-78%	Hsieh et al., 2011
	Oxacillin/Dicloxacillin/Cloxacillin/ Nafcillin	Boiling	Water/Milk	8-64%	Grunwald and Petz, 2003
	Penicillin G	Boiling	Milk	32%	Konecny 1978
	Ampicillin	Grilling/Roasting	Meat	2.3-100%	O'Brien et al., 1981
	Penicillin G	Boiling	Water/Oil	80-90%	Rose et al. 1997c
	Cefuroxime/Cefquinome/Cephalexin/ Cephalonium/Cephapirin/Cefoperazoe/ Amoxycillin/Ampicillin/PenicillinG/ Cloxacillin	Boiling	Milk	0.1-100%	Roca et al., 2011
	Penicillin G	Boiling	Meat	8.2-59.7%	Shahani et al. 1956
Tetracyclines	Tetracycline/Oxytetracycline/ Doxycycline/Chlortetracycline	Boiling/Roasting/ Microwave	Meat	42-100%	Abou-Raya et al., 2013

Oxytetracycline	Frying/Baking/ Smoking	Seafood	25-93%	Du et al., 1997
Tetracycline	Boiling/Microwave	Meat	56-81.8%	Gratacós-Cubarsí et al., 2007
Doxycycline/Oxytetracycline/ Tetracycline/Chlortetracycline	Boiling	Water	8-99%	Hsieh et al., 2011
Oxytetracycline	Boiling/Microwave /Frying	Meat	2-95%	Ibrahim and Moats, 1994
Doxycycline	Boiling/Microwave /Roasting	Meat	35-100%	Javadi, 2011
Oxytetracycline	Frying/Water/Oil Bath	Seafood/Buff er	60%	Kitts et al., 1992
Oxytetracycline	Boiling/Microwave	Meat	50-59%	Nguyen et al., 2015
Oxytetracycline	Grilling/Roasting	Meat	4.3-74%	O'Brien et al., 1981
Oxytetracycline	Boiling/Microwave /Roasting/Frying/ Grilling/Braising	Water/Oil/Me at	25-99%	Rose et al. 1996
Oxytetracycline	Canning	Meat	100%	Scheibner, 1972a
Oxytetracycline	Boiling/Baking/	Seafood	17-80%	Uno et al., 2006a

		Frying			
Macrolides	Erythromycin/Spiramycin/Tylosin	Boiling	Milk	0-93%	Zorraquino et al., 2011
	Ivermectin	Boiling	Milk	1.1-1.5%	Imperiale et al., 2009
	Ivermectin	Boiling/Frying	Meat	45-50%	Slanina et al., 1989
Aminoglycosides	Gentamicin/Kanamycin/Neomycin/Streptomycin	Boiling	Milk	17-98%	Zorraquino et al., 2009
Amphenicols	Chloramphenicol/Florfenicol/Thiamphenicol	Boiling/Microwave	Water /Meat	2-80%	Franje et al., 2010
	Chloramphenicol	Canning	Meat	100%	Epstein et al., 1988
	Chloramphenicol	Grilling/Roasting	Meat	0-100%	O'Brien et al., 1981
	Chloramphenicol	Boiling	Seafood	6-29%	Shakila et al., 2006
Quinolones	Ciprofloxacin/Norfloxacin/Flumequine/Oxolinic acid/Enrofloxacin	Boiling	Milk	0.01-12.71%	Roca et al., 2010
	Oxolinic acid	Boiling/Baking/Frying	Seafood	20-50%	Uno et al., 2006b

	Enrofloxacin	Boiling/Microwave /Roasting/Frying/ Grilling	Meat	77.04%	Lolo et al., 2006
Lincosamides	Lincomycin	Boiling	Water	0-15%	Hsieh et al., 2011
	Lincomycin	Boiling	Milk	0-5%	Zorraquino et al., 2011
Sulfonamides	Sulfamethoxazole/Sulfadiazine/ Sulfaquinoxaline/Sulfamonomethoxine	Boiling/Microwave /Roasting	Meat	2-61%	Furusawa and Hanabusa, 2002
	Sulfamethazine	Canning	Meat	50%	Epstein et al., 1988
	Sulfamethoxazole/Sulfamethazine	Boiling	Water	3-10%	Hsieh et al., 2011
	Sulphadimidine	Grilling/Roasting	Meat	0-7.6%	O'Brien et al., 1981
	Sulfamethazine/Sulfachloropyridazine/ Sulfadiazine/Sulfadimethoxine/Sulfam -erazine/Sulfapyridine/Sulfathiazole/ Sulfaquinoxaline	Boiling	Milk	0-85.1%	Roca et al., 2013
	Sulfamethazine	Boiling/Frying	Water/Oil	3-99%	Rose et al. 1995b
	Sulfadimethoxine	Frying/Baking/ Smoking	Seafood	7.5-63.5%	Xu et al., 1996

Nitrofurans	Furazolidone	Frying	Meat	0	Shit et al., 2008
Others	Dimetridazole (DMZ and its metabolite RNZ)	Boiling/Frying	Water/Oil	0-60%	Rose et al. 1998
	Levamisole	Boiling/Microwave /Frying/Grilling	Water/Oil/Meat	13%-99%	Rose et al. 1995a
	Levamisole	Frying	Meat	11-42%	Cooper et al., 2011
	Oxfendazole	Boiling/Microwave /Frying/Braising	Water/Oil/Meat	5-100%	Rose et al. 1997a

A classic sterilization procedure (120°C for 15-20 min) induced significant decrease of β-lactams antibiotics in milk and water (Hsieh et al., 2011; Roca et al., 2011). When heated in tissue, high degradation of ampicillin was also found by long-time roasting (O'Brien et al., 1981).

The low stability of β-lactams under heating is reported mainly due to the high ring strain of the small β-lactone ring, which makes it susceptible to hydrolysis (Baertschi and Alsante, 2005). Ester bonds of cephapirin and cephuroxime are unstable in biological media, which make them more susceptible to heating than other β-lactams antibiotics, even at relatively low temperatures e.g. 60-80°C (Roca et al., 2011).

2.3.2.2 Tetracyclines

Tetracyclines (TCs) are a class of broad-spectrum antibiotics including tetracycline, oxytetracycline (OTC), doxycycline (DOC), and chlortetracycline (CTC) and residues have commonly been reported in food (Myllyniemi et al., 1999).

Under heat treatments, the degradation percentages of tetracyclines range from 2% to 100%. Studies have demonstrated that DOC is the most heat stable of the four compounds, while OTC is the least heat stable both in a chicken matrix (Abou-Raya et al., 2013) and in a buffer system (Hassani et al., 2008). OTC appears to be very heat-labile, as it can be almost completely degraded during boiling for half an hour in water (Rose et al., 1996). However, when heated in oil at a high temperature, the degradation was lesser than that obtained in water (Rose et al., 1996). The author indicated that this might be due to the hydrolysis of OTC in water.

Hsieh et al. (2011) demonstrated that in water, OTC was found to degrade more at 100°C than at 121°C. Kitts et al. (1992) found the thermal kinetics of OTC to be pseudo-first-order under 100°C, and first-order at higher temperatures (110-140°C) (Hassani et al., 2008). This may explain why there was more degradation of OTC at 100°C than at 121°C. For other TCs, the thermal stability was temperature-dependent, and higher degradation was detected under higher temperature (121°C).

Studies investigating TC degradation in chicken and pig demonstrated that the type of food matrix and cooking method affects degradation (Nguyen et al., 2015; Gratacós-Cubarsí et al., 2007), and the results of one study indicated that the presence of fat as a matrix component might lead to decreased reduction in residues following thermal treatment (Gratacós-Cubarsí et al., 2007). Both studies showed a larger reduction in OTC residues in pig in comparison to chicken, and a larger reduction with microwave treatment in comparison to boiling treatment. Further studies are needed to explore the effect of specific matrix components on degradation.

Kitts et al. (1992) investigated the degradation kinetics of OTC in salmon muscle as well as buffer systems of varying pH. In both the salmon muscle and the buffer systems (pH 3 and pH 6.9), the degradation rate increased at higher temperatures. This was presented as a decrease in the D value. D values were higher at pH 3 indicating slower degradation. This is supported by the findings of Xuan et al. (2009), which indicate that OTC hydrolysis in a pH neutral solution is faster than in acidic or alkaline solutions (Xuan et al., 2009).

Kühne et al. (2001) investigated the degradation of TC and 4-epitetracycline (4eTC) in animal feed along with the corresponding formation of anhydrotetracycline (ATC) and 4-epianhydrotetracycline (4eATC), known degradation products of the compounds. While there was an initial increase in TC and 4eTC concentrations, higher temperature and longer treatment time resulted in an average of 50% decrease in residues. At these same treatment conditions, there were large increases in concentration of the degradation products ATC and 4eATC: 941% and 200%, respectively. Whereas most studies inaccurately conclude that a reduction in the parent compound means the product is safer to consume, these results highlight the importance of investigating the fate of the residues as they may degrade into biologically active and sometimes toxic degradation products. Degradation products of TCs are discussed in section 3.4.

2.3.2.3 Macrolides

Macrolides are a class of antibiotics with a 12–16-atom lactone ring in their structure. The

thermal degradation percentages of macrolides were about 0-93%. Erythromycin is the most widely used antibiotic in the macrolide family, often used as an alternative to penicillin (Reeves, 2012). Erythromycin is also the most susceptible antibiotic to heat treatment in the macrolide family (Zorraquino et al., 2011). The activation energy Ea of erythromycin, the minimum energy required to start the chemical reaction, was lower than those for other macrolides, which also indicated erythromycin was more sensitive to heating than other macrolides (Li, 2010). This was proven by real heating treatment--heating in milk at 120°C for 20 min induced more than 90% reduction of residues of erythromycin, while the figure was much lower for other macrolides (Zorraquino et al., 2011). What needs to be mentioned is that the result of Zorraquino et al. (2011) was measured by the change of antimicrobial activity, which cannot be taken to indicate the structural degradation of the compound.

2.3.2.4 Aminoglycosides

Aminoglycosides are a class of antibiotics with an aminocylitol ring linked to one or more amino sugars by a glycosidic linkage (Zorraquino et al., 2009). Due to the significant post-antibiotic effect of aminoglycosides, many aminoglycosides are banned in food-producing animals (Reeves, 2012). However, some aminoglycosides are permitted for use in dairy cows (Reeves, 2012). Thus, numerous studies have been done to investigate the degradation of aminoglycosides in milk. Almost all the aminoglycosides are heat-labile in milk. Heating at 120°C for 20 min in milk led to the reduction in residues by more than 95% (Konecny, 1978; Zorraquino et al., 2009). Interestingly, when heating in water at 121°C, all the aminoglycosides showed only slight changes in bioactivity (Traub and Leonhard, 1995).

2.3.2.5 Amphenicols

Amphenicols are a class of broad-spectrum antibiotics including chloramphenicol, florfenicol and thiamphenicol. In water, all amphenicols are relatively stable during heating. Indeed, boiling

(30-60min) and microwave heating (5min) were shown to result in less than 10% degradation for these three chemicals (Franje et al., 2010). Even when the treatment time was prolonged to 2 hours, degradation only increased slightly (Franje et al., 2010). However, when heated in meat tissue, chloramphenicol is much less stable and degradation is almost 5 times greater (O'Brien et al., 1981; Shakila et al., 2006). The bioactivity of chloramphenicol in beef was also shown to decrease by 70% after roasting for 2h according to the MIC test (O'Brien et al., 1981). Higher degradation of chloramphenicol in meat has been suggested to be a result of its lipophilic nature (Reeves, 2012). Franje et al. (2010) proposed that the greater degradation might result from the low water binding capacity of meat after heating, as Clarke et al. (1987) ever reported that low water binding capacity could increase the degradation of antibiotics. Franje et al. (2010) investigated the changes in the parent compound peak and the appearance of new peaks during the heating of three amphenicols in different media, and results varied amongst food matrices. In water, boiling induced more new peaks for florfenicol than the other two drugs, which indicated that florfenicol had more kinds of degradation products than the other two amphenicols. This could be explained by the structural differences of the three amphenicols, as the active fluorine group is more susceptible to nucleophilic substitution than the hydroxyl group (Franje et al., 2010). However, the total new peak area of florfenicol was lower than the other two drugs, which indicated that only a small percentage of florfenicol degraded during heating, while the other two amphenicols suffered high degradation in quantity.

2.3.2.6 Quinolones

The degradation of quinolones was proven to obey the first-order kinetics and was temperature-dependent (Roca et al., 2010). Both the MIC test and the chromatographic analysis indicated that quinolones were heat-stable in water and milk (Traub and Leonhard, 1995; Roca et al., 2010). Roca et al. (2011) also compared the activation energy (Ea) and collision frequency (lnA) for quinolones with β-lactam antibiotics. Low Ea and lnA values indicated a lower

degradation rate for quinolones than for β-lactam antibiotics. The same author also reported that the quinolone ring was more stable than the covalent bonds, which may explain why quinolones are stable during heating. Ciprofloxacin and norfloxacin were slightly less heat-stable than flumequine, oxolinic acid and enrofloxacin in water and milk (Roca et al., 2010). However, oxolinic acid showed more degradation when heated in black tiger shrimp tissue than in milk (Uno et al., 2006b; Roca et al., 2010). Furthermore, Junza et al. (2014) found that enrofloxacin was less stable than ciprofloxacin, as enrofloxacin could degrade into ciprofloxacin during heating. Also, Junza et al. (2014) reported that one degradation product of ciprofloxacin combined with lactose in milk when heated at 120°C for 60min. The conclusions of Uno et al. (2006b), Roca et al. (2010) and Junza et al. (2014) indicated that the heating media was important to the degradation of quinolones.

2.3.2.7 Sulfonamides

Sulfonamides were proven to obey the first-order kinetics by Zhao et al. (2011) with high coefficient of regression (0.933-0.990) and then later proved by Roca et al. (2013). The degradation percentages range from zero to 99%.

Zhao et al. (2011) heated the hen eggs with six spiked sulfonamides (0.1mg/kg) using a water bath, and the results showed that high degradation of all six sulfonamides happened under high temperature with long heating time (100°C, 20min). Sulfadiazine and sulfadimethoxine showed the shortest and the longest half-life respectively among the six compounds (Zhao, Wu and Zhang, 2011).

Roca et al. (2013) conducted the experiment in milk and they also found that the thermal degradation of sulfonamides was time-dependent. The same author calculated the standard molar enthalpy and entropy of activation of sulfonamides. The entropy of activation was found to be negative, which indicated sulfonamides were not very susceptible to thermal treatments. High Ea and lnA indicated that the molecules needed high temperature to achieve activation energy, and

when heated at high temperature, the high collisions of molecules would have enough energy to induce reaction (Roca et al., 2013). This theory explained the real degradation of sulfamerazine, sulfamethazine, sulfadiazine and sulfaquinoxaline in milk—the reaction rate was slow under low temperature and quickly increased under high temperature. Then, the high collisions between molecules had enough energy to break the pre-existed bond, which induced high degradation (Roca et al., 2013). In contrast, sulfadimethoxine and sulfathiazole showed low collision frequency, which was indicated by the low rate of reaction and low degradation. This was also confirmed by the heating experiment in milk (Roca et al., 2013). Furthermore, Roca et al. (2013) compared the k of sulfonamides with the other antibiotics in their former studies. The values for sulfonamides were similar to those for penicillins but higher than those for quinolones. This conclusion was also proven by the heating experiments in milk (Roca et al., 2010; 2011; 2013).

2.3.2.8 Other antibiotics (teicoplanin, polymixin B, vancomycin, oxfendazole and lasalocid)

Other antibiotics reported in the literature are partly heat-labile or stable. For example, the MIC test indicated that polypeptide antibiotics such as teicoplanin and polymixin B were partially heat-stable, but vancomycin was remarkably heat-stable (Traub and Leonhard, 1995). Oxfendazole was almost heat-stable in water, but heating in oil at high temperature thoroughly destroyed the residue (Rose et al. 1997a). Lasalocid was stable in neutral and acid matrix but when the pH increased to 10, heating in oil at 100°C completely broke down the residues (Rose et al. 1997b). Unfortunately, there was no literature available for the kinetic studies of these antibiotics.

2.3.2.9 Discussion on the influence of the family of antibiotics

Zorraquino et al. (2011) observed that, although compounds within a class of antibiotics may share similarities in terms of chemical structure and bioactivity, their thermal stability within their group might greatly differ (Zorraquino et al., 2011). The present review also confirms that,

based on the current literature, antibiotics cannot be ranked for thermal stability merely based on their class, as the heating environment namely the matrix and pH also has great impact on the degradation of antibiotics. In a specific condition, antibiotics in the same family have similar thermal property. Quantitative relationships describing the effect of heat on the residues are yet to be established.

2.3.3 Degradation kinetics

To date, only a few studies have attempted to model degradation kinetics of antibiotics during thermal food processing. Amongst these studies, the first-order model is dominant and has been validated for 28 antibiotic compounds (See section 3.2). Degradation rates, k, are scarce in the literature, and are mostly limited to the studies by Roca et al. (2010, 2011, 2013) on penicillin, sulfonamides and quinolones. In the present study, degradation rates were computed from other studies to allow for some critical discussion. Therefore, when available, experimental time series were computed as described in the section 2.2 to derive k values. To date, there is no report available for the degradation kinetics of amphenicols, macrolides and aminoglycosides in food. We hypothesized that their degradation also follows first-order kinetics, but this should be further confirmed in the future. The resulting k values are summarized in Table S1 (Supplementary Information).

2.3.3.1 Influence of time on the degradation

To date, first-order-kinetics have been applied by several researchers to model experimental datasets (Hassani et al., 2008; Fuliaş et al., 2010; Roca et al., 2010; 2011; 2013). Good regression coefficients, ranging from 0.703 to 1.0 for β-lactams, quinolones, sulfonamides and tetracyclines (Fuliaş et al., 2010; Roca et al., 2010; 2011; 2013; Hassani, Lázaro et al. 2008), which indicates that this model is relatively suitable to study thermal kinetics for these compounds. The existing literature does not contain sufficient data to validate the applicability of

this model to the degradation of other antibiotics including macrolides, aminoglycosides, amphenicols and lincomycin.

2.3.3.2 Influence of temperature on the degradation

Temperature has been demonstrated to have an effect on the degradation rate of antibiotics. For example, comparing the k value for β-lactams and sulfonamides in milk at 60-65°C with those at 120°C, k values at 120°C are significantly higher than those at 60-65°C (P = 0.0013 for β-lactams ; P = 0.008 for sulfonamides) (Figure 2.1 and Figure 2.2). Although the degradation percentage and k values for most sulfonamides in this review are lower than other antibiotics, the k values for sulfonamides increase significantly with the temperature. Thus, the thermal degradation of β-lactams and sulfonamides is confirmed to be temperature-dependent. Comparing the figure for macrolides, k values also showed an up-trend when temperature increased (P = 0.124), but it is not as significant as β-lactams and sulfonamides. Lincomycin only degraded under high temperature in milk, as the k value is zero at about 60°C (Figure 2.1 and Figure 2.2). Comparing the k value for antibiotics in water at 100°C, the figure for β-lactams and tetracyclines are higher than the other antibiotics, while k value for levamisole is the lowest one (Figure 2.3). Temperature has been demonstrated to have an effect on the degradation rate of tetracyclines. In model buffer systems of varying pH, the degradation rate increased as the temperature was increased from 60-100°C at both pH 3.0 and pH 6.9 (Kitts, Yu et al. 1992). Similarly, in a system of pH 9.06, the degradation rate increased as the temperature increased from 25-60°C (Xuan et al. 2009). Tetracycline residues in animal feed were shown to increase within the first 30 minutes at 100°C, but decreased by approximately 50% at 133°C (Kühne et al. 2001).

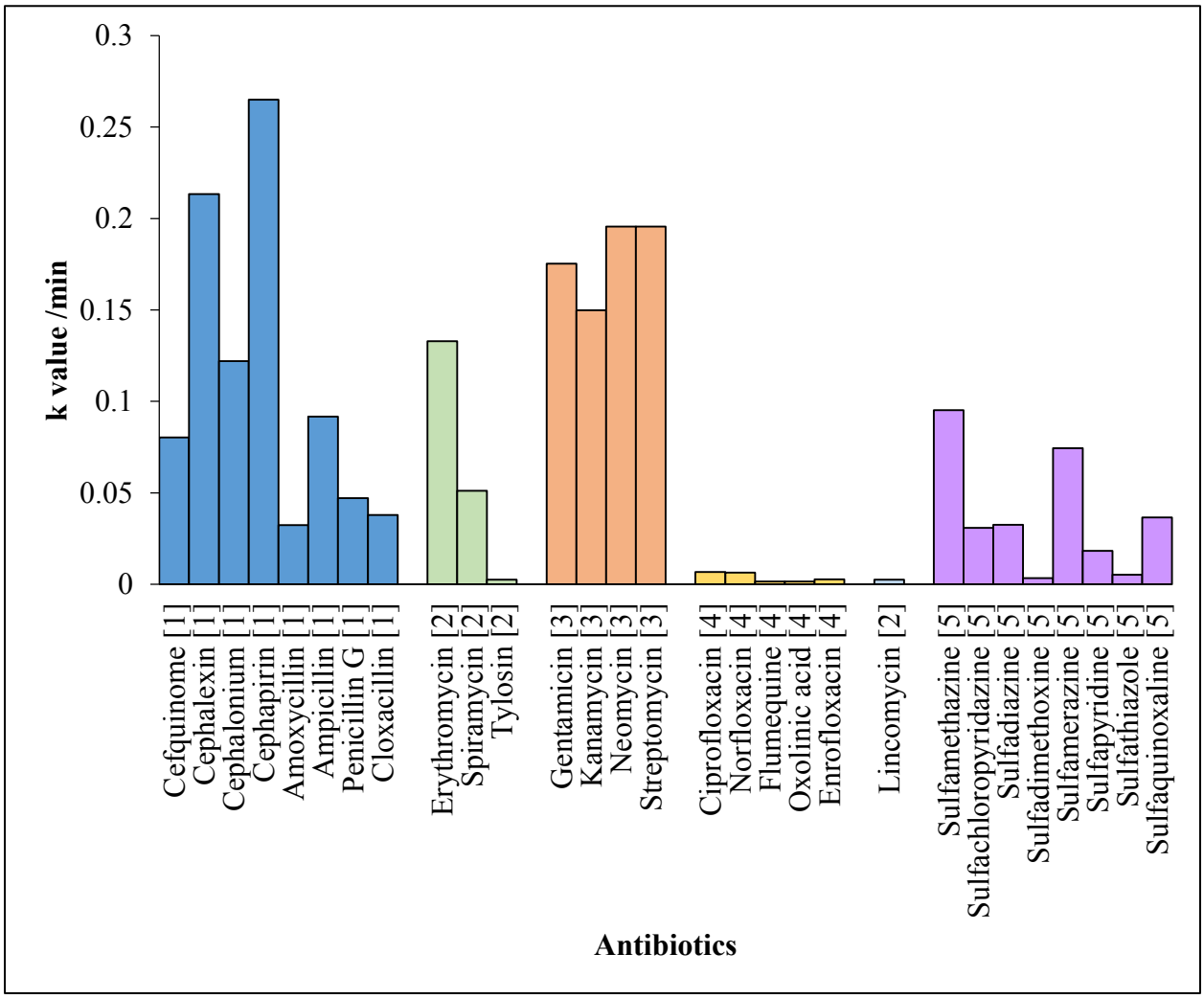


Figure 2.1. k values for selected antibiotics at 120°C derived from literature data on milk. (Zorraquino et al., 2009[3]; Roca et al., 2010[4]; Roca et al., 2011[1]; Zorraquino et al., 2011[2]; Roca et al., 2013[5])

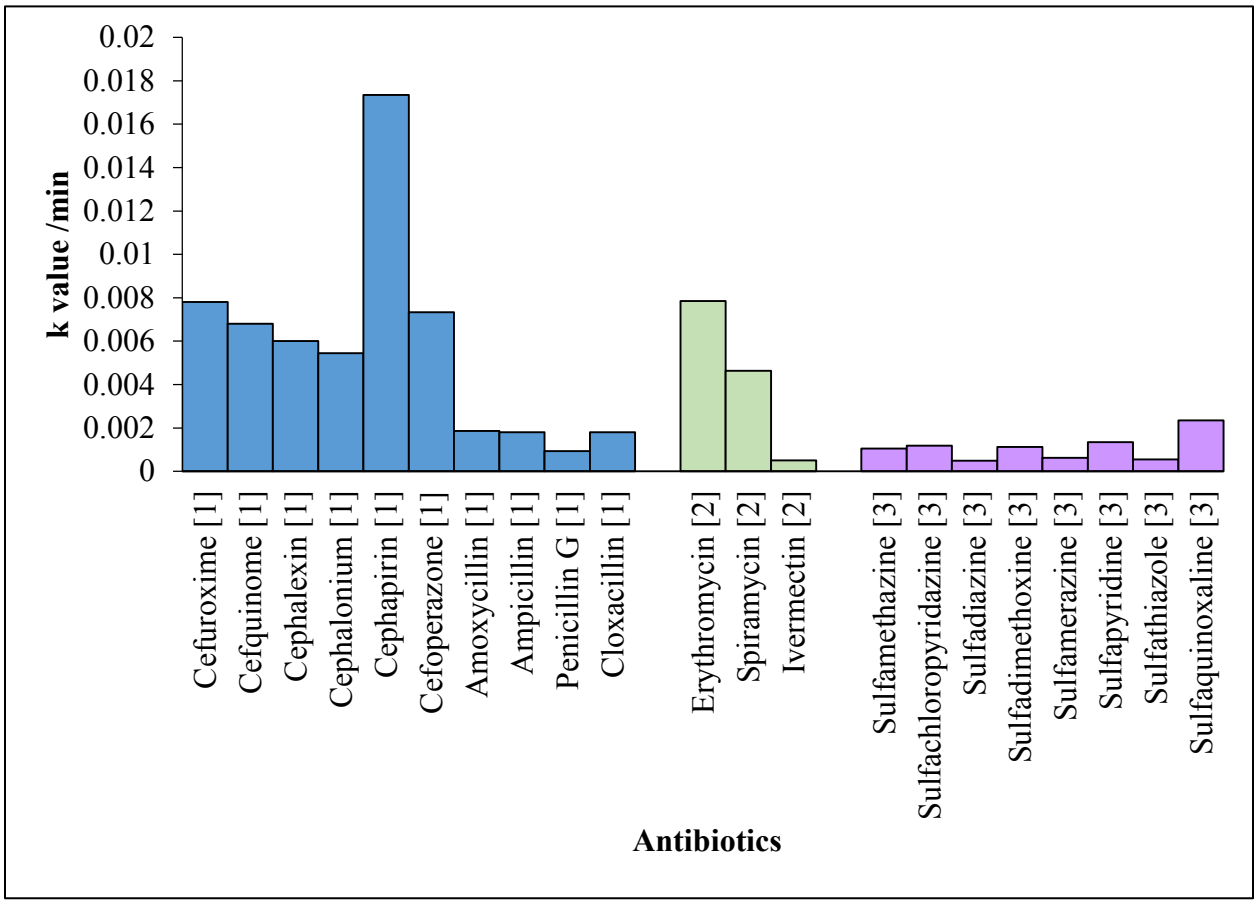


Figure 2.2. k values for selected antibiotics at 60-65°C derived from literature data on milk (Roca et al., 2011[1]; Zorraquino et al., 2011[2]; Roca et al., 2013[3]).

2.3.3.3 Influence of matrix on the degradation

Although the exact effect is still unclear, it has been demonstrated that the food matrix does indeed have an effect on the degradation of antibiotic residues. For example, it has been reported that high fat content meat increased the efficiency of microwave heating which result in the higher degradation of antibiotics than low-fat meat (Gratacós-Cubarsí et al., 2007). Shahani et al. (1956) and Grunwald and Petz (2003) pointed out that penicillins degraded more in water than in milk under thermal treatment. Nonetheless, an assessment of the k values for penicillin G in water and in milk at 120°C revealed they are similar. In another study on amphenicols (Franje et al., 2010), k values in soybean sauce were found to be about 4 times greater than those in water

at 100°C ($P < 0.01$). The effect of the food matrix on the degradation of tetracyclines also remains unclear as studies have yet to investigate the effect of specific food matrix components that may influence degradation. Xuan et al. (2009) tested the effect of Ca^{2+} on the degradation of OTC and found that it leads to a slower rate of hydrolysis, as well as a deviation from the simple first-order kinetic model. This is likely a result of the interaction of tetracyclines with divalent metal ions (Samanidou, Nikolaidou et al. 2007).

The pH may also affect the degradation rate. For example, OTC degraded more rapidly under neutral environment than acid one when heated at 60°C to 100°C (Kitts et al., 1992). Similarly, TC and doxycycline were reported to show higher degradation at 130°C under neutral environment than at pH 4.0 (Hassani et al., 2008).

Compared with solid matrix, the liquid matrix itself can affect the degradation rate, as solvent shows higher ion strength than solid matrix (Mollica et al., 1978). Thus, we can conclude that the physical and chemical properties of the food matrix can affect the thermal degradation of antibiotic residue. Finally, Fedeniuk et al. (1997) demonstrated that the thermal degradation of antibiotics can also be influenced by food additives.

Due to the insufficient data, it is difficult to determine whether thermal degradations of other antibiotics, not mentioned in the present section, are affected by the food matrix. Thus, exploring the influence of matrix on degradation is highly recommended in future studies for the purpose of risk assessment.

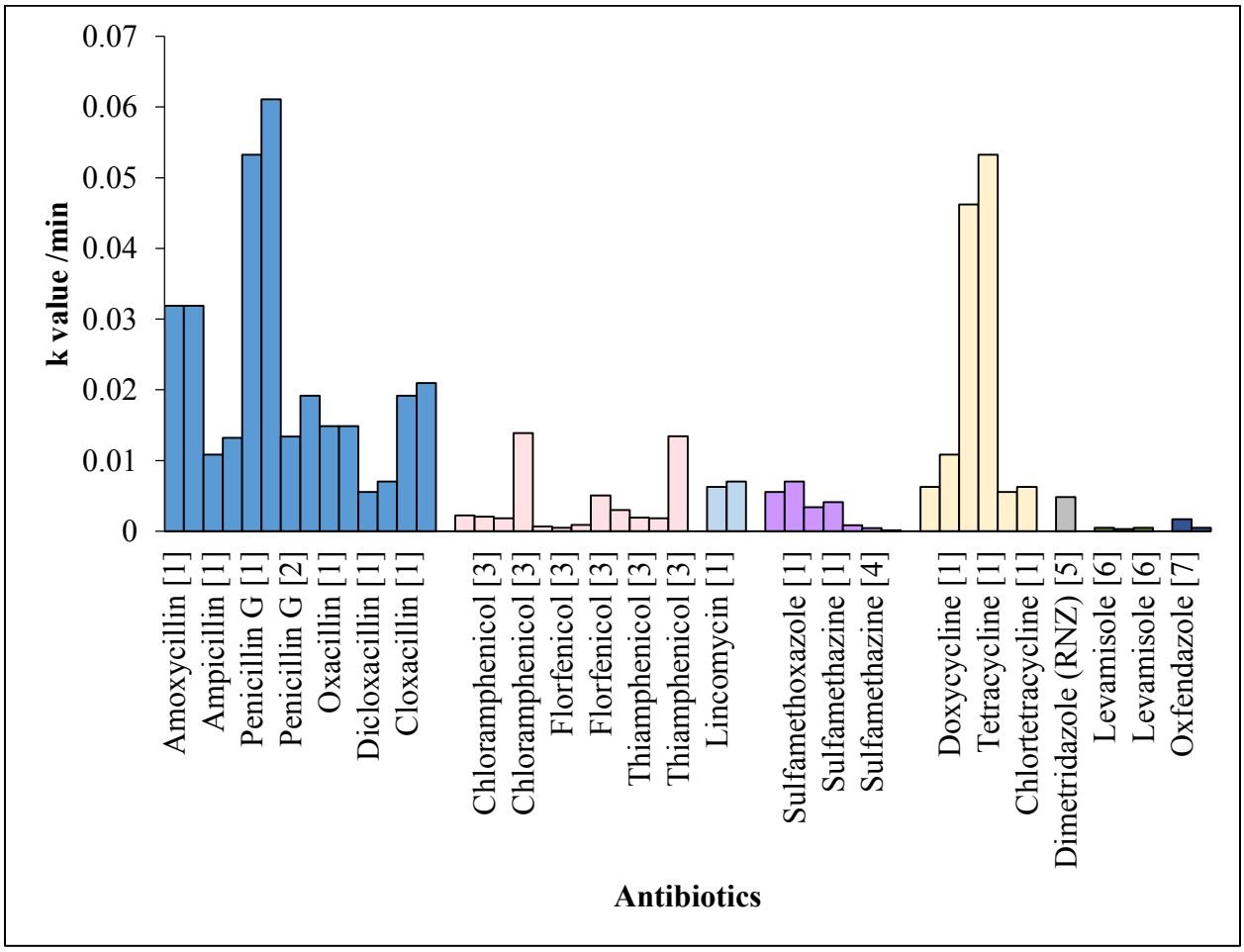


Figure 2.3. k values for selected antibiotics at 100°C derived from literature data on water (Rose et al. 1995a[6]; Rose et al. 1995b[4]; Rose et al. 1997a[7]; Rose et al. 1997c[2]; Rose et al. 1998[5]; Franje et al., 2010[3]; Hsieh et al., 2011[1]).

2.3.3.4 Influence of cooking methods on the degradation

Ibrahim and Moats (1994) studied the influences of different cooking methods on OTC in lamb meat. Boiling in a plastic bag for 30 min and microwave heating (98-102°C) for 8 min could destroy 95% and 60.5% of residue, respectively. Frying was less effective, which reduced only 3.6% (frying 4 min) and 17.3% (frying 8 min). However, frying was efficient in reducing more than half of OTC in seafood. Frying at 100°C for 15 min broke down 60% of OTC in salmon muscle (Kitts et al., 1992). Similar result was found in channel catfish. Indeed, Du et al. (1997) measured the effect of three common cooking methods on OTC in channel catfish and the result

was influenced by the treatment dose. When OTC was heated in a chicken and pig matrix, there was a larger reduction in residues with microwave treatment in comparison to boiling treatment (Nguyen et al., 2015; Gratacós-Cubarsí, Fernandez-García et al. 2007). Comparing the data for milk processing, long-time boiling is better than short-time ultra-high temperature treatment in breaking the antibiotic residues in milk (Grunwald and Petz, 2003; Zorraquino et al., 2009; Zorraquino et al., 2011; Roca et al., 2010; Roca et al., 2011; Roca et al., 2013).

The cooking method can also affect the k values as Franje et al. (2010) reported that microwave heating induced faster degradation of amphenicols than boiling. Furthermore, industrial processing such as canning can achieve relatively higher temperature than common home cooking, which in turn induces higher degradation. Cooking in a water bath at 100°C for 30min only broke down 29% of chloramphenicol in shrimp (Shakila et al., 2006). However, Epstein et al. (1998) reported that canning could completely destroy the chloramphenicol residues. Hsu and Epstein (1993) concluded that the degradation of levamisole only occurred under severe heating but not during domestic cooking. Rose et al. (1995a) also found similar results. All these studies indicated that cooking methods affect the degradation of antibiotics. Unfortunately, it is not possible to do a correlation analysis of the influence of cooking methods on degradation, due to the limitation of reports. Further investigation on whether the influence of cooking methods on the degradation is due to the temperature difference is also highly recommended.

Other food processing methods such as fermentation and cold storage can also affect the percentage of antibiotic residues in food. Epstein et al. (1988) found that sulfamethazine was partially degraded during the procedure of sausage emulsion, even though sulfamethazine was reported to be stable during cooking. Alfredsson and Ohlsson (1998) reported that long-term cold storage (at -20°C for more than 1 month) induced significant decrease (about 35%-55%) of five sulfonamides. However, the storage at -20°C for one week did not change the drug level significantly.

2.3.4 Identification of degradation products

To date, few studies have successfully identified the degradation by-products of antibiotics in food induced by thermal or food processing. Junza et al. (2014) reported that enrofloxacin and difloxacin degraded to ciprofloxacin and sarafloxacin respectively, two common antibiotics. The degradation products of antibiotics may represent a potential threat to human health. For example, penicilloic acid, a degradation product of penicillin, may induce allergic reaction in sensitive populations (Sullivan et al., 1981). There is more information in the literature on the formation and identity of the degradation products of tetracyclines than those of other antibiotic families (Hsieh et al., 2011).

Thus, tetracycline can degrade into their 4-epimers and anhydro products under certain conditions. 4eTC is biologically active, albeit at a much lower level than the parent compound, and it can also convert back to the parent compound (Fritz and Zuo, 2007). The antimicrobial activity of tetracycline is thought to be dependent on certain structural requirements, and the reduction in biological activity is thus attributed to structural changes at important carbon positions (Halling-Sørensen et al., 2002；Blasco et al., 2009). TCs follow different degradation pathways depending on the pH of the medium (Loftin et al., 2008). A dilute acid medium favours the formation of 4eTCs and anhydro-TCs (Samanidou et al., 2007; Xuan, Arisi et al. 2009)., while a strong acid medium favours the formation of anhydro-TCs which can undergo cleavage and lactonization to produce apo-derivatives (Samanidou et al., 2007). The formation of two unidentified compounds from TC and eTC, was reported following thermal treatment of chicken and pork residues (Gratacós-Cubarsí et al., 2007). The formation of unidentified compounds from TC and OTC following treatment at 100°C was reported (Hsieh et al., 2011). It is not clear whether they correspond to degradation products observed in previous studies (4-epimers, anhydro-TCs, apo-TCs) or to new products not previously identified. Further investigation into the degradation products is thus required.

Thus far, information regarding the toxicity of TC degradation products is limited. Anhydrotetracyclines are known to be toxic, exhibiting renal toxicity, which is reported to be reversible (Frimpter et al., 1963; Benitz and Diermeier 1964). Using the Ames test, heated CTC was found to induce mutagenicity in comparison to the control, while heated DOC was not (Hsieh et al., 2011). Nguyen et al. studied the effects of OTC degradation products α-apo-OTC and β-apo-OTC on male rats following oral exposure for 90 days. No adverse effects were observed for α-apo-OTC, while for β-apo-OTC toxic effects including liver and kidney damage, hepatocyte degeneration, and hepatocyte necrosis was observed (Nguyen et al., 2015). Due to the limited information on the profile of TC degradation products under different treatment conditions, there are uncertainties as to whether toxic degradation products are formed in significant amounts under typical domestic cooking procedures. Concerning the possible adverse effect of degradation products, there is thus a need to define these profiles, as well as any unidentified degradation products, and to further investigate the possibility of acute or chronic toxicity. It is urgent to have a good understanding of the degradation products of antibiotic residues in food, because even if the parent compound was shown to be destroyed completely by using quantitative analysis, the degradation products may combine with the food matrix (e.g. one thermal degradation product of ciprofloxacin combined with lactose of milk during heating) or even react with the human body (e.g. penicilloic acid, one of the major degradation products of penicillin), could cause delayed contact allergy (Levine, 1960).

2.4. Conclusions

The present literature review explored the fate of antibiotic residues in food during thermal processing. To quantify degradation, two major techniques have been applied: liquid chromatography-based methods and microbiological tests. As the degradation products may also display some antimicrobial activity, microbiological tests cannot be considered accurate analytical methods for quantifying antibiotic residues' degradation. Coupling advanced mass spectrometry techniques to liquid chromatography separation can provide insight about the

identity of the degradation by-products.

The various studies presented in the literature confirm that the thermal degradation of β-lactams, quinolones, sulfonamides and tetracyclines can be described using a first-order kinetic model. Degradation rates, k, derived for this model for liquid matrix (water) at 100°C, followed the general trend amongst antibiotic classes: β-lactams = tetracyclines (most heat-labile) > lincomycin > amphenicols > sulfonamides > oxfendazole > levamisole (most heat-stable).

The thermal degradation of β-lactams, quinolones, sulfonamides, macrolides, tetracyclines and aminoglycosides are temperature-dependent, and under certain temperatures, prolonged heating time helps to induce more degradation. Furthermore, the food matrix composition and physico-chemistry, (e.g. pH, fat content), the cooking methods, and the presence of food additives were shown to be parameters possibly influencing the degradation of antibiotics. Further studies are needed in this field to systematically understand the impact of these parameters and the profile of the degradation products throughout heating procedures.

According to the *Codex Alimentarius* commission (2010), dietary intake assessments for contaminants should account for the effect of food processing and cooking. Thermal processing usually results in a decrease in the concentration of parent antibiotic residues in food, but degradation by-products have not been properly characterized to date. As some of these products were shown to be hazardous, further investigation is needed to determine their impact on food safety and human health. It is therefore currently difficult to definitively conclude whether or not antibiotic degradation during food processing is necessarily beneficial in terms of food safety, thus only partially satisfying *Codex Alimentarius*' requirements.

CHAPTER 3 APPLICATION OF PYROLYSIS-GC-MS IN STUDYING THERMAL DEGRADATION OF AMPHENICOLS AND OTHER DRUG RESIDUES

3.1 Introduction

Gas chromatography (GC) is a critical technique in food analysis for decades, especially for the analysis of sterols, oils, short chain fatty acids, aroma components as well as many contaminants, such as pesticides, industrial pollutants, and certain types of drugs in foods (Lehotay & Hajšlová, 2002). In parallel, high performance liquid chromatography (HPLC) rose to a dominant position for the analysis of polar, thermo-labile, and non-volatile chemicals (Lehotay & Hajšlová, 2002). These compounds are not easily measured using GC, although strategies (e.g. derivatization) exist to quantify veterinary drugs, herbicides and natural toxins with GC.

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) is an instrumental analysis method in which the sample is heated to decomposition in an inert atmosphere or a vacuum to produce smaller molecules. Then, all these smaller molecules are separated by gas chromatography and detected by the mass spectrometry (Halket & Zaikin, 2006). Py-GC-MS has been dominantly used to identify thermal degradation products of polymers in material science. However, in recent decades it has been reported that the inorganic salts, nucleic acids and other large molecular weight organics, which are outside of the analysis capacity of general GC, can be analyzed through the pyrolysis-GC (Lehotay & Hajšlová, 2002). It was also reported for the identification of environmental contaminants in aquatic system (Kruge & Permanyer, 2004) and carcinogens in food during cooking (Yaylayan, 2006). Thus, this technique appears as a promising technique to study the degradation products of contaminants during the cooking or thermal processing of food. To the best of our knowledge, Py-GC-MS has not been applied to date for the study the thermal degradation of human and veterinary drugs.

Applying the Pyrolysis GC-MS as a fast analysis method to study the thermal degradation products of pharmaceutical contaminants is the main purpose of this chapter. This technique was applied to chloramphenicol and florfenicol as they are the main focus of this thesis (See Chapter

1). Other pharmaceutically active compounds detected in food, including lincomycin hydrochloride, diphenhydramine hydrochloride, gemfibrozil and carbamazepine were also tested. Lincomycin is a linosamide antibiotic. Due to its potential adverse health effect, lincomycin is not authorized to use in food producing animals in the United States and Canada (Arrioja-Dechert, 2002). However, lincomycin was detected in vegetables (Hu et al., 2010). Diphenhydramine is an antihistamine drug, which is commonly used to treat allergies (Berninger et al., 2011). The consumption of diphenhydramine was high in Canada estimated at 6 tons in 2007 (McLaughlin & Belknap, 2008). Berninger et al. (2011) pointed that 2-15% of diphenhydramine was excreted unchanged. Thus, diphenhydramine was frequently detected in both water and soil (Stackelberg et al., 2004; Topp et al., 2012). Gemfibrozil is a common drug to regulate the blood lipid (e.g. cholesterol) in both human and animals (Rodney et al., 1976). Carbamazepine is typically used for the treatment of epilepsy and neuropathic pain. Both the gemfibrozil and carbamazepine are cost-effective and used by the public for a long time and they are frequently detected in water system (Vieno et al., 2007; Sacher et al., 2008). Diphenhydramine, carbamazepine and gemfibrozil were also detected in fish in several rivers of USA (Ramirez et al., 2009).

3.2 Materials & Method

3.2.1 Materials

The chloramphenicol, florfenicol, lincomycin hydrochloride, diphenhydramine hydrochloride, gemfibrozil and carbamazepine standards were purchased from Sigma-Aldrich (St Louis, USA). All detailed information of these compounds is shown in Table 3.1.

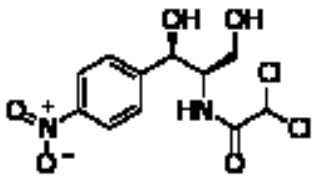
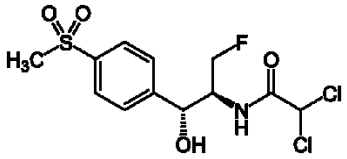
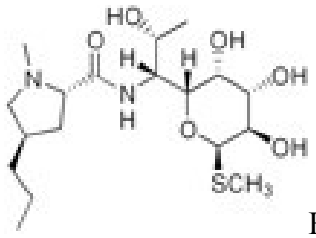
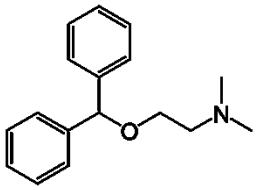
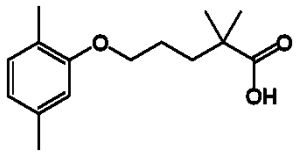
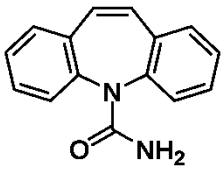
3.2.2 Pyrolysis GC-MS analysis

In all experiments, chemicals were ground in a small mortar before analysis. A Varian CP-3800

gas chromatograph coupled to a Saturn 2000 ion trap detector interfaced to a CDS Pyroprobe 2000 unit through a valved interface (CDS 1500) was used for the pyrolysis analysis. A mass of 0.5±0.1 mg of the ground pure standard was introduced into a quartz tube (0.3 mm thickness), plugged with quartz wool, and inserted into the coil probe of the instrument. GC separation was performed on a fused silica HP-5 MS column (50 m length × 0.2 mm i.d. × 0.33 μm film thickness; J&W Scientific). The analysis model was similar to the one of Yaylayan et al. (2003). The pyroprobe was set at the desired temperature (250°C) at a heating rate of 50 °C s^{-1}. The melting point of the six chemicals in this study is lower than 210 °C (See Table 3.1), and the temperature was set to be 250°C to maximize the thermal degradation. The temperature in the GC column was initially set at -5 °C for 12 min (using CO_2 cooling), then increased to 50 °C at a rate of 50 °C min^{-1}, finally raised to 250 °C at a rate of 8 °C.min^{-1} and kept at 250°C for 5 min. A constant flow of 1.5 mL min^{-1} was used during analysis. Capillary direct MS interface temperature was 250°C; ion source temperature was 180°C. The ionization voltage was 70 eV, and the electron multiplier was 2047 V. The m/z range analyzed was 29-500 amu. The chromatograms were recorded and the identity and purity of individual peaks were determined using NIST AMDIS version 2.1 software. Purity (%) of a peak is calculated by the software which indicates whether the selected peak in the chromatogram contains impurities at one specific retention time (e.g. if the purity is 100, it means that there is no other fragments come out at the same retention time, and the peak corresponds to only one fragment). Another parameter, the mass match (‰) calculated by the software reflects how well the recorded MS matches the theoretical spectra for each proposed structure. The probability (%) was also given by the software which indicate the probability of the fragments formation.

Quality assurance procedure included procedural blanks processed together with the samples. Procedural blanks consisted of empty quartz tubes plugged with quartz wool and inserted into the coil probe. Then the empty tube was burnt using the same method as the standards. All the experiments were triplicated.

Table 3.1 Properties of 6 chemical standards

Name	Mass (average molecular weight)	Melting point (ChemSpider)	Structure	Standard Purity
Chloramphenicol	323.1	171°C		99.8%
Florfenicol	358.2	153°C		99%
Lincomycin Hydrochloride	443	156-158°C	 HCl	95%
Diphenhydramine Hydrochloride	291.8	161-162°C	 HCl	98%
Gemfibrozil	250.3	62°C		99%
Carbamazepine	236.3	204-206°C		98%

3.3 Results and discussions

All the detectable chromatogram peaks were analyzed using the NIST AMDIS database. The

chromatograms for a typical blank and for each compound are presented in Figure 3.1 to 3.7. The peak height was scaled by the highest peak in the chromatogram (i.e. the highest was scaled as 100). The identity of degradation products was done by comparing the chromatogram obtained for each compound with the procedural blank. Peaks occurring in the blanks were excluded from this analysis. Peaks that only existed in standards with the purity higher than 50 % were marked by numbers on the chromatograms. The possible structure and the probability of each peak were shown in Table 3.2 to 3.6. In many cases, the structure given by the software with high probability was inconsistent with the parent compound, for the suggestion of the software contained elements not present in the parent compound. The top five probabilities of each peak are shown in Table 3.2 to 3.6, and the one that had high match with the parent compound is highlighted and potential structure was given in tables.

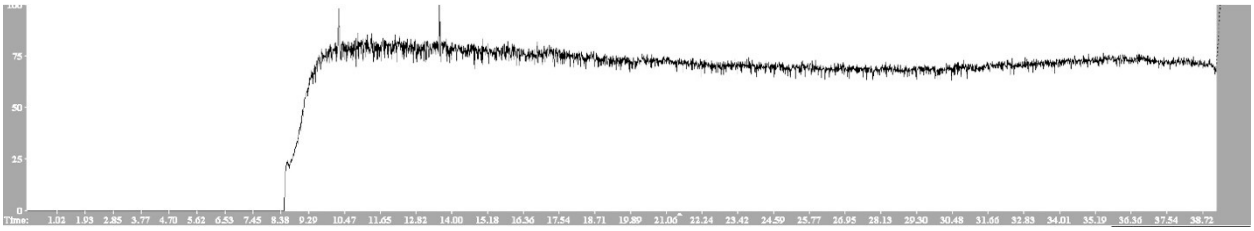


Figure 3.1 Typical GC-MS chromatogram for procedural blanks

3.3.1 Py-GC-MS of Chloramphenicol

Figure 3.2 depicts the GC-MS chromatogram obtained for the degradation of chloramphenicol. There was no match data for peaks 3, 6, 7 and 9 in the NIST AMDIS database. Peak 1, 2, 4, 5, 8, 10 and 11 corresponded to degradation products of chloramphenicol with high match in mass spectrum and the detailed information was shown in Table 3.2. In the reviewed literatures, the thermal degradation of chloramphenicol was about 5-20% in water but higher in tissue (up to 100%) (Franje et al., 2010). The degradation products (peak 4 and 5) found in this study were same as those reported by Franje et al. (2010). This result indicated that Py-GC-MS can be an

effective tool to explore the thermal degradation of chloramphenicol or at least a labor-saving and time-saving method which minimize the steps of sample pretreatments.

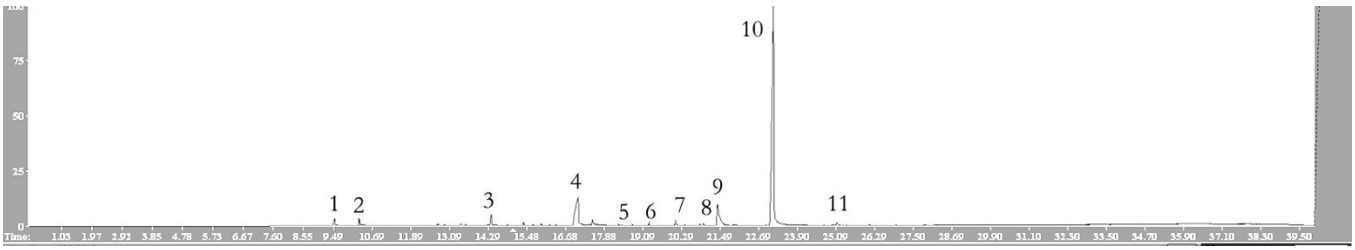
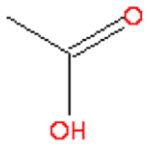
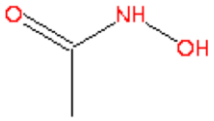
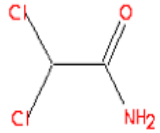
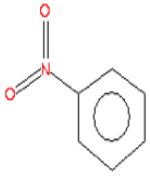
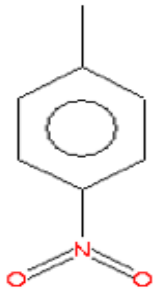
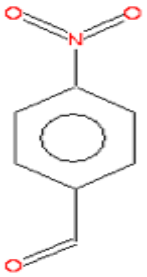
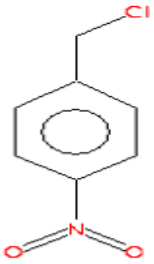


Figure 3.2 GC chromatogram of Chloramphenicol

Table 3.2 Degradation products of Chloramphenicol

Peak	Retention time (min)	Possible degradation products	Mass match (‰)	Probability (%)	Structure*
1	9.525	**Acetic acid**	824	55.1	
		Acetic acid	810	55.1	
		Ammonium acetate	794	16.8	
		Acetic acid anhydride with formic acid	799	15.5	
		Ethyl acetate	731	2.44	
2	10.285	**Acetohydroxamic acid**	820	65.5	
		Methylglyoxal	864	10.8	
		Acetic anhydride	911	6.74	
		2-Propanone,1-hydroxy-	729	5.16	
		2-Propanone,1-hydroxy-	715	5.16	
4	16.959	**Acetamide, 2,2-dichloro**	737	95.9	
		Acetamide, 2,2-dichloro	711	95.9	
		Acetamide, 2,2,2-trichloro	591	2.07	
		N-dl-Alanylglycine	686	0.62	
		L-cysteine sulfinic acid	627	0.41	
5	18.333	**Benzene, nitro**	930	97.2	
		Benzene, nitro	909	97.2	
		Diazo benzene, 4-nitro-	754	2.25	
		Benzoic acid, 2-nitro-	657	0.19	
		Benzene acetic acid, α,4-dihydroxy-	607	0.04	

8	20.873	**Benzene, 1-methyl-4-nitro**	877	71.8	
		Benzene, 1-methyl-4-nitro	874	71.8	
		Benzene, 1-methyl-3-nitro	857	24.0	
		Benzene, 1-methyl-3-nitro	825	24.0	
		s-Benzyl-N-carbobenzoxy-L-cystein e	685	0.57	
10	23.181	2-Nitrosobenzamide	752	40.6	
		4-Nitro-N-(3-pyridylmethylene) benzhydrazide	643	7.33	
		Methanone, (4-ritrophenyl)	629	4.00	
		Benzoic acid, 2-nitro-, methylester	629	3.69	
		Benzaldehyde, 4-nitro	633	3.26	
11	25.145	**Benzene, 1-(chloromethyl)-4-nitro**	917	86.6	
		Benzene, 1-(chloromethyl)-4-nitro	903	86.6	
		Benzene, 1-(chloromethyl)-3-nitro	837	10.6	
		Benzene, 1-(chloromethyl)-3-nitro	816	10.6	
		Carbonoc hloridic acid	750	1.33	

* The structure is corresponding to the highlight degradation product.

3.3.2 Py-GC-MS of Florfenicol

All numbered peaks in Figure 3.3 were identified using NIST AMDIS database. However, very few peaks were corresponding to the parent compound. Peak 3 and 14 seemed to be the only peaks that correspond to the florfenicol (Table 3.3). In reviewed literature, florfenicol showed low degradation in water (2-15%), but high degradation in tissue (up to 80%) (Franje et al., 2010). Theoretically, the temperature was high enough to induce degradation when compared the heating condition with former studies in literatures. Also, indication of degradation, such as change in color of the compound following heating, was present in this experiment. However, in

this study, there were very few degradation products of florfenicol under 250 °C, which indicated that the degradation products of florfenicol were non-volatile.

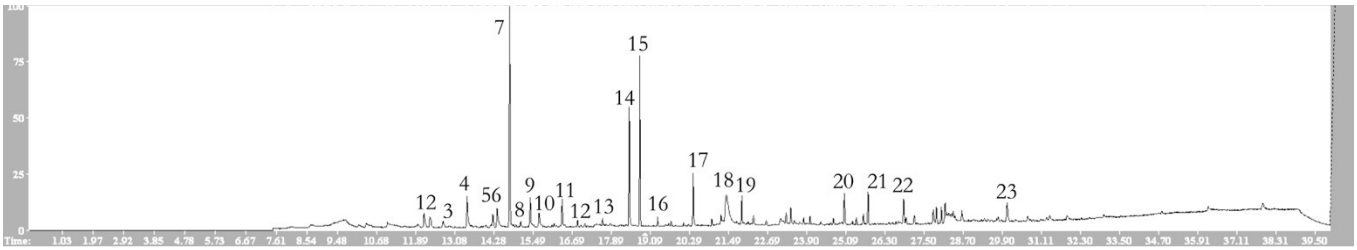
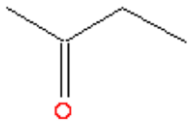


Figure 3.3 GC chromatogram of Florfenicol

Table 3.3 Degradation products of Florfenicol

Peak	Retention time (min)	Possible degradation products	Mass match (‰)	Probability (%)	Structure*
3	12.737	5,9-dodecadien-2-one 6,10-dimethyl-(e e))-	775	60.8	
		2-Butanone	811	16.6	
		1-propen-2-ol-acetate	748	4.96	
		1,3-propanediol-acetal	715	3.80	
		Acetic acid	738	3.50	
14	18.453	**2-Nonen-1-ol-**	878	21.1	
		2-Nonen-1-ol-(E)-	859	15.7	
		2-Nonen-1-ol-(E)-	848	15.7	
		2-Nonen-1-ol-(E)-	844	15.7	
		2-Decen-1-ol-	843	7.16	

* The structure corresponds to the highlighted degradation product.

3.3.3 Py-GC-MS of Carbamazepine

For carbamazepine, peaks 1, 2, 3, 4 and 5 in Figure 3.4 may be the interfering residues of the GC column, as their spectrum is unrelated to the parent compound. Two new peaks were identified (peak 6 and 7) in this experiment, but none of the structures in the NIST AMDIS database were

related to the carbamazepine (structures not shown). It may be because that the volatile degradation products of carbamazepine are not in the database in this study.

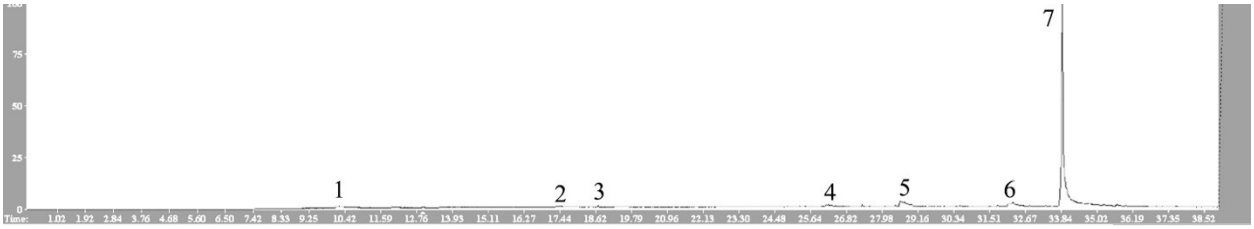


Figure 3.4 GC chromatogram of Carbamazepine

3.3.4 Py-GC-MS of Lincomycin hydrochloride

Figure 3.5 showed the chromatogram of lincomycin hydrochloride. There is no matching data for peak 1, 2, 9, 11, 12, 13, 14, 16, and 17 (not shown in Table 3.4). Peak 7 and 15 were identified to be the contaminants form the GC column, because the match of peaks with database was high, but all these structures were not corresponding to the parent compound (Table 3.4). There was no match data for peak 3, 4, 10 in the NIST AMDIS database. Peak 5, 6, 8 were correspond to the degradation products of licomycin hydrochloride with high match in mass spectrum and the detailed information was shown in Table 3.4.

Lincomycin has been reported to be stable under general cooking conditions. Boiling at 60 °C for 30 min induced no significant degradation (Zorraquino et al., 2011). When heated at 100 °C to 120 °C, the degradation percentage was only 9-15% (Hsieh et al., 2011). In this experiment, heating was conducted at a high temperature of 250 °C, which led to the release of the methanethiol moieties from the parent compound, a reaction that only happened at high temperature. The methanethiol may produce an unpleasant smell during heating, undergoes further degradation into various thio-compounds and is also reported to be harmful to human health (Devos, 1990).

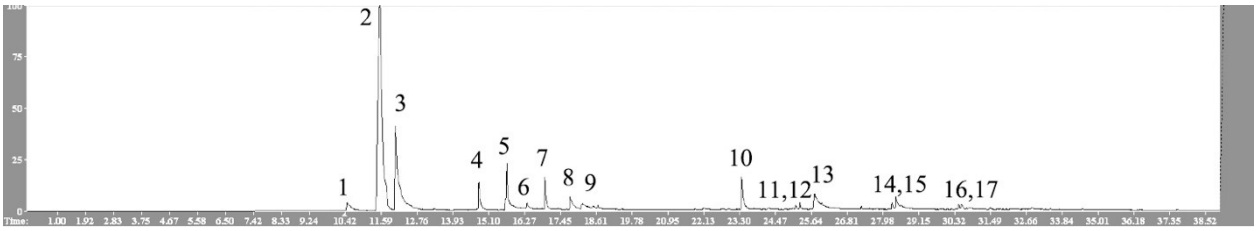
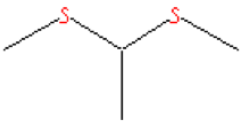
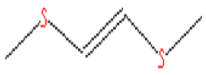


Figure 3.5 GC chromatogram of Lincomycin hydrochloride

Table 3.4 Degradation products of Lincomycin hydrochloride

Peak	Retention time (min)	Possible degradation products	Mass match (‰)	Probability (%)	Structure*
2	11.569	Dimetridazole	834	26.6	
		3-Methyl-thiophene-2-carboxamide	617	25.6	
		2-Amino-4-methylthiazole-5-carbo-xylic acid phenylamide	590	5.16	
		Benzene, 1-fluoro-nitro	559	3.95	
5	15.704	2,3,7,8,12,13,17,18-Tetracyl-10-methoxymethyl-7,8-dihydro-21 H, 23H-porphine	642	70.5	
		Carbonodithioic acid, S,S-dimethyl ester	760	9.16	
		Ethane, 1,1-bis(methylthio)-	792	3.89	
		5-Gemaspiro |4,4| nona-1,3,6,8 tetraene	431	2.75	
		Benzo | 1,2-b:4,5-b| bisbenzofuran-6,12-dione, 2,3,8,9-tetrakis |(trimethylsilyl) oxy|-	501	1.77	
6	16.356	**Dimethyltrisulfide**	798	86.4	
		Bis-(methylthio)-phosphine	727	6.32	
		5-Methylmethanethiosulphonate	587	0.54	
		4-Methyl-2-pyrimidinethiol	587	0.50	
		3-|(Cyclohexl-methyl-amino)-methyl|-3 H-benzo oxazole-2-one	593	0.36	
7	16.942	2,3,7,8,12,13,17,18-Tetracyl-10-me	623	30.4	

		thoxymethyl-7,8-dihydro-21 H, 23 H-porphine			
		2(1 H)-Pyridinone, 1,6-dimethyl-	788	19.0	
		2(1 H)-Pyridinone, 1,4-dimethyl-	856	8.09	
		5-Gemaspiro |4,4| nona-1,3,6,8 tetraene	417	6.52	
		2(1 H)-Pyridinone, 1,5-dimethyl-	853	3.75	
8	17.757	**Ethene, 1,2-bis(methylthio)-**	872	75.8	
		2-Methyl-1,3-dithiacyclopentane	768	14.2	
		Benzenepentanenitrile	751	1.17	
		Ethaneperoxoic acid, 1-cyano-1-phenyloctyl ester	708	0,77	
		Ethaneperoxoic acid, 1-cyano-1,4-diphenyloctyl ester	739	0.66	
15	28.407	Benzophenone	882	63.8	
		2,5-Dimethyl-7,7-diphenyl-3-aza-4, 6-dioxabicyclo|3.2.0| hept-2-ene	810	13.3	
		1,2,4,5-Tetraxane,3,3,6,6-tetraphen yl-	832	6.08	
		4-Acetyl-7,7-diphenyl-6-oxa-2,4-di azabicyclo |3.2.0| hept-2-ene	773	5.13	
		Benzophenone dimethylketal	759	3.42	

* The structure corresponds to the highlighted degradation product.

3.3.5 Py-GC-MS of Diphenhydramine hydrochloride

The degradation of diphenhydramine hydrochloride shown in Figure 3.6. The identity for peak 2 and 5 was not corresponded to the parent compound (not shown in Table 3.5). Peak 1, 3, 4, 6 and 7 detailed in Table 3.5.

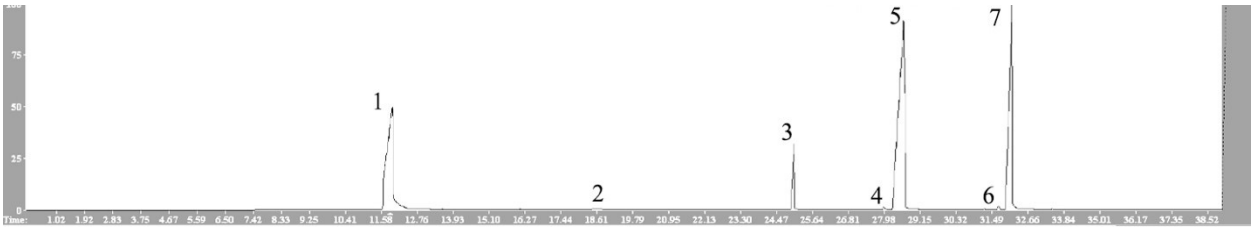
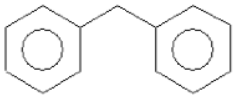
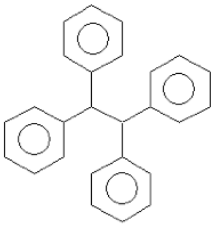
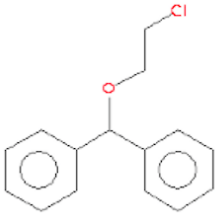
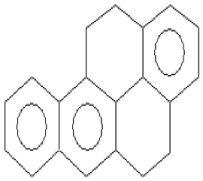


Figure 3.6 GC chromatogram of Diphenhydramine hydrochloride

Table 3.5 Degradation products of Diphenhydramine hydrochloride

Peak	Retention time (min)	Possible degradation products	Mass match (‰)	Probability (%)	Structure*
1	11.968	2-Propanol , 1-hydrazino	721	47.2	
			783	12.6	
		Hydrazine, 2(methoxyethyl)-	663	12.1	
		Ethanol, 2-(2-methoxyethoxy)-	619	2.90	
		1 H-1,2,3-Triazol-1-amine, 4-phenyl-N-(phenylmethylene)-	619	2.05	
		Tromethamine			
3	25.050	1,1 Biphenyl, 4-methyl-	760	15.0	
		1,1 Biphenyl, 3-methyl-	760	13.8	
		Diphenylmethane	758	12.8	
		1,1Biphenyl, 2-methyl-	747	9.26	
		1,1 Diphenyl, 2-propanol	744	7.46	
4	27.968	Benzene, 1-dimethylamino-4phenylmethyl-	634	13.5	
		Benzeneacetamide, α-phenyl-	695	9.49	
		Benzeneacetic acid, α-phenyl-	863	6.89	
		Felbinac	868	5.14	
		Benzene, 1,1,1,1-(1,2-ethanediylidene) tetrakis-	843	4.14	
6	31.716	**Benzhydryl 2-chloroethylether**	817	88.8	
		1,1-Biphenyl, 4-bromo-4-methyl	684	3.56	
		3-Bromodiphenylmethane	673	3.56	
		1,1-Biphenyl, 3-bromo-5-methyl	663	1.0	
		Ethyl 1-(diphenylmethyl) piperidine-4-carboxylate	594	0.61	
7	32.139	**4,5,11,12-Tetrahydrobenzo|a|pyrene**	705	9.69	
		3,8-Azo-4,7-methanocyclobuta|b|naphthalene	704	8.93	
		2,2-Biquinoline	768	7.89	
		6,6-Biquinoline	754	7.89	
		Benzo|c|phenanthrene, 5,8-dimethyl	576	7.28	

* The structure corresponds to the highlighted degradation product.

3.3.6 Py-GC-MS of Gemfibrozil

For gemfibrozil, peak 1, 2, 3 and 7 were identified inconsistent to the parent compound, and

there was no match data in software for peak 8 (Figure 3.7). The identity of degradation products of gemfibrozil was shown in Table 3.6.

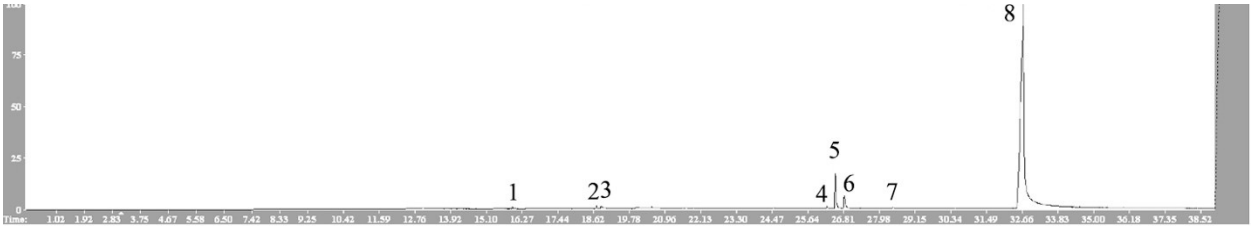
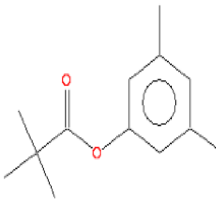
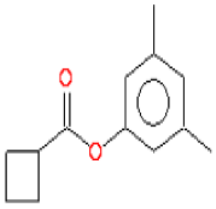
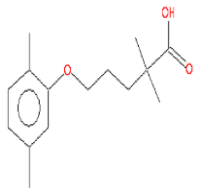


Figure 3.7 GC chromatogram of Gemfibrozil

Table 3.6 Degradation products of Gemfibrozil

Peak	Retention time (min)	Possible degradation products	Mass match (‰)	Probability (%)	Structure*
4	26.271	**2,2 Dimethylpropanoic acid, 3,5-dimethylphenylester**	820	51.3	
		Valeric acid, 3,5-dimethylphenylester	786	33.1	
		Phenol, 3,5-dimethyl methylcarbamate	850	2.42	
		Butyric acid, 3,5-dimethylphenylester	861	1.95	
		5-Chlorovaleric acid, 3,5-dimethylphenylester	853	1.53	
5	26.548	**Cyclobutanecarboxyic acid, 3,5-dimethylphenylester**	850	94.6	
		3-Methylbut-2-enoic acid, 3,5-dimethylphenylester	689	1.66	
		Tricyclo |6.3.0.0(2,4)| undec-8-ene, 3,3,7,11-tetramethyl	592	0.40	
		Piperidin-4-one,3-(2-furanylmethylene)-1,2,5 trimethyl-	603	0.37	
		1,4-Dimethyl-8-isopropyidenetricyclo	589	0.21	

6	26. 834	Cyclobutanecarboxyic acid, 3,5-dimethylphenylester	804	68.8	
		5-Chlorovaleric acid, 3,5-dimethylphenylester	741	4.83	
		3-Chlorovaleric acid, 3,5-dimethylphenylester	736	3.89	
		Gemfibrozil	708	3.89	
		3-Methylbut-2-enoic acid, 3,5-dimethylphenylester	706	2.44	

* The structure corresponds to the highlighted degradation product.

3.4 Conclusions

In this study, Py-GC-MS was successfully applied for the first time to detect a range of volatile thermal degradation products for various pharmaceutical residues encountered in food products. For example, acetic acid, acetohydroxamic acid and 2,2-dichloroacetamide (chemicals commonly in drug synthesis) were identified as three of the volatile degradation products of chloramphenicol. Some of these residues are known to be toxic at high dose. For example, very high dose (750 mg/kg) of acetohydroxamic acid may cause leg deformities (USP, 1997). Similarly, the methanethiol moieties degraded from lincomycin hydrochloride are also reported to be harmful to human health (Devos, 1990). The present study does not explore which dose of these residues would be available in the food or whether they would be taken up by the human body, but it underlines how essential is the understanding of degradation products for food safety. There is no information in the literature about the thermal stability of diphehydramine. McEneff et al. (2013) reported the change of gemfibrozil and carbamazepine concentrations in mussel during thermal treatment, and surprisingly, the concentration of two compounds increased with heating (steaming). In the present study, results indicated that the diphenhydramine hydrochloride, gemfibrozil and carbamazepine all decompose into volatile compounds during heating at 250 °C. The temperature used in this study is higher than the general cooking, except for roasting and grilling processes.

Py-GC-MS is fast and simple to implement and a run only takes about one hour, two if a blank run is completed between each sample. In the present study, replicate analyses were performed for all compounds and results were reproducible. As observed in figures 3.2 to 3.7, chromatograms are overall simple to interpret. Altogether, these results suggest that Py-GC-MS is an effective and simple technique to study the thermal degradation of several pharmaceutical contaminants and to identify the volatile degradation products. Structural elucidation could not be achieved though for the new peaks obtained for carbamazepine, underlining the limitations associated with the mass spectrometry database.

In future studies, the low temperature pyrolysis (e.g. below 200°C) using Py-GC-MS should be explored, which can simulate the general cooking condition in order to give a good idea about the fate of pharmaceutical residues in food that had undergone food processing. Also, new mass spectrometry databases should be added for further identification of the degradation compounds.

CHAPTER 4. THERMAL DEGRADATION KINETICS OF AMPHENICOLS

4.1 Introduction

As introduced in Chapter 2, the degradation of antibiotics is generally assessed by measuring the change in either the antimicrobial activity or the concentration of parent antibiotic residue in the food or the food extracts using analytical techniques based on chromatography. Earlier studies used microbiological assays to determine the reduction in antibiotic residues, which determined the difference in microbial activity before and after treatment, from which a degradation percentage in the biologically active compound was calculated (O'Brien et al., 1981; Shakila et al., 2006; Franje et al., 2010). As the degradation products of antibiotics may be bioactive, biological tests may not always reflect the real concentration of the parent compound (Traub and Leonhard, 1995), the utilization of techniques based on chromatography, especially HPLC, have become more and more popular in analyzing the thermal degradation of antibiotics.Although the limit of detection (LOD) of HPLC-UV instruments is generally reported to be higher than for HPLC-MS (Evaggelopoulou & Samanidou, 2013; Vue et al., 2002), both techniques are able to monitore the degradation chloramphenicol and florfenicol under controlled conditions at relatively high concentrations (>10 ppm).

As the first-order kinetics were applied for most of antibiotics in the reviewed literature, the amphenicols are hypothesized to follow the first-order kinetic, which will be confirmed in this chapter. The thermal degradation of chloramphenicol and florfenicol will be analyzed by HPLC (Waters-2795) coupled with Photodiode Array Detector (PAD) (Waters-996) and HPLC (Agilent 1290) coupled to an Agilent 6460 Triple Quad liquid chromatograph-tandem mass spectrometer. First, the applicability of the first-order degradation model (See Equation 3 and Equation 4 in Chapter 2) was assessed. Then the effect of different parameters such as temperature (from 60 °C to 180 °C) and matrix (water and oil) on the degradation rate and the differences between the two analytical methods will be explored.

4.2 Materials and methods

4.2.1 Materials

Standards of chloramphenicol and florfenicol were obtained from Sigma–Aldrich (St. Louis, MO, USA). Detailed physico-chemical information about the compound was presented in Table 3.1. Bisphenol A (BPA) (purity = 99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA) and was used as an internal standard for both HPLC-PAD and HPLC-QqQ-MS/MS. Acetonitrile, methanol and water (all HPLC grade) were purchased from Fisher Company (Fisher Scientific, IOWA, USA). Corn oil (Selection, QC, Canada) was purchased in local market.

Stock standard solutions of chloramphenicol and florfenicol were prepared in methanol at a final concentration of 5000 mg/L (5000 ppm). BPA standard solution was prepared in methanol at a final concentration of 50 mg/L (50 ppm). Working standard solutions of chloramphenicol and florfenicol were diluted by HPLC grade water into 50 mg/L (50 ppm) before the heating experiment. The BPA standard stock solution was diluted by methanol into 2 mg/L (2 ppm).

4.2.2 Method validation

Instrument linearity, LOD and extraction recovery were assessed in each experiment. The linearity of instrument response was determined with diluted standard solution at five concentrations ranging from 20 to 1000 ng/L for HPLC-QqQ-MS/MS (Agilent 1290) and from 10 to 100 mg/L for HPLC (Waters-2795). The LOD was calculated as three times the standard deviation (3σ) of blanks divided by the slope of calibration curves. The extraction recovery of each amphenicol in oil was calculated as detailed in section 4.3.3.4.

4.2.3 Experimental procedure

4.2.3.1 Kinetic studies at 100° C in water by HPLC-QqQ-MS/MS

The degradation kinetics of the two compounds were first explored at 100°C in water at two nominal concentrations (50 ppm and 500 ppb) to validate the kinetic model (1st order). A diluted

standard solution (0.5 mL) for each amphenicol (500 ppb or 50 ppm) in water was transferred in 2mL-HPLC glass vials (n=5). Each vial was closed by the screw cap and hold by a floating rack. Vials were heated in water bath at 100 °C, for 30 min (t_{30}), 60 min (t_{60}), 120 min (t_{120}) and 240 min (t_{240}). One vial was left as the unheated control (t_0). After heating, vials were taken out of the water bath and wiped dry, and then they were cooled down rapidly in cold water. For the 50 ppm experiment, an aliquot of each sample was diluted 100 times in methanol containing BPA (2 ppm) as internal standard. For the 500 ppb experiment, the internal standard was added and vials were quantified as such.

Antibiotic concentrations were quantified using an Agilent 1290 high performance liquid chromatographic system using an Agilent C8 analytical column (Zorbax Eclipse Plus 150 mm × 2.1 mm, 5 μm). The HPLC and Agilent 6460 Triple Quad liquid chromatograph-tandem mass spectrometer were interfaced with an electrospray ionization source operating in the negative ion mode. The multiple reaction monitoring (MRM) parameters are listed in the Table 4.1. Mobile phases were 80:20 v/v water/acetonitrile (A) and 100 % acetonitrile (B) with a flow rate of 0.3 mL min^{-1} and a gradient profile as follows: 0-1 min is 100 % A; 1-2 min A is decreased to 80% and B is increased to 20%; 2-3 min B is raised to 35 %; 3-4min B is increased to 50%; 4-5 min B is increased to 75% and then adjusted to 100 % for a further 5 min. Then B is decreased to 50% for 4 min; 14-15 min B is decreased to 25%; 15-16 min A is increased to 100% again.

The applicability of the first-order degradation model was assessed through the examination of the plot of the natural logarithm of the concentrations as a function of time (See Equation 3 in Chapter 2). The first-order degradation rate constant (k) was determined as the slope (absolute value) of the linear fit using Sigmaplot version 12.0 (SSI, CA, USA). The model was considered acceptable when at least two of three replications fit the model with the R^2 for the linearity larger than 0.85 and P values lower than 0.05.

Table 4.1 Multiple reaction monitoring (MRM) parameters of HPLC-QqQ-MS/MS

Amphenicol	MRM	Collision Energy (V)	Fragmentor energy (V)
Chloramphenicol	321.0>152.0	10	115
	321.0>257.0	2	115
Florfenicol	355.8 > 185.0	12	90
	355.8 > 336.0	5	90

4.2.3.2 Kinetic studies at 60 °C and 80 °C in water by HPLC-QqQ-MS/MS

After validation of the first-order kinetic model, degradation studies in water (50ppm) were conducted at 60 °C and 80 °C using only three time-point series (0, 30 and 60 min). Samples were treated in the same way as 3.2.3.1 and analyzed by HPLC-MS/MS. First-order degradation rate constant (k) were again determined from the linear fit (slope) of the plot the natural logarithm of the concentrations as a function of time (See Equation 2-5 in Chapter 2).

4.2.3.3 Kinetic studies at 60 °C and 80 °C and 100 °C in water by HPLC-PAD

In the initial phase of this work, kinetics was investigated using HPLC-PAD. The 50 ppm standard solution was used and the experimental conditions had been set as above.

Antibiotic concentrations were quantified using a Waters 2795 HPLC system consisting of a binary solvent manager, autosampler, Photodiode Array Detector (Waters 996) (PAD) and a C8 analytical column (Zorbax Eclipse Plus 150 mm × 2.1 mm, 5 μm). HPLC working condition is similar to the one of McEneff et al. (2013). Mobile phases were 80:20 v/v water/acetonitrile (A) and 100 % acetonitrile (B) with a flow rate of 0.3 mL min^{-1} and a gradient profile as follows: 0-2 min is 100 % A; 2-3 min A is decreased to 80% and B is increased to 20%; 3-4 min B is raised to 35 %; 4-5min B is increased to 50%; 5-6 min B is increased to 75% and then adjusted to 100 % for a further 5 min. Then B is decreased to 50% for 4 min; 15-16 min B is decreased to 25%; 16-18 min A is increased to 100% again. The injection volume was 10 μL. The wavelength for

quantification of chloramphenicol in this study is 277 nm, under which the compound shows the maximum absorbance (λ_{Max}), and this wavelength is same as the one in the study of Chen et al. (2011). The λ_{Max} of florfenicol was reported to be 266 nm (Ma et al., 2014), however, in our case, the maximum absorbance wavelength is 227 nm for florfenicol.

4.2.3.4 Kinetic studies in oil by HPLC-PAD

In another experiment, the degradation kinetics of amphenicols were explored in oil. Therefore, a standard solution (5 µL) for each amphenicol (5000 ppm) was transferred in 2 mL-HPLC glass vials. Then, 495µL corn oil was added into each vial and mixed well by Vortex mixer (Scientific Industries, NY, USA). Samples were heated at 60 °C, 80 °C and 100 °C for 30 min and 60 min in a water bath as described above. In addition, samples in oil were wrapped in aluminum paper and heated at higher temperatures (140 °C and 180 °C) in an accurate laboratory oven (Fisher Scientific) for 30 min and 60 min. After heating, all the samples were cool down rapidly by cool water.

All samples in oil were then extracted with methanol. 0.5 mL of methanol was added in each vial and mixed by Vortex mixer for 1 min. Then, vials were cooled in freezer for 1 h to let the oil and methanol separated. The upper methanolic extract was collected. The extraction was repeated another two times. Extracts were combined in a pre-weighted glass vial, and the weight of extracts were recorded. Then the volume of extraction ($V_{Extraction}$) was calculated by dividing the density of methanol (0.792 g/mL). Then equal weight BPA standard solution (50 ppm) was added as the internal standard. For the control, 5 µL amphenicol standard solution (5000 ppm) was added in a pre-weighted glass vial, methanol was added to a final weight of $W_{Extraction}$. Then, equal weight BPA standard solution (50 ppm) was added as the internal standard. The recovery of the extraction was calculated as follows:

$$Recovery\ (\%) = \frac{M_{\ Extracted\ amphenicol}}{M_{\ Control}} \times 100 \qquad \text{(Equation 6)}$$

where $M_{\text{Extracted amphenicol}} = C_{\text{Extracted amphenicol}} \times V_{\text{Extraction}} \times 2$;

and $M_{\text{Control}} = C_{\text{Control}} \times V_{\text{Extraction}} \times 2$;

Then, the Equation 6 can be simplified to Equation 7:

$$Recovery\ (\%) = \frac{C_{\ Extracted\ amphenicol}}{C_{\ Standard}} \times 100 \qquad \text{(Equation 7)}$$

where the $\frac{C_{\ Extracted\ amphenicol}}{C_{\ Standard}}$ is equal to the $\frac{Peak\ area\ ratio\ of\ extracted\ amphenicol}{Peak\ area\ ratio\ of\ standard}$;

Peak area ratio = Peak area of amphenicol / Peak area of BPA.

For this experiment, samples were analyzed by HPLC-UV under the same condition as section 4.2.3.3. Similarly, k for section 4.2.3.3 and section 4.2.3.4 was determined as the slope of the curve, which achieved by plotting the natural logarithm of the concentrations as a function of time. All the experiments were triplicated. Statistical study was done by Sigmaplot version 12.0 (SSI, CA, USA) and SPSS version 22.0 (IBM, NY, USA)

4.3 Results and discussions

4.3.1 Linearity of equipment, equipment LOD and extraction recovery

The average extraction recovery for chloramphenicol and florfenicol is 95.6±1.8 % and 93.8±3.0 %, respectively (n=3). Detailed information for linearity and LOD is given in Table 4.2. The LOD for HPLC-QqQ-MS/MS is much lower than the one for HPLC-UV. Nonetheless, both techniques were suitable for this study as LODs were much lower than the experimental concentrations.

Table 4.2 Linearity of equipment and limit of detection (LOD)

Equipment	Antibiotics	R^2	*P*	Average R^2	LOD (ppb)	Average LOD (ppb)
HPLC-MS/MS	Chloramphenicol	0.9645	0.0179	0.965 ± 0.01	2.61	3.29 ± 0.68
		0.9755	0.0016		3.29	
		0.9549	0.0041		3.98	
	Florfenicol	0.9650	0.0028	0.950 ± 0.02	3.63	3.64 ± 0.33
		0.9615	0.0032		3.98	
		0.9232	0.0392		3.32	
HPLC-UV	Chloramphenicol	0.9953	0.0001	0.997±0.002	234.5	202.87 ± 26.09
		0.9954	0.0001		203.5	
		0.9988	0.0001		170.6	
	Florfenicol	0.9985	0.0007	0.980± 0.032	185.5	190.00 ± 10.11
		0.9992	0.0001		204	
		0.9431	0.0059		180.5	

4.3.2 Thermal degradation kinetic of amphenicols at 100°C in water

Degradation studies using the methodology present in 4.2.3.1 (HPLC-QqQ-MS/MS) confirmed the degradation of amphenicols at 100°C in water. The logarithm of the concentration vs time was plotted in Figure 4.1 to Figure 4.4. In Figure 4.1 there are only four points for each line due to the data (t_{60}) missing during the analysis. However, four points are still enough to verify the model.

The degradation rate constants k derived from the curves in Figure 4.1 to Figure 4.4 are shown in Table 4.3. There is one k for chloramphenicol and one for florfenicol at 50 ppm that not accepted for the first-order model as the R^2 is lower than 0.85 (Table 4.3), and these k values will be excluded for further analysis. The other two k values for chloramphenicol and florfenicol at 50 ppm fit the first-order model well (Table 4.3). Thus, the degradation kinetic of chloramphenicol

at 50 ppm fit the first-order model. The logarithm of the concentration-time data points for the two amphenicols showed the linearity with R^2 ranging from 0.9372 to 0.9870 (500 ppb) and R^2 ranging from 0.9172 to 0.9980 (50 ppm), with p values below 0.03. These results indicate that the first-order kinetic model is an appropriate model for the thermal degradation of both chloramphenicol and florfenicol.

Table 4.3 k for amphenicols at 100 °C in water derived from Figure4.1 to Figure 4.4

Amphenicols	Slope (-k)	P	R^2	Slope (-k)	P	R^2
		50 ppm			500 ppb	
Chloramphenicol	- 0.0011*	0.3858	0.6295	- 0.0027	0.0011	0.9811
	- 0.0022	0.0027	0.9945	- 0.0026	0.0033	0.9612
	- 0.0015	0.0010	0.9980	- 0.0021	0.0068	0.9372
Florfenicol	- 0.0032	0.0104	0.9172	- 0.0026	0.0063	0.9405
	- 0.0036	0.0027	0.9661	- 0.0045	0.0048	0.9504
	- 0.0084*	0.0282	0.8415	- 0.0033	0.0282	0.9870

Note: * indicates the k which is not accepted and excluded for further study.

The t-test (SPSS) showed that the difference between k for 50ppm and k for 500ppb was not significant for both chloramphenicol ($P = 0.18$) and florfenicol ($P = 0.93$). This result indicates that the k for chloramphenicol and florfenicol were not affected by the initial concentration, which further proves that the thermal degradation of chloramphenicol and florfenicol follows a first-order kinetic (Upadhyay, 2007).

The k values for chloramphenicol at 100 °C range from 0.0015 to 0.0027 (min^{-1}), which indicates that 60 min boiling can induce about 9.0-16.2% degradation of chloramphenicol. This result is is comparable with the results from the former study done in water by GC-MS (Franje et al., 2010). The average k value for florfenicol at 100°C is about 0.0034 (min^{-1}), which indicates

that 60 min boiling can induce 15.6-27.0% degradation of florfenicol. The degradation percentage of florfenicol in this study is also comparable with the one of Franje et al. (2010).

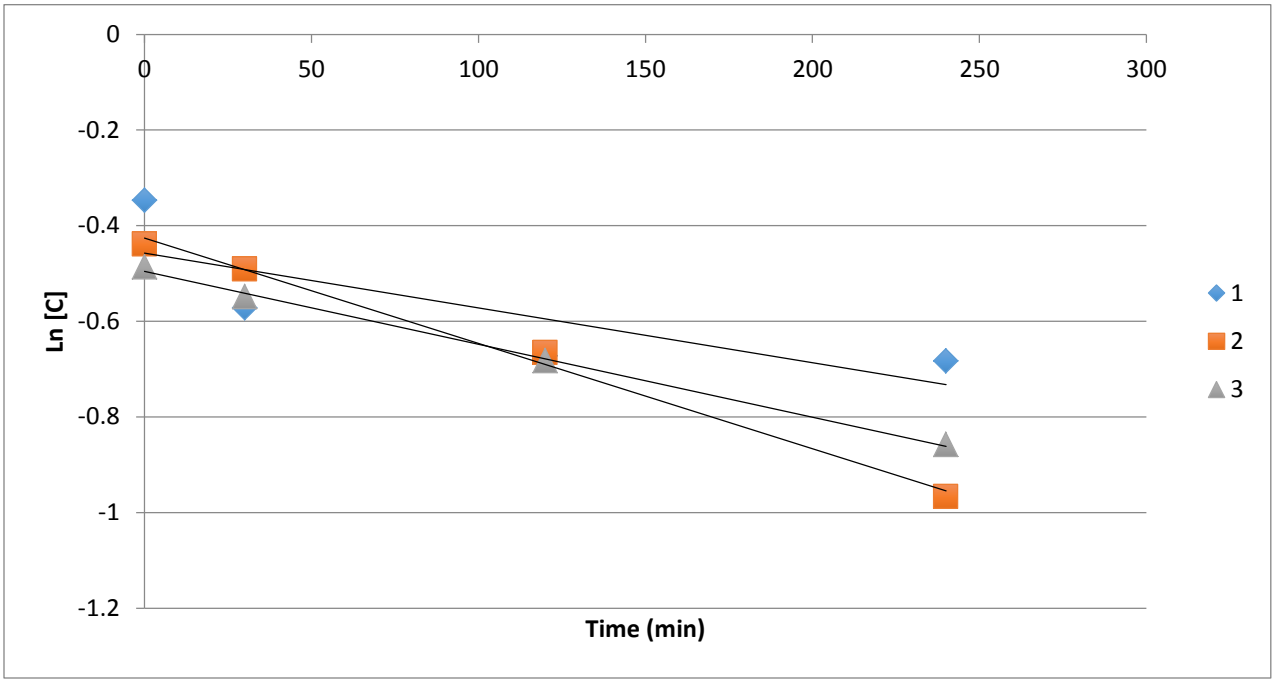


Figure 4.1 Thermal kinetic of Chloramphenicol at 100 °C in water (50 ppm). Note: 1, 2 and 3 correspond to each of the three replicates.

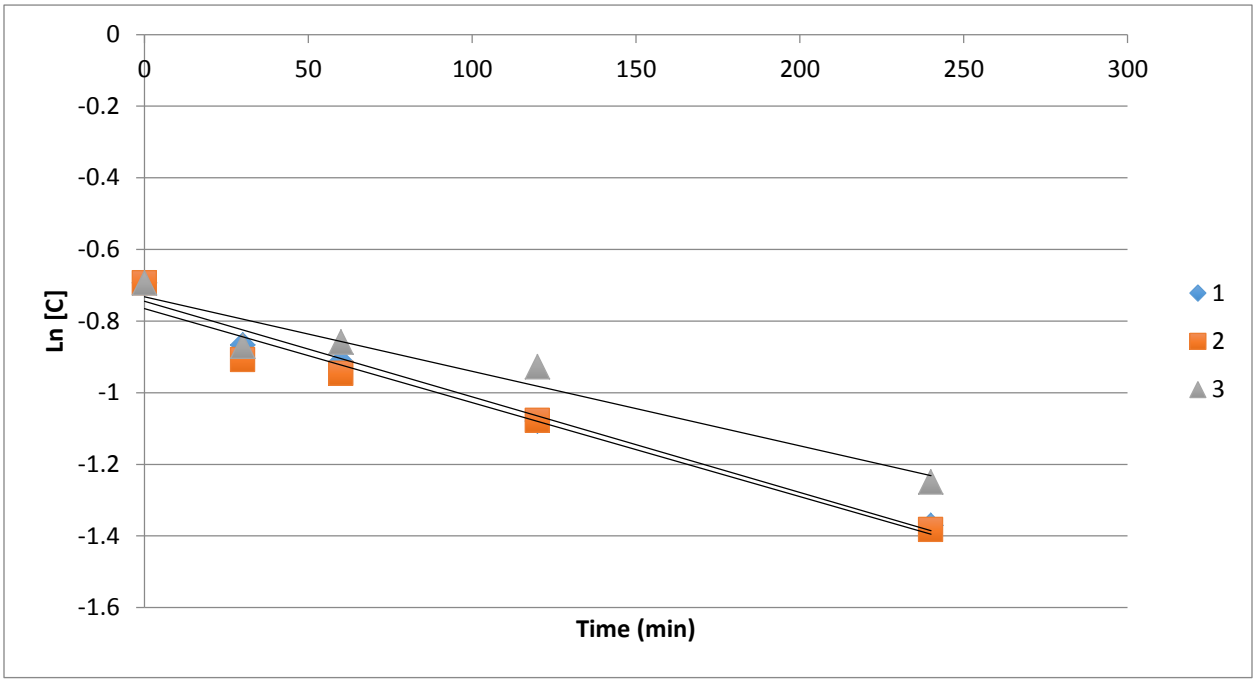


Figure 4.2 Thermal kinetic of Chloramphenicol at 100 °C in water (500 ppb). Note: 1, 2 and 3 correspond to each of the three replicates.

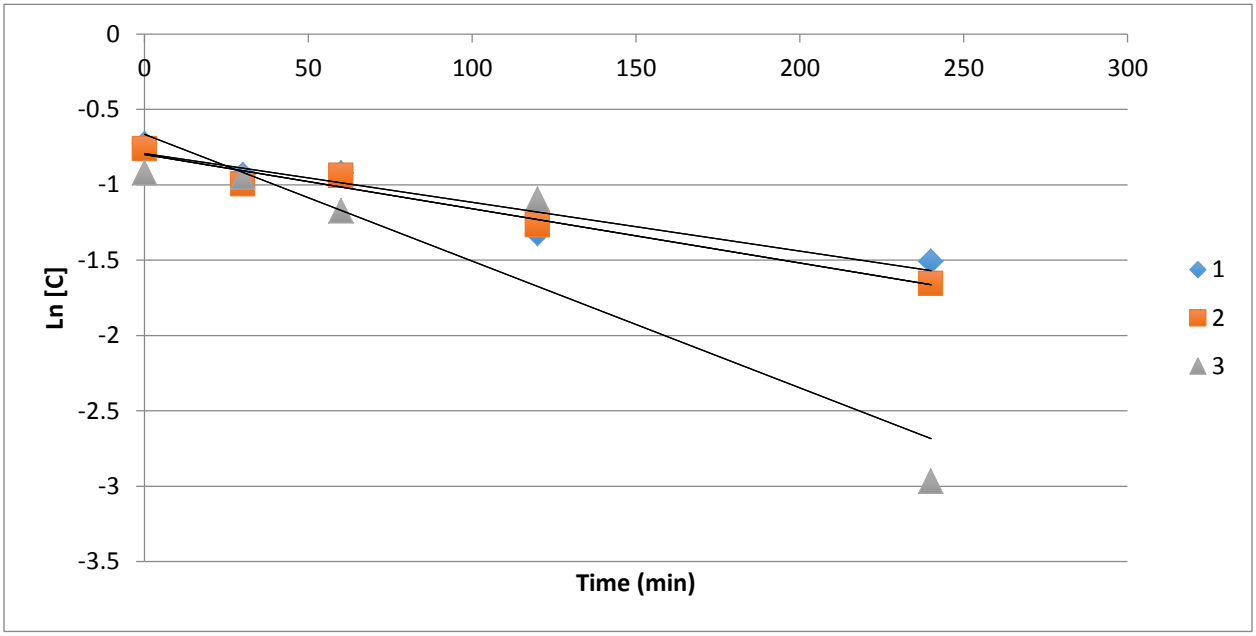


Figure 4.3 Thermal kinetic of Florfenicol at 100 °C in water (50ppm). Note: 1, 2 and 3 correspond to each of the three replicates.

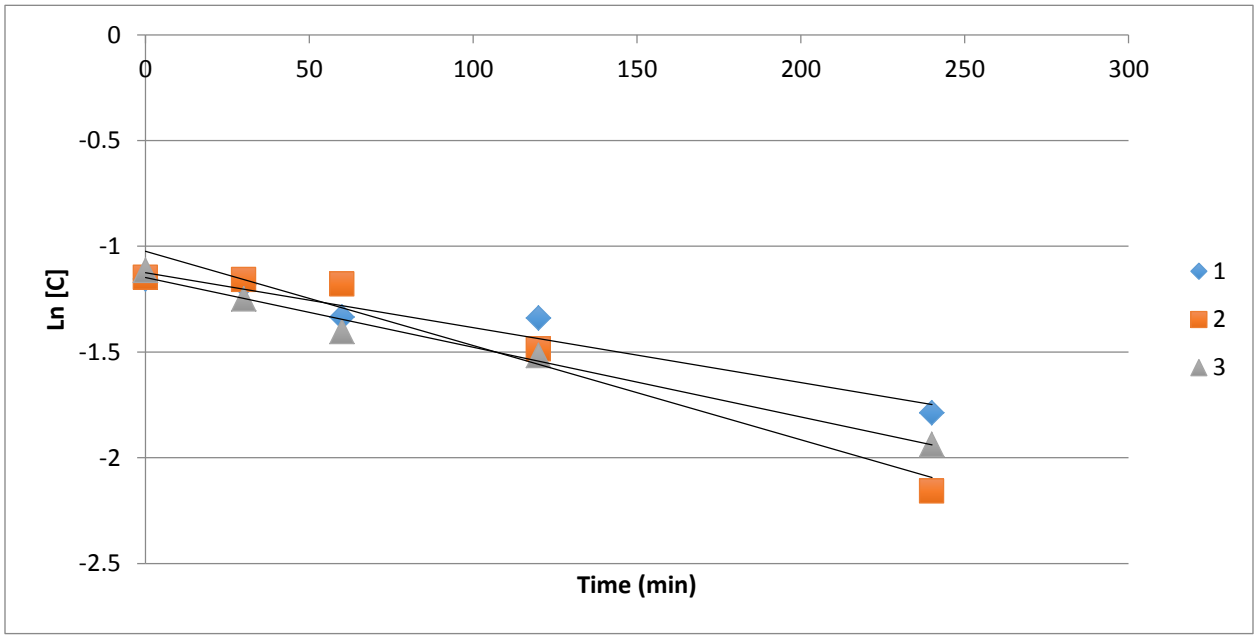


Figure 4.4 Thermal kinetic of Florfenicol at 100 °C in water (500ppb). Note: 1, 2 and 3 correspond to each of the three replicates.

Table 4.4 K for amphenicols (50ppm) under different temperatures (in water)

Amphenicols	Equipment	60 °C	80 °C	100 °C
Chloramphenicol	HPLC-MS/MS	0.0022	0.0024	-
		0.0057	0.0022	0.0022
		0.0025	0.0016	0.0015
Average k		0.0035 ± 0.0016	0.0021 ± 0.0003	0.0019 ± 0.0004
Chloramphenicol	HPLC-PAD	0.0001	0.0006	0.0019
		0.0001	0.0009	0.0027
		0.0005	0.0019	0.0035
Average k		0.0002 ± 0.0001	0.0011 ± 0.0005	0.0027 ± 0.0006
Florfenicol	HPLC-MS/MS	0.0023	0.0018	0.0032
		0.0032	0.0032	0.0036
		0.0014	0.0019	-

Average k		0.0023 ± 0.0007	0.0023 ± 0.0006	0.0034 ± 0.0002
Florfenicol	HPLC-PAD	0.0010	0.0005	0.0038
		0.0005	0.0002	0.0039
		0.0005	0.0010	0.0056
Average k		0.0007 ± 0.0002	0.0006 ± 0.0003	0.0044 ± 0.0008

4.3.3 Comparison of k obtained using HPLC-MS/MS and HPLC-UV

In the initial phase of this work, kinetics was investigated using HPLC-PAD as the operating cost is lower than HPLC-QqQ-MS/MS. The k values for chloramphenicol and florfenicol at different temperatures and matrix using both tools are shown in Table 4.4 and Table 4.5. When comparing the k for chloramphenicol obtained at different temperatures and under different analysis equipment (two-way ANOVA-Tukey test), results indicated that there was no significant difference between the rates obtained for the 3 tested temperatures (P = 0.54) (Table 4.4). However, the k values achieved from HPLC-MS/MS were significantly different from those achieved form HPLC-UV (P = 0.04). Conversely, for florfenicol, both the temperature (P < 0.01) and the equipment (P = 0.046) showed significant effect on the k.

4.3.4 Influence of temperature and matrix on k

Comparing the results achieved by HPLC-PAD, the k (in water) increased slightly with the temperature rising (Figure 4.5 & Figure 4.6), however, there is no significant difference between 60 °C and 80 °C for chloramphenicol (P=0.13, one-way ANOVA) and florfenicol (P=0.85, one-way ANOVA), respectively. This maybe because that amphenicols showed less degradation in water under low temperature (Mitchell et al., 2015).

When heated in oil, the k for chloramphenicol at 180 °C is significant higher than those for other temperatures (P=0.015, one-way ANOVA). However, the differences between 60 °C, 80 °C, 100 °C and 140 °C are not significant (P=0.8, one-way ANOVA) (Table 4.5). This result

indicates that the k increases with temperature at a very low rate under 140 °C but significantly increase at 180°C. For florfenicol, the highest k value was shown at 140 °C, and there is no significant difference between the k values at 60 °C, 80 °C and 100 °C (*P*=0.3, one-way ANOVA).

Table 4.5 K for amphenicols under different temperatures (in oil)

Amphenicols	60 °C	80 °C	100 °C	140°C	180°C
Chloramphenicol	0.0006	0.0025	0.0014	0.0015	0.0058
	0.0010	0.0021	0.0028	0.0029	0.0052
	0.0010	0.0036	0.0036	0.0065	0.0092
Average k	0.0009	0.0027	0.0026	0.0036	0.0067
	± 0.0002	± 0.0006	± 0.0009	± 0.0021	± 0.0018
Florfenicol	0.0026	0.0014	0.0014	0.0049	0.0021
	0.0030	0.0018	0.0016	0.0064	0.0033
	0.0034	0.0026	0.0030	0.0090	0.0055
Average k	0.0030	0.0019	0.0020	0.0068	0.0036
	± 0.0003	± 0.0005	± 0.0007	± 0.0017	±0.0014

When comparing the k values for chloramphenicol and florfenicol from HPLC-PAD between water and oil (Figure 4.5 & Figure 4.6), both the two compounds showed higher k value in oil than in water at 60 °C and 80 °C. However, the difference between water and oil at 100 °C is not significant (*P*=0.91, t-test). Conversely, for florfenicol, the k in water at 100 °C is significant higher than the value in oil at 100 °C (*P*=0.03, t-test).

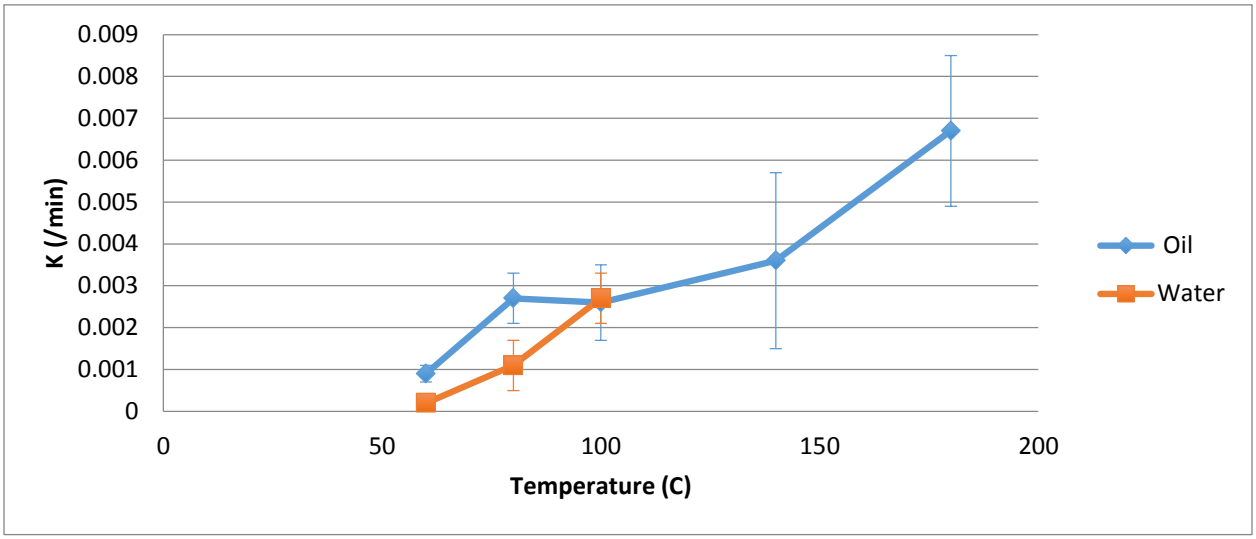


Figure 4.5 k for chloramphenicol under different thermal treatments analyzed by HPLC-UV

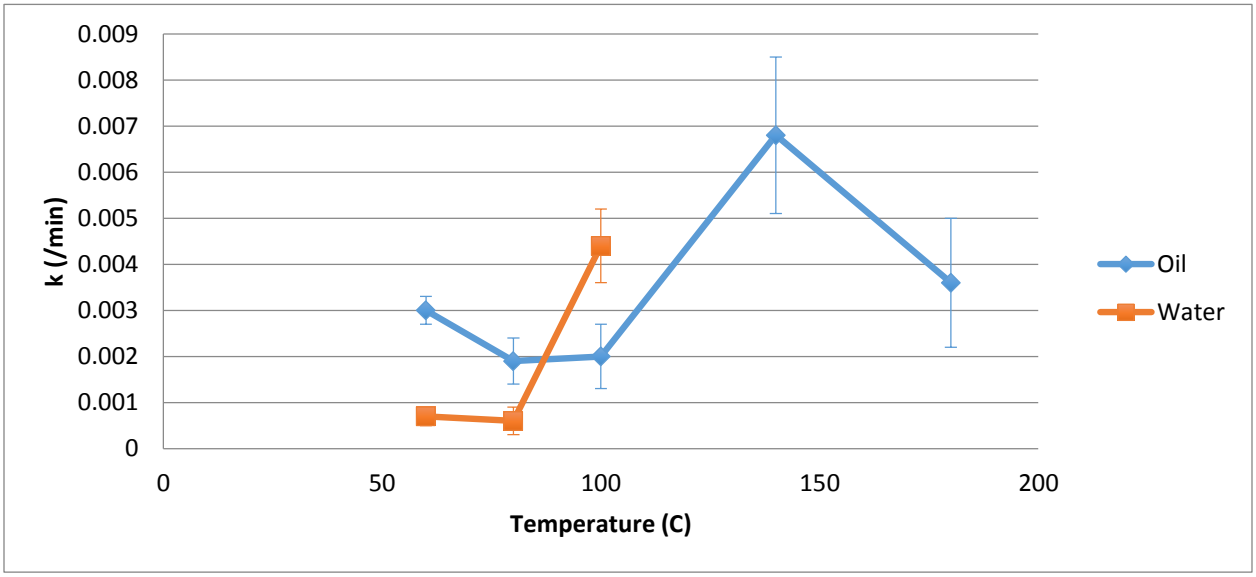


Figure 4.6 k for florfenicol under different thermal treatments analyzed by HPLC-UV

4.4 Conclusions

As hypothesized, the thermal degradation kinetics of chloramphenicol and florfenicol follow the first-order kinetic model, and the initial concentration showed no significant influence on the degradation at 100°C. Overall, heating amphenicols in water or oil at 100 °C for one hour can

induce about 9% to 21% degradation of chloramphenicol and about 15.6% to 27% degradation of florfenicol. Degradation rate constants, k, increased with temperature. When heated in oil, k for chloramphenicol at 180 °C was about four times higher than at 100 °C. Similarly, for florfenicol, k at 140 °C is about three times as high as the k at 100 °C.

In the present work, experiments were repeated with two different analytical instruments to quantify antibiotic concentrations. k for chloramphenicol and florfenicol were different in these two sets of experiments, and the exact reason of this practical discrepancy could not be identified. Nonetheless, since HPLC-QqQ-MS/MS is more sensitive and selective than HPLC-PAD, results obtained with HPLC-MS/MS are considered to be more credible.

k values for both the two amphenicols achieved by HPLC-MS/MS at 60°C in this study were comparable with the result from another study (Mitchell et al., 2015). So far, no literature has reported the degradation kinetics of amphenicol at high temperature (100 °C), and this study is the first one to explore the thermal degradation kinetic of amphenicols at 100 °C, which is close to the cooking condition. Furthermore, this study is the first to report the thermal degradation rate of amphenicols as well as the influences of temperature and matrix on the degradation rate. Thermal treatments, especially at high temperatures, can induce significant degradation of chloramphenicol and florfenicol. Thus, it is very important to explore the degradation products to do the risk assessment from a food safety perspective.

CHAPTER 5. IDENTIFICATION OF DEGRADATION PRODUCTS OF CHLORAMPHENICOL IN WATER AND IN BLUE MUSSEL

5.1 Introduction

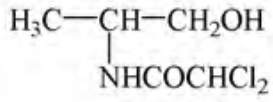
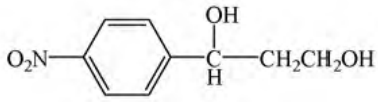
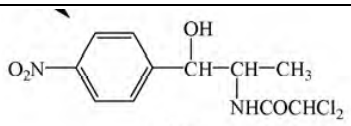
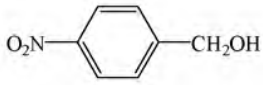
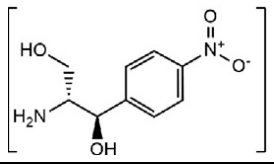
Chloramphenicol was used as a veterinary drug in various places (Allen, 1984). As chloramphenicol can induce adverse health effects on human (e.g. anemia) (Kemper, 2008), it has been banned in countries such as the United States, Canada and European Union for use in food-producing animals for several years (Shakila et al. 2006; EFSA, 2014). Nevertheless, it can still be detected in the environment and in fish (Na et al., 2013; Tittlemier et al., 2007; Sheridan et al., 2008).

As discussed in the earlier sections, it is essential to understand the fate of antibiotic residues in food during processing and identify any degradation products (See Table 5.1). To date, a few studies have explored the degradation of chloramphenicol in matrices such as water, salt water, soybean sauce, chicken and shrimp (Franje et al., 2010; Shakila et al., 2006). Mitchell et al. (2015) also explored the degradation of chloramphenicol in water from an environmental perspective by HPLC-QqQ-MS/MS (i.e. low resolution mass spectrometry). The temperature range explored in this earlier study was low, ranging from 25℃ to 60℃, and is not completely relevant to domestic cooking conditions. Franje et al. (2010) reported the possible structure of the degradation products of chloramphenicol in water and chicken meat by GC-MS. Interestingly, the degradation products suggested from the low resolution MS were different in water and in chicken meat, suggesting that the matrix may influence the thermal degradation. To date, no study has investigated the degradation of chloramphenicol in mollusks.

To date, all the five degradation products of chloramphenicol reported have been generated from investigations based on low resolution mass spectrometry. It is unclear whether the differences between the identified degradation products in water and in food matrix are due to different degradation pathways or to the limitations of the low resolution mass spectrometry technique in identifying compounds in complex matrices. In the end, the unambiguous determination of the

chemical formula (the first step of structural elucidation) of untargeted degradation products can only be achieved through the measurement of accurate mass, especially in complex food matrices (Dunn et al., 2013). Besides, a single molecular formula obtained using accurate mass measurements is not sufficient to elucidate the structure of molecules (Kind & Fiehn 2006; Kind & Fiehn 2007). In this context, tandem mass spectrometry (MS/MS or MS^n) has emerged as a strategy to further identify mass fragments and therefore molecules (Dunn et al., 2013). High resolution and accuracy of mass spectrometer can help to reduce the search space size (number of potential molecular formula), therefore, the high resolution accurate mass spectrometry (HRAM) is more and more favorable (Dunn et al., 2013).

Table 5.1 Degradation products of chloramphenicol in different food matrix reported in reviewed literatures

Molecular formula	Exact mass	Structure	Reference (Matrix)
C5H9NO2Cl2	185.0010		Franje et al., 2010 (In water)
C9H11NO4	197.0688		Franje et al., 2010 (In water)
C11H12N2O3Cl2	290.0225		Franje et al., 2010 (In water)
C7H7NO3	153.0426		Franje et al., 2010 (In chicken meat)
C9H12N2O4	212.0797		Mitchell et al., 2015 (In water)

In this chapter, the degradation products of chloramphenicol in water will be investigated using ultra-high performance liquid chromatography (UHPLC) coupled with hybrid quadruple time-of-flight (QTOF) high resolution mass spectrometer. This approach was anticipated to produce a more comprehensive and accurate screening of degradation products as compared to low resolution mass spectrometry investigations. Then, a comparison of chloramphenicol degradation was then performed between water and cooked tissue media. For this purpose, blue mussels were raised under controlled laboratory conditions in artificial sea water spiked with chloramphenicol. Mussels were selected as (i) they can be easily raise in laboratory, (ii) chloramphenicol residue may be encountered in these mollusks due to their very high bioaccumulation, and (iii) because they have a low biotransformation potential (therefore the parent compound is expected to remain dominant in mussels after a 3-day exposure) (Smolders et al., 2003). Another group of blue mussels were raised in antibiotic-free artificial sea water and later tissues were spiked by chloramphenicol standard solution. The results between chloramphenicol-fed and chloramphenicol-spiked mussels were compared to verify the low biotransformation potential of mussels. Tissue extraction was conducted using Accelerated Solvent Extraction (ASE™-350), and chloramphenicol and its degradation products of in mussels were then analyzed by UHPLC-QTOF-MS/MS. Chromatograms were then explored to conclude whether the thermal degradation products of chloramphenicol in cooked mussel tissues are the same as those in water.

5.2 Materials and methods

5.2.1 Materials

Chloramphenicol was purchased from Sigma–Aldrich (St. Louis, MO, USA). Detailed information was shown in Table 3.1. Acetonitrile, methanol and water (all HPLC grade) were purchased from Fisher Company (Fisher Scientific, IOWA, USA). Artificial sea salt was purchased from Aquatica Aquarium (Montreal, QC, CA). Live blue mussels were obtained from

Allen's Fisheries LTD. (NL, CA).

Stock standard solutions of chloramphenicol were prepared in HPLC grade water at a final concentration of 5000 mg/L (5000 ppm) and further diluted into working solutions (100 ppm and 50 ppm) with HPLC grade water.

5.2.2 Thermal degradation experimental procedures

5.2.2.1 Thermal degradation of chloramphenicol in water

One mL of diluted chloramphenicol standard solution (50 ppm) was transferred in three 2 mL-HPLC-glass amber vials. Then, vials were closed by the screw cap and hold by a floating rack. Two of them were heated in water bath at 100 °C for 1 and 4 hours respectively. The unheated one was left as the control. After heating, vials were taken out from the water bath and cooled down rapidly by cool water. The water blanks (HPLC grade water) was heated for 4h. This experiment was repeated 5 times. UHPLC-QTOF "QC samples" were prepared combining 50μL of sample from each experimental vial (including blanks, heated and unheated samples).

5.2.2.2 Thermal degradation of chloramphenicol in mussels

Two tanks of mussels (20 mussels in each) were exposed to artificial seawater (sea salt was dissolved in distilled water to a salinity of 33 g/kg) (McEneff et al., 2013). Chloramphenicol standard solution (5000 ppm) was added into one tank to a final concentration of 100 ppm in artificial sea water (**CHL-mussel**). The other tank was kept as the control (**BL-mussel**). The experiment was carried out over 72 h and tanks were exposed to light for 10 hours per day. Aeration was produced by an aquarium air pump, and the water temperature was kept between -1-5°C. After exposure, mussels (lived ones) were taken out of the tank, opened and soft tissues (CHL-mussels and BL-mussels separately) were blended. Then, 12 g of homogenized BL-mussel tissues was spiked with the chloramphenicol standard solution to a final concentration of 50 μg/g (**SP-CHL-mussel**).

Two grams of homogenized mussel tissues from each group (CHL-mussel, BL-mussel and SP-CHL-mussel) were transferred into 10-mL glass vials and closed with cap (n=6 for each group). Then, all these samples were heated in water bath at 100 °C for 1 hour. After heating, vials were taken out and cooled down rapidly in cold water.

5.2.2.3 Extraction of mussel samples by ASE™–350

All tissue samples (raw BL-mussel, cooked BL-mussel, raw CHL-mussel, cooked CHL-mussel and cooked SP-CHL-mussel) were extracted by the accelerated solvent extraction Dionex ASE™ -350 (Thermo Fisher Scientific, CA, USA). This technique can enhance the solubility of extracts and also makes the extraction less time-consuming (Ridgway et al., 2007). The extraction process was modified based on the method of McEneff et al. (2013). Although salts may have influence the later HPLC analysis, a desalting pretreatment may lead to an unwanted loss of untargeted metabolites (Dunn et al., 2013). Thus, desalted pretreatment was not applied in this study.

Cooked samples were taken out from the vial and the vial was rinsed with several drops of methanol (up to 0.5mL), then mixed with about 3 g of Ottawa sand (Fisher Scientific, CA, USA) by a stainless spatula and transferred into a 5-mL stainless steel extraction cell with cellulose filter paper placed in both sides of the cell. Methanol was used as the extraction solvent. ASE extraction conditions are detailed in Table 5.2. After extraction, the extract was collected in a pre-weighted glass vial. The volume of extract (V) is calculated by dividing the density of methanol (0.792 g/mL). Extracts were filtered with a filter syringe (0.22 μm, PTFE), and 1mL extract was transferred into a HPLC glass amber vial for analysis. Raw mussel samples were treated in the same procedure as cooked ones. Procedure blanks was conducted the as the cooked ones without any tissue in 10-mL glass vials.

Table 5.2 Working conditions of Dionex ASE™-350

Pressure	1500 psi
Temperature	40 ℃
Static time	5 min
Cycles	3
Rinse volume	60%

5.2.2.4 Extraction efficiency of chloramphenicol in blue mussel

Twelve grams of raw BL-mussel were homogenized in a blender (Cuisinart, ON, CA), and 6 g of them was heated the same way as in section 5.3.2.2. Then, both raw and cooked tissues were spiked by chloramphenicol standard solution (5000ppm) to a final concentration of 50 μg/g (50 ppm). The spiked tissue were homogenized again. Two grams of samples were mixed with about 3 g of Ottawa sand (Fisher Scientific, CA, USA) by a spetruler and then transferred into a 5-ml stainless steel extraction cell. Extraction conditions were identical as in section 5.3.2.4 (Table 5.2). Extraction recoveries were calculated based on the Equation 8:

$$Recovery\ (\%) = \frac{M_{Extracted\ chloramphenicol}}{M_{\ Control}} \times 100 \qquad \text{(Equation 8)}$$

where $M_{Extracted\ chloramphenicol} = C_{Extracted\ chloramphenicol} \times V$;

$M_{Control} = C_{Control} \times V$.

Then, the Equation 8 can be simplified to Equation 9:

$$Recovery\ (\%) = \frac{C_{\ Extracted\ chloramphenicol}}{C_{\ Control}} X100 \qquad \text{(Equation 9)}$$

where the $\frac{C_{\ Extracted\ chloramphenicol}}{C_{\ Control}}$ is equal to the $\frac{Peak\ area\ \ of\ extracted\ chloramphenicol}{Peak\ area\ of\ the\ control}$.

The extraction samples and the control were analyzed by HPLC-QTOF-MS (detailed in formation was in section 5.3.2.5). The experiment was triplicated.

5.2.2.5 UHPLC-QTOF-MS/MS Analysis

Identification of the degradation products was carried out with an Agilent 1290 Infinity LC System coupled to an Agilent 6540 Accurate-Mass QTOF System (Agilent Technologies, Palo Alto, CA, USA). The 6540 QTOF was operated in the same run combining two modes, the full-scan MS mode at 5 Hz acquisition rate, and the Automatic MS/MS mode with the preferred masses for fragmentation based on the literature (Franje et al., 2010; Mitchell et al., 2015). In the literatures, the degradation products of chloramphenicol had been identified by positive ion mode. To avoid missing any possible degradation products, both positive (ESI+) and negative ionization (ESI-) modes in the electrospray were tested in this study for water samples. For the mussel samples, only the positive ionization mode (ESI+) was applied as it was earlier deemed sufficient. The UHPLC and QTOF working condition was detailed in Table 5.3 and Table 5.4.

Table 5.3 Working conditions of UHPLC

Column	ZORBAX Eclipse Plus C18, RRHD, 2.1 x 100 mm, 1.8 μm
Mobile phase	A: H2O + 0.1% formic acid B: Acetonitrile + 0.1% formic acid
Injection volume	10 μL
Column temperature	25°C
Solvent gradient	0-1min: 5% B 1-15min: 5% B to 100% B 15-20 min: 100% B 20-25 min: 5% B
Run time	25 min

5.2.2.6 Data analysis

The thermal degradation products of chloramphenicol were identified by Agilent workstation series software. The chromatogram was first aligned by Agilent Masshunter Profinder (version B 06.00) (applying the "*Common organic extraction*" mode), which reduces the acquired data size and complexity through the removal of redundant and non-specific information by identifying

the important variables (features) associated with the data. Then the new peaks, candidates as degradation products, were filtered by a statistical comparison of the heated samples with unheated ones using Agilent Masshunter Mass Profiler (version B.07.01). The tolerance for RT and mass difference was set to 0.1min and 10ppm, respectively. The potential molecular formulas for selected peaks were generated using Agilent Qualitative analysis software (version B.07.00) based on the auto MS/MS information. Finally, the structure of degradation products of chloramphenicol was identified by Agilent Masshunter Molecular Structure Correlator (version B.07.00) based on the Chemspider online database (Royal Society of Chemistry, 2009). The one with highest match score (percentage of match based on the MS/MS fragments) was reported.

Table 5.4 Working conditions of Agilent 6540 Accurate-Mass QTOF

Ionization modes	Both Positive (ESI+) and negative (ESI-); (different runs)
Drying gas temperature	350°C
Drying gas flow	10 L/min (nitrogen)
Sheath gas temperature	400°C
Sheath gas flow	12 L/min (nitrogen)
Capillary voltage	4000 V
Hozzle voltage	0 V(+) /1500 V (-)
Acquisition type	Auto MS/MS (data-dependent)
Mass range	65–1100 m/z for both MS and 50–1100 m/z MS/MS
Transients	1161/spectrum for MS and 1128/spectrum for MS/MS
Acquisition rate	5 spectra/sec (combined MS and MS/MS)
Precursors/cycle	1
Active exclusion	Exclude after 2 spectra, release after 0.1 min
Collision energy	20 EV
Flow rate	0.2 mL /min
Preferred list of precursors (m/z)	463.1468/ 345.00213/ 325.01724/ 323.02019/ 307.02527/ 305.05407/ 291.03035/ 287.0872/ 269.0782/ 251.0663/ 213.08756/ 213.0863/ 198.07665/ 195.0765/ 186.00887/ 177.0654/ 165.0660/ 154.05043/ 130.0080

Mode	Extended dynamic range (2 GHz)

5.3 Results and discussions

5.3.1 Thermal degradation of chloramphenicol in water

The chromatograms of chloramphenicol are extracted at *m/z* 323.0200 (achieved by analyzing the control samples in Agilent Masshunter profiler) from the total ion chromatogram (TIC) for different experiment groups (Figure 5.1). As expected, chloramphenicol concentration decreased over time. The degradation rate, k, for chloramphenicol in water in this experiment was calculated based on 3 time points: 0, 1 hour, 4 hours and ranged from 0.0018 to 0.0025 min^{-1}. This range was similar to those obtained in Chapter 4 (0.0011-0.0022 min^{-1}). Again, this result confirms the degradation of chloramphenicol in water and the degradation percentage ranges from 6.6 % to 15% (heating 1h at 100 ºC in water).

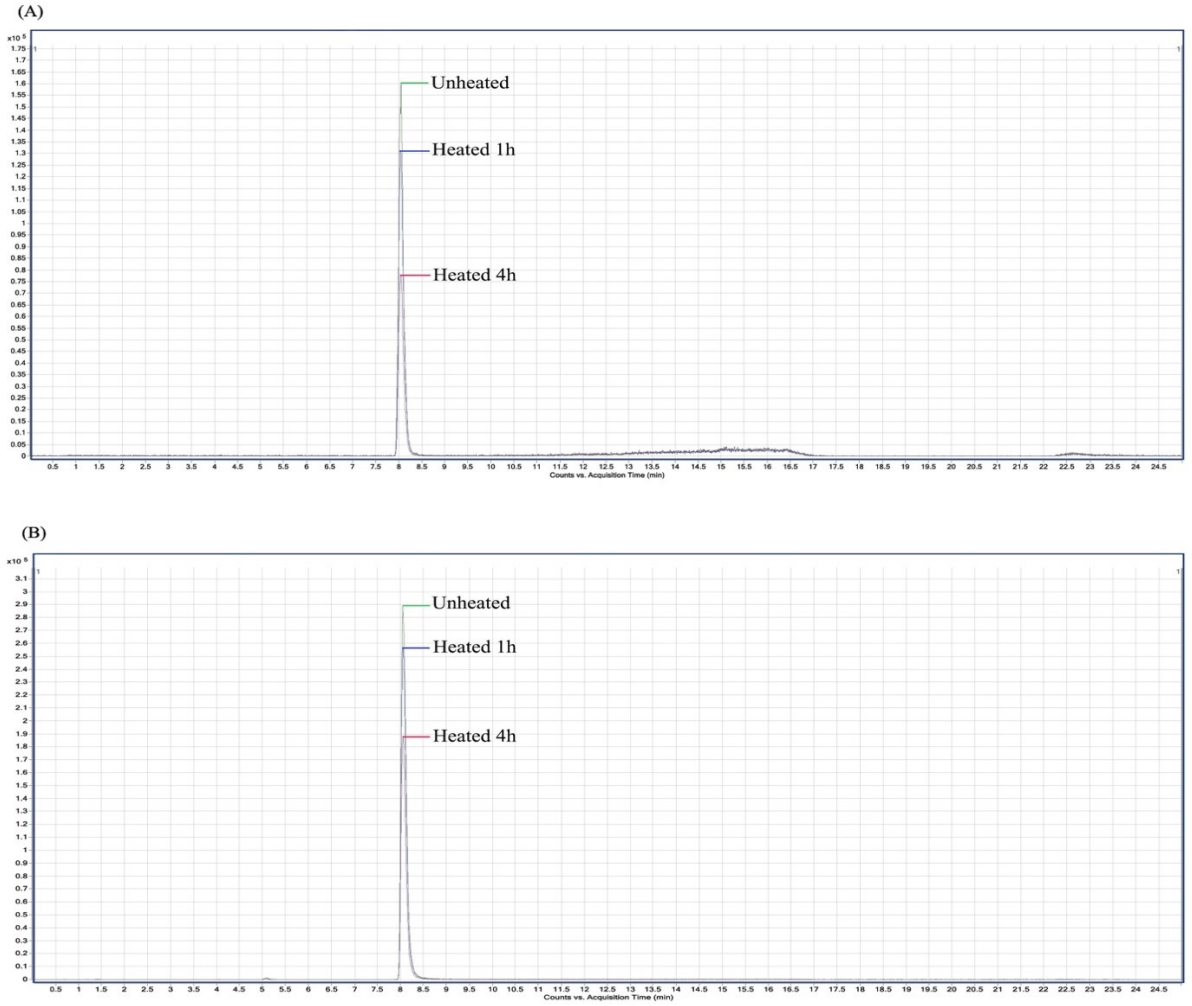


Figure 5.1 Chromatogram of chloramphenicol before and after heating in water (overlap). Note : (A) positive ion mode, (B) negative ion mode. (Chromatograms were extracted at *m/z* 323.0200)

5.3.1.1 Filtration of new peaks for further identification

The total ion chromatograms (TIC) of chloramphenicol in water before and after thermal degradation are shown in Figure 5.2. The filtration of new peaks was conducted in both positive (ESI+) and negative ionization (ESI-) modes. After the alignment of the full scan chromatograms, the "water blank" group, the "unheated control" group, "heated 1h" group and "heated 4h" group were compared with each other using Agilent Masshunter Mass Profiler. Peaks present in the water blank group were ignored in the further identification steps. Peaks for degradation products

of chloramphenicol were identified by overlapping the full-scan chromatograms for heated 1h and the chromatograms for heated 4h with those for unheated samples, respectively. However, the standard solution may also degrade during storage, we cannot exclude that all the peaks existed in unheated samples.

The abundance difference between two compared groups were measured by Agilent Masshunter Mass Profiler software based on the value of *Log 2* (Abundance for feature in heated sample /Abundance for feature in unheated sample) (Figure 5.3). When the log difference was lower than 0.6, i.e. the peak size ratio for the same feature in two different groups was smaller than 2 (possibility of insignificant difference), the peak was excluded from further identification.

Remarkably, the identity for new peaks after 1h heating is same as those for new peaks after 4h. The only difference was that peaks in the heated 4h group were larger than in heated 1h group (confirming further degradation). The peaks in the heated 4h group were further analyzed for compound identification.

Often, the data treatment led to the identification of various new features within a narrow range of retention times (RT, e,g, difference less than 0.1 min) with individual predicted masses differing by multiples of 18 amu. This mass difference is expected to correspond to the sequential loss of H_2O molecules during mass spectrometry. In that case, these new features were considered to arise from one single new degradation product structure.

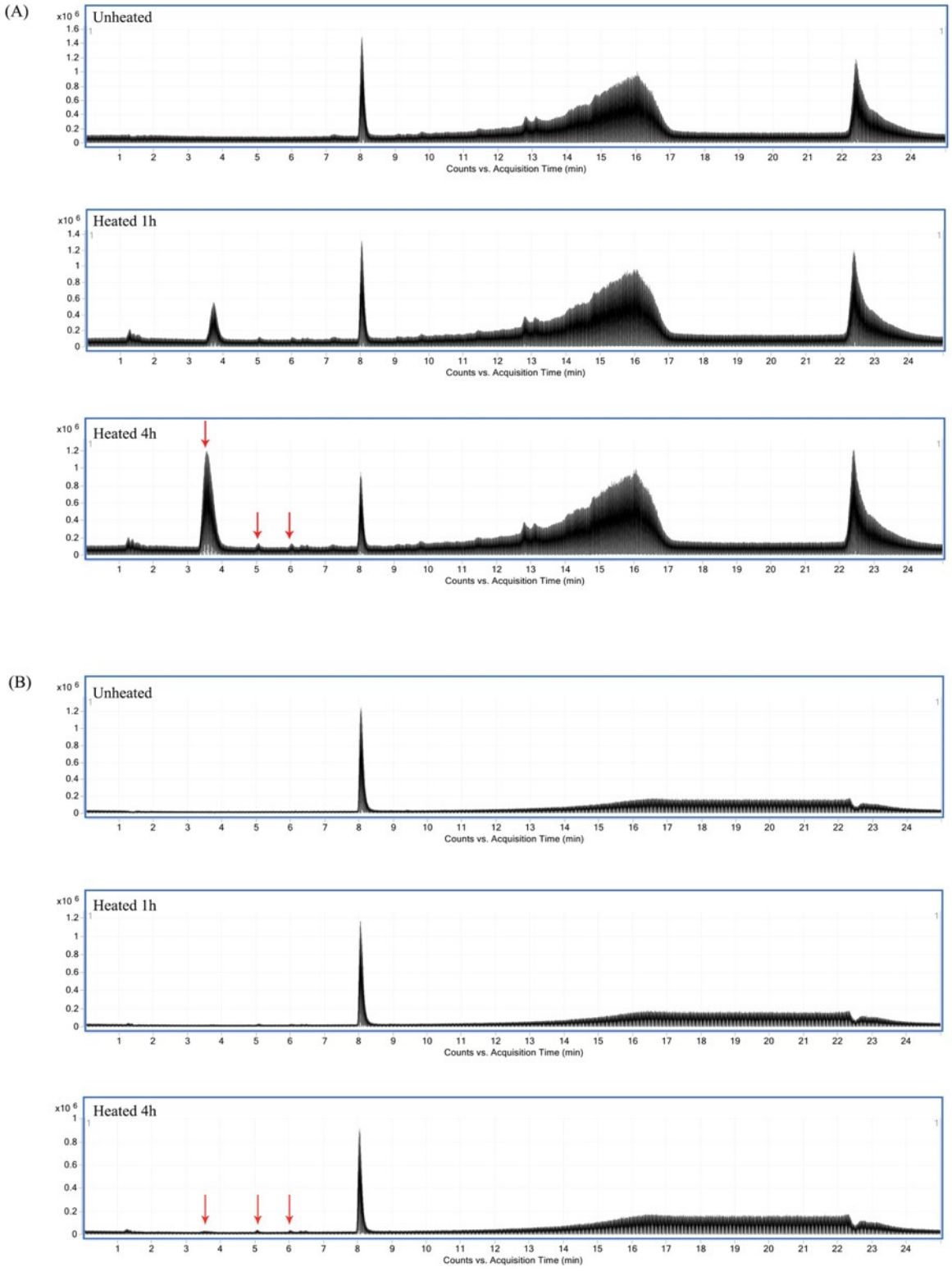


Figure 5.2 TIC of chloramphenicol in water under different experiment conditions. Note: (A) positive ion mode, (B) negative ion mode.

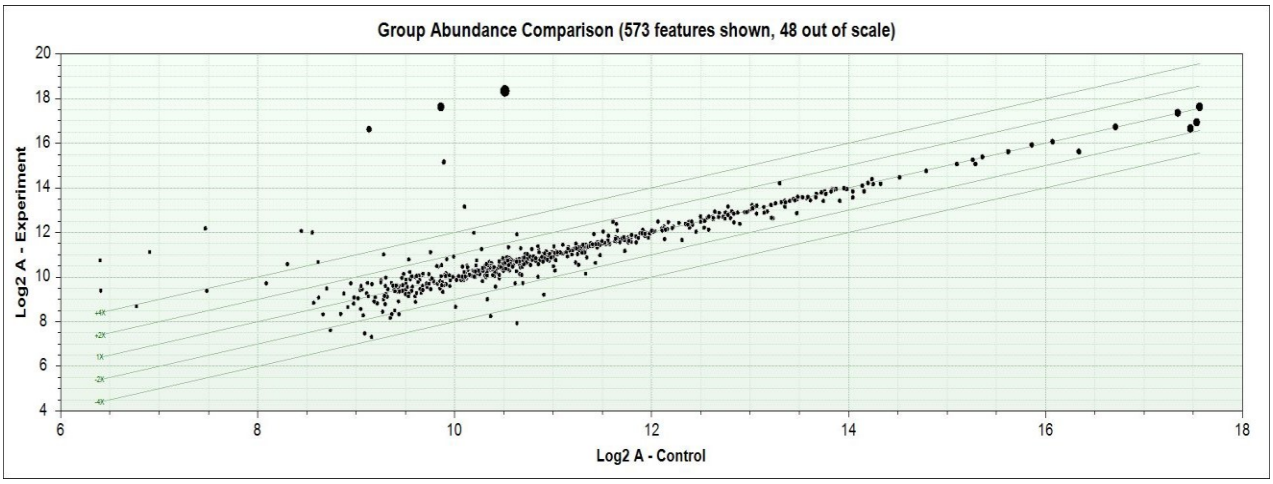


Figure 5.3 Group abundance comparison for heated 4h (experiment) and unheated (control) chloramphenicol in water. Note: The X-axis and Y-axis are the log 2-fold of abundance of features in control and in experiment groups respectively. A point on the 1:1 line refer to the features exist in both groups in the same amount. Points which are above the 1:1 line refer to the features that exist in greater amounts in the "experiment group". On the contrary, points below the 1:1 line refer to the features that are present in greater amounts in the control samples.

Table 5.5 Identity of new peaks for heated 4 h samples

RT (min)	*m/z*	RSD*
3.826	213.0864	0.57
5.066	287.0875	0.12
6.042	251.0662	0.07

Note: *RSD refers to the relative standard deviation of the abundance of features in one group (n=5).

There were more new peaks found using positive electrospray ionization mode (ESI+) than using the negative ionization mode (ESI-), and peaks found using ESI- mode were all recorded at the same RT in ESI+ mode (Table 5.5). Sometimes, the *m/z* of the fragment in one specific peak under positive ion mode is different from the one under negative ion mode due to the difference in ionization, but the same RT indicates that these fragments belong to same compound. Thus, identification of the degradation products for chloramphenicol in ESI+ was sufficient. Altogether,

there were 3 new peaks (highlight by red arrow in Figure 5.2.A&B) found to be degradation products. The detailed information for selected peaks is shown in Table 5.5. The information in Table 5.5 was aligned by Agilent Masshunter Profinder, thus the RT and m/z indicated an average value. The RSD is the relative standard deviation of the abundance of features in one group (5 replications). All the RSD values in Table 5.5 are lower than 0.5%, which indicates that the result of this experiment is highly reproducible (n=5), and the samples was well prepared.

5.3.1.2 Identification of degradation products of chloramphenicol

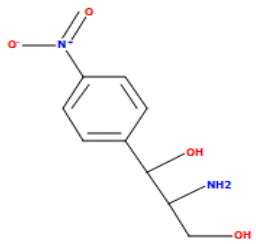
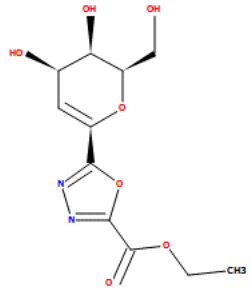
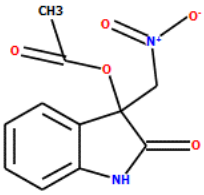
The molecular mass for new peaks (Table 5.5) were generated by Agilent Qualitative analysis software based on the auto MS/MS information and the molecular formula of chloramphenicol. The formula, a possible structure proposed by the software and the match score (percentage match) was shown in Table 5.6.

There are currently five degradation products in total proposed in the literature by former studies (Table 5.1). Compared with former studies, there was only one degradation product ($C_9H_{12}N_2O_4$) that matched those reported earlier, and other compounds are first reported. As there are numerous probabilities for molecular structure at one exact mass, the result should be confirmed by comparing the identity with standard in future study.

5.3.2 Thermal degradation of chloramphenicol in blue mussels

The average extraction efficiency of chloramphenicol in raw and cooked mussels is 43.9 ± 0.4% (n=3) and 40.5 ± 5% (n=3), respectively. The recovery is lower than the one in former study that extracted mussel under similar condition (McEneff et al., 2013). This may be due to relatively high moisture content in the present mussel samples as compared to McEneff et al. (2013), and there might have been some loss of chloramphenicol during the sample transfer. Alternatively, there might have been some matrix effect in the UHPLC-QTOF analysis. Nonetheless, the recovery is not the key point for the qualitative analysis purpose of this study.

Table 5.6 Identification of degradation products of chloramphenicol in water

RT (min)	Molecular formula	m/z	Mass difference (ppm)	Structure	Match score (%)
3.826	C9H12N2O4	213.0864	1.8		98.07
5.066	C11H14N2O7	287.0875	0.3		80.5
6.042	C11H10N2O5	251.0662	0.2		85.8

There was no peak corresponding to chloramphenicol in the raw BL-mussel and cooked BL-mussel samples, indicating that the initial blue mussels used in this study are chloramphenicol-free. When comparing the cooked CHL-mussels with the raw CHL-mussels, the average degradation percentage for one-hour heating (34.9 ± 13%, n=6) (calculated from peak area) was significantly higher than the one in water (12.8 ± 1.6%, n=5; section 5.4.1) (P = 0.013, t-test). Similarly, one-hour heating induced 65.4 ± 7.4% (n=5) degradation in cooked SP-CHL-mussels (compared with raw spiked mussel based on the peak area), which is

significant higher than in the cooked CHL-mussels (P = 0.01, t-test). This number is not straightforward to explain as it chloramphenicol metabolites in the mussels may need to be accounted for in this degradation "mass balance". Nonetheless, this result indicates that simply spiking a food sample (e.g. SP-CHL-mussel) with a standard solution of a contaminant cannot reflect the actual thermal degradation of the residues in real food samples. Instead, real contaminated samples or samples contaminated under controlled laboratory exposure conditions (e.g. CHL-mussels in this study) are more relevant to study contaminant degradation.

Overall, the degradation of chloramphenicol in mussel tissues was greater than in water. Franje et al. (2010) reported that chloramphenicol degraded more in chicken meat than in water. Shakila et al. (2006) reported the boiling 30 min in shrimp can induce 29% degradation of chloramphenicol. All these results indicate that chloramphenicol degraded more in food matrix than in water. This may be because that protein-bound drugs might be protected from degradation under thermal treatment in meat; in the other hand, a drug which is not protein-bounded, will be heating-liable in meat (Rose et al., 1995b; Fedeniuk et al. 1997).

Accurate *m/z* corresponding to the new compounds in Table 5.5 were extracted from the full-scan chromatograms of mussel samples. No peak could be detected for any of the compounds at the same RT (i.e. peak to noise ratio was lower than 3). For example, the chromatogram extracted at m/z 251.0662 corresponding to C11H10N2O5 for sample in water (heated 1h) was overlapped with the one in mussel (heated 1h) (Figure 5.4). As can be observed in this diagram, there was no corresponding peak in the cooked mussel sample. Thus, the thermal degradation products of chloramphenicol in water were not observed in mussels. Actual degradation products of chloramphenicol in mussel remain to be identified through further data treatment.

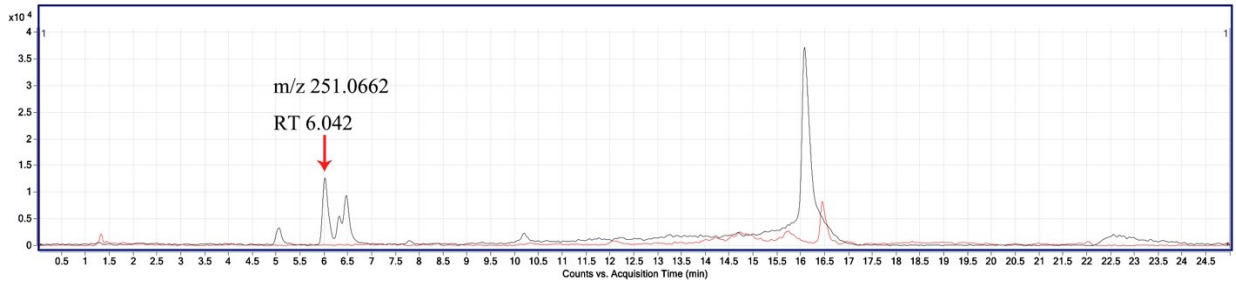


Figure 5.4 Chromatogram extracted at m/z 251.0662 for different samples. Note: The black chromatogram is the cooked chloramphenicol water solution (1h); The red chromatogram is the cooked CHL-mussel (1h).

5.4 Conclusions

The degradation of chloramphenicol in water at 100 ºC was also explored using high-resolution mass spectrometry e.g. UHPLC-QTOF-MS/MS (AIM 3). Degradation rates (k) were confirmed to range from 0.0018 to 0.0025 min^{-1}. The positive ion mode was selected for analyzing the degradation of chloramphenicol in mussel, as it offered more peaks for degradation products. When heated in mussels, chloramphenicol degraded more than when it was heated in water. Also, the degradation products of chloramphenicol in water did not exist in cooked CHL-mussel, which indicated that thermal degradation reactions in water are different from those in actual food matrices. This work suggests that relying only on the "water model" will not be sufficient to understand the actual thermal degradation products of antibiotics in real food.

There was a significant difference between the degradation in CHL-mussel and SP-CHL-mussel, which indicates the spiking method cannot always reflect the real degradation of antibiotic in food matrix.

Using UHPLC-QTOF, two new additional degradation products of chloramphenicol in water were identified. However, the structure of these degradation products should be confirmed using a standard, and the toxicity of degradation products are highly recommended to investigate in

future study. The degradation products and metabolites of chloramphenicol in mussel tissues should also be investigated in future for a food safety purpose.

CHAPTER 6. GENERAL CONCLUSIONS

This work reviewed the effected of heating on the degradation of antibiotics, based on which three aims was set for investigation.

First, Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was applied as a fast and effective analysis method to identify the volatile degradation products of various human and veterinary drugs (AIM 1). My study explored the fate of three veterinary antibiotics and three human medicines, commonly reported in food or in the environment. For chloramphenicol, lincomycin hydrochloride, diphenhydramine hydrochloride and gemfibrozil, a range of volatile degradation products were identified. Results indicated that florfenicol does not degrade into volatile substances. Py-GC-MS was a fast and simple method to use, although limitations exist in relation to data treatment. For example, degradation products of carbamazepine could not be matched with any structure of the database. Some of the degradation products of chloramphenicol and lincomycin hydrochloride are reported to be harmful to human health, which justify the exploration of the thermal degradation of these contaminants.

Then, this work investigated the thermal kinetics of chloramphenicol and florfenicol (AIM 2) in water at 100 °C, which has never been reported before. Results indicate that both chloramphenicol and florfenicol follow the first-order kinetic at 100 °C in water. The k for chloramphenicol at 180 °C in oil is about four times as high as k at 100 °C (in oil), and for florfenicol, the k in oil at 140 °C is about three times as high as the k at 100 °C (in oil). At lower temperatures (60 °C and 80 °C), both the two compounds showed higher k values in oil than in water.

The degradation of chloramphenicol in water at 100 ºC was also explored using high-resolution mass spectrometry e.g. UHPLC-QTOF-MS/MS (AIM 3). Degradation rates (k) were confirmed to range from 0.0018 to 0.0025 min^{-1}. The positive ion mode was selected for analyzing the degradation of chloramphenicol in mussel, as it offered more peaks for degradation products. When heated in mussels, chloramphenicol degraded more than when it was heated in water. Also,

the degradation products of chloramphenicol in water did not exist in cooked CHL-mussel, which indicated that thermal degradation reactions in water are different from those in actual food matrices. This work suggests that relying only on the “water model” will not be sufficient to understand the actual thermal degradation products of antibiotics in real food.

In this study, there are nine new degradation products of chloramphenicol in water that was identified. However, these degradation products should be confirmed as a standard, and it is highly recommended that the toxicity of degradation products to be investigated in future study. The degradation products and metabolites of chloramphenicol in mussel tissues should also be investigated in future for a food safety purpose.

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APPADIX

Appendix Table 1. Detailed information for Reviewed Literatures

Antibiotic family	**Antibiotics**	**Degradation percentage, [heating condition]**	**References**	***K* value/min**
β-lactams	Cefuroxime	22.1, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.0078**
β-lactams	Cefuroxime	1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cefuroxime	100, [120°C, 20min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cefuroxime	8.6, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cefquinome	16.2, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.0068**
β-lactams	Cefquinome	1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cefquinome	79.9, [120°C, 20min, M/Boiling]	Roca et al., 2011	8.022E-02
β-lactams	Cefquinome	1.1, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cephalexin	17.8, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00601**
β-lactams	Cephalexin	1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cephalexin	98.6, [120°C, 20min, M/Boiling]	Roca et al., 2011	2.134E-01
β-lactams	Cephalexin	3.8, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cephalonium	17.8, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00545**
β-lactams	Cephalonium	1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cephalonium	91.3, [120°C, 20min, M/Boiling]	Roca et al., 2011	1.221E-01
β-lactams	Cephalonium	1.9, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cephapirin	41.2, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.01734**
β-lactams	Cephapirin	1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cephapirin	99.5, [120°C, 20min, M/Boiling]	Roca et al., 2011	2.649E-01
β-lactams	Cephapirin	3.8, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cefoperazone	19.8, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00733**
β-lactams	Cefoperazone	1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cefoperazone	100, [120°C, 20min, M/Boiling]	Roca et al., 2011	NA

β-lactams	Cefoperazone	16.8, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Amoxycillin	38, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	3.187E-02
β-lactams	Amoxycillin	38, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	3.187E-02
β-lactams	Amoxycillin	76, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	9.514E-02
β-lactams	Amoxycillin	75, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	9.242E-02
β-lactams	Amoxycillin	6.3, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00187**
β-lactams	Amoxycillin	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Amoxycillin	47.6, [120°C, 20min, M/Boiling]	Roca et al., 2011	3.231E-02
β-lactams	Amoxycillin	0.5, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Ampicillin	15, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	1.083E-02
β-lactams	Ampicillin	18, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	1.323E-02
β-lactams	Ampicillin	72, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	8.486E-02
β-lactams	Ampicillin	71, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	8.252E-02
β-lactams	Ampicillin	3.3, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00181**
β-lactams	Ampicillin	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Ampicillin	84, [120°C, 20min, M/Boiling]	Roca et al., 2011	9.163E-02
β-lactams	Ampicillin	2.1, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Ampicillin	7.8, [Maximum to 56°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	4.6, [Maximum to 45°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	2.7, [Maximum to 44°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	3.9, [Maximum to 35 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	30.4, [Maximum to 71°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA

β-lactams	Ampicillin	33.6, [Maximum to 90 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	2.3, [Maximum to 52°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	13.3, [Maximum to 58 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	81.8, [Maximum to 90°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	77.2, [Maximum to 92 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	25.3, [Maximum to78°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	34.8, [Maximum to 87 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	27.8, [Maximum to 62°C, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	77.6, [Maximum to 73 °C, Total 210min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	79.4, [Maximum to 78°C, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	78.9, [Maximum to 82 °C, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	79.9, [NA, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	83.2, [NA, Total 210min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	87.3, [NA, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	100, [NA, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	100, [Maximum to 96°C, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA

β-lactams	Ampicillin	100, [Maximum to 93 °C, Total 210min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	85.9, [Maximum to 99°C, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Ampicillin	100, [Maximum to 98 °C, Total 180min, TM/Roasting]	O'Brien et al., 1981	NA
β-lactams	Penicillin G	55, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	5.323E-02
β-lactams	Penicillin G	60, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	6.109E-02
β-lactams	Penicillin G	57, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	5.626E-02
β-lactams	Penicillin G	50, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	4.621E-02
β-lactams	Penicillin G	80, [100°C, 120min, W/Boiling]	Rose et al. 1997c	1.341E-02
β-lactams	Penicillin G	90, [100°C, 120min, W/Boiling]	Rose et al. 1997c	1.919E-02
β-lactams	Penicillin G	90, [140°C, 120min, O/Boiling]	Rose et al. 1997c	1.919E-02
β-lactams	Penicillin G	100, [180°C, 120min, O/Boiling]	Rose et al. 1997c	NA
β-lactams	Penicillin G	8.2, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00093**
β-lactams	Penicillin G	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Penicillin G	61, [120°C, 20min, M/Boiling]	Roca et al., 2011	4.708E-02
β-lactams	Penicillin G	0.8, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Penicillin G	8.2, [62°C, 30min, M/Boiling]	Shahani et al. 1956	NA
β-lactams	Penicillin G	59.7, [121°C, 30min, M/Boiling]	Shahani et al. 1956	NA
β-lactams	Penicillin G	32, [100°C, 30min, M/Boiling]	Konecny 1978	NA
β-lactams	Oxacillin	20, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	1.488E-02
β-lactams	Oxacillin	20, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	1.488E-02
β-lactams	Oxacillin	70, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	8.026E-02
β-lactams	Oxacillin	69, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	7.808E-02
β-lactams	Oxacillin	59, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	5.944E-02
β-lactams	Oxacillin	13, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	9.284E-03

β-lactams	Oxacillin	42, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	3.632E-02
β-lactams	Oxacillin	8, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	5.559E-03
β-lactams	Dicloxacillin	8, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	5.559E-03
β-lactams	Dicloxacillin	10, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	7.024E-03
β-lactams	Dicloxacillin	50, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	4.621E-02
β-lactams	Dicloxacillin	55, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	5.323E-02
β-lactams	Dicloxacillin	70, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	8.026E-02
β-lactams	Dicloxacillin	15, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	1.083E-02
β-lactams	Dicloxacillin	35, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	2.872E-02
β-lactams	Dicloxacillin	20, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	1.488E-02
β-lactams	Cloxacillin	25, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	1.918E-02
β-lactams	Cloxacillin	27, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	2.098E-02
β-lactams	Cloxacillin	70, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	8.026E-02
β-lactams	Cloxacillin	78, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	1.009E-01
β-lactams	Cloxacillin	6.9, [63°C, 30min, M/Boiling]	Roca et al., 2011	0.00181**
β-lactams	Cloxacillin	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cloxacillin	53.1, [120°C, 20min, M/Boiling]	Roca et al., 2011	3.786E-02
β-lactams	Cloxacillin	0.6, [140°C, 0.067min, M/Boiling]	Roca et al., 2011	NA
β-lactams	Cloxacillin	64, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	6.811E-02
β-lactams	Cloxacillin	15, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	1.083E-02
β-lactams	Cloxacillin	28, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	2.190E-02
β-lactams	Cloxacillin	26, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	2.007E-02
β-lactams	Nafcillin	63, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	6.628E-02
β-lactams	Nafcillin	16, [90°C, 15min, W/Boiling]	Grunwald & Petz, 2003	1.162E-02
β-lactams	Nafcillin	17, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	1.242E-02
β-lactams	Nafcillin	16, [90°C, 15min, M/Boiling]	Grunwald & Petz, 2003	1.162E-02
Macrolides	Erythromycin	21, [60°C, 30min, M/Boiling]	Zorraquino et al., 2011	7.857E-03

Macrolides	Erythromycin	93, [120°C, 20min, M/Boiling]	Zorraquino et al., 2011	1.330E-01
Macrolides	Erythromycin	30, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2011	NA
Macrolides	Spiramycin	13, [60°C, 30min, M/Boiling]	Zorraquino et al., 2011	4.642E-03
Macrolides	Spiramycin	64, [120°C, 20min, M/Boiling]	Zorraquino et al., 2011	5.108E-02
Macrolides	Spiramycin	35, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2011	NA
Macrolides	Tylosin	NS[60°C, 30min, M/Boiling]	Zorraquino et al., 2011	0.000E+00
Macrolides	Tylosin	5, [120°C, 20min, M/Boiling]	Zorraquino et al., 2011	2.565E-03
Macrolides	Tylosin	5, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2011	NA
Macrolides	Ivermectin	1.5, [65°C, 30min, M/Boiling]	Imperiale et al., 2009	5.038E-04
Macrolides	Ivermectin	1.1, [75°C, 0.25min, M/Boiling]	Imperiale et al., 2009	NA
Macrolides	Ivermectin	47, [Interanl 60 °C, Total 10min, TM/Frying]	Slanina et al., 1989	NA
Macrolides	Ivermectin	50, [Interanl 71 °C, Total 13min, TM/Frying]	Slanina et al., 1989	NA
Macrolides	Ivermectin	48, [Interanl 77 °C, Total 16min, TM/Frying]	Slanina et al., 1989	NA
Macrolides	Ivermectin	45, [Interanl 78 °C, Total 9min, TM/Boiling]	Slanina et al., 1989	NA
Aminoglycosides	Gentamicin	97, [120°C, 20min, M/Boiling]	Zorraquino et al., 2009	1.753E-01
Aminoglycosides	Gentamicin	20, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2009	NA
Aminoglycosides	Kanamycin	95, [120°C, 20min, M/Boiling]	Zorraquino et al., 2009	1.498E-01
Aminoglycosides	Kanamycin	17, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2009	NA
Aminoglycosides	Neomycin	98, [120°C, 20min, M/Boiling]	Zorraquino et al., 2009	1.956E-01
Aminoglycosides	Neomycin	40, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2009	NA
Aminoglycosides	Streptomycin	98, [120°C, 20min, M/Boiling]	Zorraquino et al., 2009	1.956E-01
Aminoglycosides	Streptomycin	26, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2009	NA
Amphenicols	Chloramphenicol	6.5, [100°C, 30min, W/Boiling]	Franje et al., 2010	2.240E-03

Amphenicols	Chloramphenicol	11.7, [100°C, 60min, W/Boiling]	Franje et al., 2010	2.074E-03
Amphenicols	Chloramphenicol	20, [100°C, 120min, W/Boiling]	Franje et al., 2010	1.860E-03
Amphenicols	Chloramphenicol	6.7, [100°C, 5min, W/Microwave]	Franje et al., 2010	1.387E-02
Amphenicols	Chloramphenicol	10, [100°C, 30min, W+Salt/Boiling]	Franje et al., 2010	3.512E-03
Amphenicols	Chloramphenicol	18, [100°C, 60min, W+Salt/Boiling]	Franje et al., 2010	3.308E-03
Amphenicols	Chloramphenicol	20, [100°C, 120min, W+Salt/Boiling]	Franje et al., 2010	1.860E-03
Amphenicols	Chloramphenicol	5, [100°C, 30min, S/Boiling]	Franje et al., 2010	1.710E-03
Amphenicols	Chloramphenicol	41, [100°C, 60min, S/Boiling]	Franje et al., 2010	8.794E-03
Amphenicols	Chloramphenicol	78, [100°C, 120min, S/Boiling]	Franje et al., 2010	1.262E-02
Amphenicols	Chloramphenicol	40, [100°C, 30min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Chloramphenicol	59, [100°C, 60min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Chloramphenicol	80, [100°C, 120min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Chloramphenicol	6, [100°C, 10min, TS/Boiling]	Shakila et al., 2006	NA
Amphenicols	Chloramphenicol	12, [100°C, 20min, TS/Boiling]	Shakila et al., 2006	NA
Amphenicols	Chloramphenicol	29, [100°C, 30min, TS/Boiling]	Shakila et al., 2006	NA
Amphenicols	Chloramphenicol	9, [121°C, 10min, TS/Boiling]	Shakila et al., 2006	NA
Amphenicols	Chloramphenicol	16, [121°C, 15min, TS/Boiling]	Shakila et al., 2006	NA
Amphenicols	Chloramphenicol	0, [Maximum to 56°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	4.9, [Maximum to 30 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	0, [Maximum to 52°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	7.1, [Maximum to 42 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	50, [Maximum to 58°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA

Amphenicols	Chloramphenicol	46.7, [Maximum to 70°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	14.2, [Maximum to 60°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	41, [Maximum to 75 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	61, [Maximum to 77 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	27.3, [Maximum to 80 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	35.7, [Maximum to 82°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	50, [Maximum to 82 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	63.5, [Maximum to 87°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	74, [Maximum to 76 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	37.4, [Maximum to 51°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	42.8, [Maximum to 59 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	100, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	100, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	46.4, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	42.8, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	66.9, [Maximum to 94°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA

Amphenicols	Chloramphenicol	70.4, [Maximum to 90 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	55.2, [Maximum to 87°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Chloramphenicol	75, [Maximum to 101 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Amphenicols	Florfenicol	2, [100°C, 30min, W/Boiling]	Franje et al., 2010	6.734E-04
Amphenicols	Florfenicol	3, [100°C, 60min, W/Boiling]	Franje et al., 2010	5.077E-04
Amphenicols	Florfenicol	10, [100°C, 120min, W/Boiling]	Franje et al., 2010	8.780E-04
Amphenicols	Florfenicol	2.5, [100°C, 5min, WMicrowave]	Franje et al., 2010	5.064E-03
Amphenicols	Florfenicol	3, [100°C, 30min, W+Salt/Boiling]	Franje et al., 2010	1.015E-03
Amphenicols	Florfenicol	4, [100°C, 60min, W+Salt/Boiling]	Franje et al., 2010	6.804E-04
Amphenicols	Florfenicol	15, [100°C, 120min, W+Salt/Boiling]	Franje et al., 2010	1.354E-03
Amphenicols	Florfenicol	9, [100°C, 30/min, S/Boiling]	Franje et al., 2010	3.144E-03
Amphenicols	Florfenicol	34, [100°C, 60min, S/Boiling]	Franje et al., 2010	6.925E-03
Amphenicols	Florfenicol	42, [100°C, 120min, S/Boiling]	Franje et al., 2010	4.539E-03
Amphenicols	Florfenicol	32, [100°C, 30min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Florfenicol	58, [100°C, 60min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Florfenicol	80, [100°C, 120min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Thiamphenicol	8.6, [100°C, 30min, W/Boiling]	Franje et al., 2010	2.997E-03
Amphenicols	Thiamphenicol	11, [100°C, 60min, W/Boiling]	Franje et al., 2010	1.942E-03
Amphenicols	Thiamphenicol	20, [100°C, 120min, W/Boiling]	Franje et al., 2010	1.860E-03
Amphenicols	Thiamphenicol	6.5, [100°C, 5min, W/Microwave]	Franje et al., 2010	1.344E-02
Amphenicols	Thiamphenicol	2, [100°C, 30min, W+Salt/Boiling]	Franje et al., 2010	6.734E-04
Amphenicols	Thiamphenicol	5, [100°C, 60min, W+Salt/Boiling]	Franje et al., 2010	8.549E-04
Amphenicols	Thiamphenicol	8, [100°C, 120min, W+Salt/Boiling]	Franje et al., 2010	6.948E-04
Amphenicols	Thiamphenicol	2, [100°C, 30min, S/Boiling]	Franje et al., 2010	6.734E-04

Amphenicols	Thiamphenicol	22, [100°C, 60min, S/Boiling]	Franje et al., 2010	4.141E-03
Amphenicols	Thiamphenicol	78, [100°C, 120min, S/Boiling]	Franje et al., 2010	1.262E-02
Amphenicols	Thiamphenicol	36, [100°C, 30min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Thiamphenicol	40, [100°C, 60min, TM/Boiling]	Franje et al., 2010	NA
Amphenicols	Thiamphenicol	65, [100°C, 120min, TM/Boiling]	Franje et al., 2010	NA
Quinolones	Ciprofloxacin	0.01, [72°C, 0.25min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Ciprofloxacin	12.71, [120°C, 20min, M/Boiling]	Roca et al., 2010	6.797E-03
Quinolones	Ciprofloxacin	0.11, [140°C, 0.067min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Norfloxacin	0.01, [72°C, 0.25min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Norfloxacin	12.01, [120°C, 20min, M/Boiling]	Roca et al., 2010	6.397E-03
Quinolones	Norfloxacin	0.11, [140°C, 0.067min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Flumequine	0.01, [72°C, 0.25min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Flumequine	2.99, [120°C, 20min, M/Boiling]	Roca et al., 2010	1.518E-03
Quinolones	Flumequine	0.02, [140°C, 0.067min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Oxolinic acid	0.01, [72°C, 0.25min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Oxolinic acid	2.99, [120°C, 20min, M/Boiling]	Roca et al., 2010	1.518E-03
Quinolones	Oxolinic acid	0.02, [140°C, 0.067min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Oxolinic acid	35, [100°C, 4min, TS/Boiling]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	45, [100°C, 8min, TS/Boiling]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	50, [100°C, 12min, TS/Boiling]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	20, [200°C, 4min, TS/Baking]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	25, [180°C, 1min, TS/Frying]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	25, [100°C, 4min, TS (shell)/Boiling]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	21, [200°C, 4min, TS (shell)/Baking]	Uno et al., 2006b	NA
Quinolones	Oxolinic acid	30, [180°C, 1min,TS (shell)/Frying]	Uno et al., 2006b	NA
Quinolones	Enrofloxacin	0.01, [72°C, 0.25min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Enrofloxacin	5.22, [120°C, 20min, M/Boiling]	Roca et al., 2010	2.681E-03

Quinolones	Enrofloxacin	0.04, [140°C, 0.067min, M/Boiling]	Roca et al., 2010	NA
Quinolones	Enrofloxacin	66.3, [NA, NA, TM/Frying]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	48.1, [100°C, 10min, TM/Boiling]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	77, [NA, 3.8min, TM/Microwave]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	incerased 151.5, [200°C, 10min, TM/Roasting]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	increased 101.1, [NA, 10min, TM/Grilling]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	64.9, [NA, NA, TM/Frying]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	62.8, [100°C, 10min, TM/Boiling]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	65.5, [NA, 3.8min, TM/Microwave]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	incerase 59.1, [200°C, 10min, TM/Roasting]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	increase61.9, [NA, 10min, TM/Grilling]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	61.5, [NA, NA, TM/Frying]	Lolo et al., 2006	NA
Quinolones	Enrofloxacin	incerase 39.6, [200°C, 10min, TM/Roasting]	Lolo et al., 2006	NA
Lincosamides	Lincomycin	9, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	6.287E-03
Lincosamides	Lincomycin	10, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	7.024E-03
Lincosamides	Lincomycin	10, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	7.024E-03
Lincosamides	Lincomycin	15, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	1.083E-02
Lincosamides	Lincomycin	NS[60°C, 30min, M/Boiling]	Zorraquino et al., 2011	0.000E+00
Lincosamides	Lincomycin	5, [120°C, 20min, M/Boiling]	Zorraquino et al., 2011	2.565E-03
Lincosamides	Lincomycin	5, [140°C, 0.167min, M/Boiling]	Zorraquino et al., 2011	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 32°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	1.5, [Maximum to 32 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA

Sulfonamides	Sulphadimidine	1.9, [Maximum to 35°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	5.4, [Maximum to 30 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 78°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 69 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 81°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	7.6, [Maximum to 80 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 81°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 84 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 86°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	6.1, [Maximum to 85 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 48°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 48 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 66°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 57 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA

Sulfonamides	Sulphadimidine	0, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0.7, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 82°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 88 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 92°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulphadimidine	0, [Maximum to 84 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Sulfonamides	Sulfamethoxazole	8, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	5.559E-03
Sulfonamides	Sulfamethoxazole	10, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	7.024E-03
Sulfonamides	Sulfamethoxazole	8, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	5.559E-03
Sulfonamides	Sulfamethoxazole	9, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	6.287E-03
Sulfonamides	Sulfamethoxazole	32, [100°C, 3min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	42, [100°C, 6min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	49, [100°C, 9min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	54, [100°C, 12min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	7, [170°C, 3min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	16, [170°C, 6min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA

Sulfonamides	Sulfamethoxazole	21, [170°C, 9min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	39, [170°C, 12min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	18, [NA, 0.25min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	26, [NA, 0.5min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	29, [NA, 0.75min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethoxazole	36, [NA, 1min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamethazine	5, [100°C, 15min°C, 9]	Hsieh et al., 2011	3.420E-03
Sulfonamides	Sulfamethazine	6, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	4.125E-03
Sulfonamides	Sulfamethazine	3, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	2.031E-03
Sulfonamides	Sulfamethazine	5, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	3.420E-03
Sulfonamides	Sulfamethazine	5, [100°C, 60min, W/Boiling]	Rose et al. 1995b	8.549E-04
Sulfonamides	Sulfamethazine	5, [100°C, 120min, W/Boiling]	Rose et al. 1995b	4.274E-04
Sulfonamides	Sulfamethazine	3, [100°C, 240min, W/Boiling]	Rose et al. 1995b	1.269E-04
Sulfonamides	Sulfamethazine	20, [180°C, 30min, O/Frying]	Rose et al. 1995b	7.438E-03
Sulfonamides	Sulfamethazine	35, [180°C, 60min, O/Frying]	Rose et al. 1995b	7.180E-03
Sulfonamides	Sulfamethazine	70, [180°C, 180min, O/Frying]	Rose et al. 1995b	6.689E-03
Sulfonamides	Sulfamethazine	90, [260°C, 30min, O/Frying]	Rose et al. 1995b	7.675E-02
Sulfonamides	Sulfamethazine	97, [260°C, 60min, O/Frying]	Rose et al. 1995b	5.844E-02
Sulfonamides	Sulfamethazine	99, [260°C, 180min, O/Frying]	Rose et al. 1995b	2.558E-02
Sulfonamides	Sulfamethazine	3.1, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00105**
Sulfonamides	Sulfamethazine	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfamethazine	85.1, [120°C, 20min, M/Boiling]	Roca et al., 2013	9.519E-02

Sulfonamides	Sulfamethazine	2.3, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfachloropyridazine	6, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00119**
Sulfonamides	Sulfachloropyridazine	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfachloropyridazine	46, [120°C, 20min, M/Boiling]	Roca et al., 2013	3.081E-02
Sulfonamides	Sulfachloropyridazine	0.4, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfadiazine	2, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00049**
Sulfonamides	Sulfadiazine	0, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfadiazine	47.9, [120°C, 20min, M/Boiling]	Roca et al., 2013	3.260E-02
Sulfonamides	Sulfadiazine	1.7, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfadiazine	40, [100°C, 3min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	51, [100°C, 6min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	60, [100°C, 9min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	61, [100°C, 12min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	6, [170°C, 3min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	2, [170°C, 6min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	2, [170°C, 9min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	4, [170°C, 12min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	19, [NA, 0.25min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	26, [NA, 0.5min, TM/Microwave]	Furusawa & Hanabusa,	NA

			2002	
Sulfonamides	Sulfadiazine	32, [NA, 0.75min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadiazine	36, [NA, 1min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfadimethoxine	3.3, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00112**
Sulfonamides	Sulfadimethoxine	0, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfadimethoxine	6.5, [120°C, 20min, M/Boiling]	Roca et al., 2013	3.360E-03
Sulfonamides	Sulfadimethoxine	0, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfadimethoxine	35.2, [160-200 °C, 120min, TS/Smoking]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	51.8, [160-200 °C, 120min, TS/Smoking]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	52.7, [160-200 °C, 120min, TS/Smoking]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	30.9, [190°C, 45min, TS/Baking]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	47, [190°C, 45min, TS/Baking]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	61.9, [190°C, 45min, TS/Baking]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	7.5, [190°C, 7-10min, TS/Frying]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	63.5, [190°C, 7-10min, TS/Frying]	Xu et al., 1996	NA
Sulfonamides	Sulfadimethoxine	42.3, [190°C, 7-10min, TS/Frying]	Xu et al., 1996	NA
Sulfonamides	Sulfamerazine	2.4, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00063**
Sulfonamides	Sulfamerazine	0, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfamerazine	77.4, [120°C, 20min, M/Boiling]	Roca et al., 2013	7.436E-02
Sulfonamides	Sulfamerazine	1.8, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfapyridine	4.3, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00134**
Sulfonamides	Sulfapyridine	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA

Sulfonamides	Sulfapyridine	30.7, [120°C, 20min, M/Boiling]	Roca et al., 2013	1.834E-02
Sulfonamides	Sulfapyridine	0.3, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfathiazole	1.7, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00055**
Sulfonamides	Sulfathiazole	0, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfathiazole	9.9, [120°C, 20min, M/Boiling]	Roca et al., 2013	5.213E-03
Sulfonamides	Sulfathiazole	0.1, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfaquinoxaline	5.7, [63°C, 30min, M/Boiling]	Roca et al., 2013	0.00235**
Sulfonamides	Sulfaquinoxaline	0.1, [72°C, 0.25min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfaquinoxaline	51.9, [120°C, 20min, M/Boiling]	Roca et al., 2013	3.659E-02
Sulfonamides	Sulfaquinoxaline	0.6, [140°C, 0.067min, M/Boiling]	Roca et al., 2013	NA
Sulfonamides	Sulfaquinoxaline	27, [100°C, 3min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	40, [100°C, 6min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	41, [100°C, 9min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	47, [100°C, 12min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	12, [170°C, 3min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	22, [170°C, 6min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	26, [170°C, 9min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	38, [170°C, 12min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	23, [NA, 0.25min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA

Sulfonamides	Sulfaquinoxaline	36, [NA, 0.5min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	36, [NA, 0.75min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfaquinoxaline	41, [NA, 1min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	28, [100°C, 3min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	37, [100°C, 6min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	43, [100°C, 9min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	45, [100°C, 12min, TM/Boiling]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	18, [170°C, 3min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	25, [170°C, 6min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	29, [170°C, 9min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	40, [170°C, 12min, TM/Roasting]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	28, [NA, 0.25min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	32, [NA, 0.5min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Sulfonamides	Sulfamonomethoxine	32, [NA, 0.75min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA

Sulfonamides	Sulfamonomethoxine	35, [NA, 1min, TM/Microwave]	Furusawa & Hanabusa, 2002	NA
Tetracyclines	Doxycycline	11.5-14.1, [97°C, 20min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	27.7-29.3, [97°C, 30min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	41.8-44.1, [97°C, 40min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	13.7-15.1, [99°C, 40min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	28.5- 26.1, [99°C, 60min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	42.2-44.5, [99°C, 80min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	17.6-18.2, [98°C, 10min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	33- 35.4, [98°C, 15min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	45.6-46.7, [98°C, 20min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Doxycycline	99, [118°C, 30min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Doxycycline	98.7, [121°C, 20min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Doxycycline	84, [135°C, 0.25min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Doxycycline	85, [140°C, 0.12min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Doxycycline	9, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	6.287E-03
Tetracyclines	Doxycycline	15, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	1.083E-02
Tetracyclines	Doxycycline	55, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	5.323E-02
Tetracyclines	Doxycycline	65, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	6.999E-02
Tetracyclines	Doxycycline	100, [100°C, 9min, TM/Boiling]	Javadi, 2011	NA
Tetracyclines	Doxycycline	35, [100°C, 24min, TM/Boiling]	Javadi, 2011	NA
Tetracyclines	Doxycycline	100, [100°C, 85min, TM/Boiling]	Javadi, 2011	NA
Tetracyclines	Doxycycline	65.5, [200°C, 25min, TM/Roasting]	Javadi, 2011	NA
Tetracyclines	Doxycycline	74.8, [200°C, 40min, TM/Roasting]	Javadi, 2011	NA
Tetracyclines	Doxycycline	84.5, [200°C, 60min, TM/Roasting]	Javadi, 2011	NA

Tetracyclines	Doxycycline	100, [NA, 3min, TM/Microwave]	Javadi, 2011	NA
Tetracyclines	Doxycycline	100, [NA, 3min, TM/Microwave]	Javadi, 2011	NA
Tetracyclines	Doxycycline	62.2, [NA, 3min, TM/Microwave]	Javadi, 2011	NA
Tetracyclines	Tetracycline	25.8-28, [97°C, 20min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	54-55.8, [97°C, 30min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	75.6, [97°C, 40min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	46-58, [99°C, 40min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	70-71, [99°C, 60min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	82-86.4, [99°C, 80min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	79.4-83.8, [98°C, 10min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	39.8-40.6, [98°C, 15min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	59.4-64.4, [98°C, 20min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Tetracycline	99, [118°C, 30min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Tetracycline	99, [121°C, 20min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Tetracycline	76, [135°C, 0.25min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Tetracycline	77, [140°C, 0.12min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Tetracycline	50, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	4.621E-02
Tetracyclines	Tetracycline	55, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	5.323E-02
Tetracyclines	Tetracycline	76, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	9.514E-02
Tetracyclines	Tetracycline	99, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	3.070E-01
Tetracyclines	Tetracycline	56, [final 97.6°C, 14min, TM/Boiling]	Gratacós-Cubarsí et al., 2007	NA
Tetracyclines	Tetracycline	57.7, [final 97.6°C, 14min, TM/Boiling]	Gratacós-Cubarsí et al., 2007	NA

Tetracyclines	Tetracycline	60.3, [final 97.6°C, 14min, TM/Boiling]	Gratacós-Cubarsí et al., 2007	NA
Tetracyclines	Tetracycline	60, [NA (final 130)°C, 440 W-0.75 + 100 W-6min, TM/Microwave]	Gratacós-Cubarsí et al., 2007	NA
Tetracyclines	Tetracycline	80.5, [NA (final 130)°C, 440 W-0.75 + 100 W-6min, TM/Microwave]	Gratacós-Cubarsí et al., 2007	NA
Tetracyclines	Tetracycline	81.8, [NA (final 130)°C, 440 W-0.75 + 100 W-6min, TM/Microwave]	Gratacós-Cubarsí et al., 2007	NA
Tetracyclines	Oxytetracycline	51.5-57.3, [97°C, 20min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	71.4-73.6, [97°C, 30min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	84.6-91.5, [97°C, 40min, TM/Boiling]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	61.2-72.1, [99°C, 40min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	87.4-89.9, [99°C, 60min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	94.6-97.6, [99°C, 80min, TM/Roasting]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	73.3-74.7, [98°C, 10min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	92-94.6, [98°C, 15min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	99-100, [98°C, 20min, TM/Microwave]	Abou-Raya et al., 2013	NA
Tetracyclines	Oxytetracycline	99, [118°C, 30min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Oxytetracycline	99, [121°C, 20min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Oxytetracycline	56, [135°C, 0.25min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Oxytetracycline	60, [140°C, 0.12min, Heating in Buffer]	Hassani et al., 2008	NA
Tetracyclines	Oxytetracycline	75, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	NA
Tetracyclines	Oxytetracycline	58, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	NA
Tetracyclines	Oxytetracycline	60, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	NA
Tetracyclines	Oxytetracycline	50, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	NA
Tetracyclines	Oxytetracycline	55.6, [190°C, 7-10min, TS/Frying]	Huang et a.l, 1997	NA

Tetracyclines	Oxytetracycline	33.3, [190°C, 7-10min, TS/Frying]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	72.5, [190°C, 7-10min, TS/Frying]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	30.6, [190°C, 7-10min, TS/Frying]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	47.3, [190°C, 7-10min, TS/Frying]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	54.7, [190°C, 7-10min, TS/Frying]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	93.2, [190°C, 7-10min, TS/Baking]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	56.4, [190°C, 7-10min, TS/Baking]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	52.9, [190°C, 7-10min, TS/Baking]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	25, [140°C, 7-10min, TS/Smoking]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	75.8, [140°C, 7-10min, TS/Smoking]	Huang et a.l, 1997	NA
Tetracyclines	Oxytetracycline	79.9, [140°C, 7-10min, TS/Smoking]	Huang et al., 1997	NA
Tetracyclines	Oxytetracycline	99, [100°C, 30min, W/Boiling]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	25, [110°C, 150min, O/Frying]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	90, [180°C, 30min, O/Frying]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	78, [Internal maximum90 °C, 20min, TM/Boil in bag]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	94, [Internal maximum98 °C, 36min, TM/Roasting]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	80, [Internal maximum100 °C, 7min, TM/Microwaving]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	75, [Internal maximum96 °C, 10min, TM/Boiling]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	75, [Internal maximum90 °C, 5+3min, TM/Microwaving]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	49, [Internal maximum84 °C, 14min, TM/Shallow frying]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	39, [Internal maximum59 °C, 8min, TM/Grilling]	Rose et al. 1996	NA

Tetracyclines	Oxytetracycline	71, [Internal maximum92 °C, 6+6min, TM/Braising]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	64.7, [Internal maximum62 °C, 24min, TM/Shallow frying]	Rose et al. 1996	NA
Tetracyclines	Oxytetracycline	100, [90-95 °C, 60min, TM/Canning]	Scheibner, 1972a	NA
Tetracyclines	Oxytetracycline	39, [100°C, 10min, TM/Boiling]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	80, [100°C, 20min, TM/Boiling]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	95, [100°C, 30min, TM/Boiling]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	2, [NA, 2min, TM/Microwave]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	12.2, [NA, 4min, TM/Microwave]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	60.5, [Final 98-102 °C, 8min, TM/Microwave]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	2.7, [NA, 2min, TM/Frying]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	3.6, [NA, 4min, TM/Frying]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	17.3, [Internal 81 °C, 8min, TM/Frying]	Ibrahim & Moats, 1994	NA
Tetracyclines	Oxytetracycline	60, [100°C, 15min, TS/Frying]	Kitts et al., 1992	NA
Tetracyclines	Oxytetracycline	90, [80°C, 144min, TS/Waterbath heating]	Kitts et al., 1992	NA
Tetracyclines	Oxytetracycline	90, [90°C, 76min, TS/Oil bath heating]	Kitts et al., 1992	NA
Tetracyclines	Oxytetracycline	90, [90°C, 31min, TS/Oil bath heating]	Kitts et al., 1992	NA
Tetracyclines	Oxytetracycline	90, [60°C, 645min, Heating in Buffer]	Kitts et al., 1992	0.0036 (pH 3.0)*
Tetracyclines	Oxytetracycline	90, [80°C, 50min, Heating in Buffer]	Kitts et al., 1992	0.0046 (pH 3.0)*
Tetracyclines	Oxytetracycline	90, [90°C, 48min, Heating in Buffer]	Kitts et al., 1992	0.0048 (pH 3.0)*
Tetracyclines	Oxytetracycline	90, [100°C, 18.7min, Heating in Buffer]	Kitts et al., 1992	0.123 (pH 3.0)*

Tetracyclines	Oxytetracycline	90, [60°C, 111.7min, Heating in Buffer]	Kitts et al., 1992	0.0206 (pH 6.9)*
Tetracyclines	Oxytetracycline	90, [80°C, 22.1min, Heating in Buffer]	Kitts et al., 1992	0.1042 (pH 6.9)*
Tetracyclines	Oxytetracycline	90, [90°C, 16.5min, Heating in Buffer]	Kitts et al., 1992	0.14 (pH 6.9)*
Tetracyclines	Oxytetracycline	90, [100°C, 12.7min, Heating in Buffer]	Kitts et al., 1992	0.181 (pH 6.9)*
Tetracyclines	Oxytetracycline	26.8, [75.7 °C, 3min, TM/Boiling]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	34.9, [91.3 °C, 6min, TM/Boiling]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	50.2, [95.1 °C, 15min, TM/Boiling]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	31.6, [92.9 °C, 0.5min, TM/Microwave]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	44.9, [95.8 °C, 1min, TM/Microwave]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	55.3, [92.4 °C, 0.5min, TM/Microwave]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	28, [75.3 °C, 3min, TM/Boiling]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	36.6, [90.5 °C, 3min, TM/Boiling]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	52.7, [95.4 °C, 3min, TM/Boiling]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	28.8, [94.2 °C, 0.5min, TM/Microwave]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	38.2, [96.7 °C, 0.5min, TM/Microwave]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	59.1, [92.5 °C, 0.5min, TM/Microwave]	Nguyen et al., 2014	NA
Tetracyclines	Oxytetracycline	7.4, [Maximum to 22°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	15.8, [Maximum to 50 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	10.4, [Maximum to 48°C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	4.3, [Maximum to 44 °C, Total 10min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	15.1, [Maximum to 58°C, Total 20min,	O'Brien et al., 1981	NA

		TM/Grilling]		
Tetracyclines	Oxytetracycline	18, [Maximum to 62 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	16, [Maximum to 85°C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	12.3, [Maximum to 73 °C, Total 20min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	24.2, [Maximum to 76°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	39, [Maximum to 83 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	27.7, [Maximum to 85°C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	22.5, [Maximum to 73 °C, Total 30min, TM/Grilling]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	17.8, [Maximum to 59°C, Total 90min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	21.4, [Maximum to 70 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	32, [Maximum to 65°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	23.1, [Maximum to 63 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	29.3, [NA, Total 90min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	28.2, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	76.3, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	23.1, [NA, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	21.8, [Maximum to 79°C, Total 90min,	O'Brien et al., 1981	NA

		TM/Roasting]		
Tetracyclines	Oxytetracycline	38.9, [Maximum to 88 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	74, [Maximum to 94°C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	58.2, [Maximum to 94 °C, Total 120min, TM/Roasting]	O'Brien et al., 1981	NA
Tetracyclines	Oxytetracycline	60, [100°C, 15min, TS/Frying]	Kitts et al., 1992	NA
Tetracyclines	Oxytetracycline	62.5, [190°C, 7-10min, TS/Frying]	Du et al., 1997	NA
Tetracyclines	Oxytetracycline	75, [190°C, 45min, TS/Baking]	Du et al., 1997	NA
Tetracyclines	Oxytetracycline	60.7, [140-160 +180-200 °C, 60+60min, TS/Smoking]	Du et al., 1997	NA
Tetracyclines	Oxytetracycline	55, [100°C, 4min, TS/Boiling]	Uno et al., 2006a	NA
Tetracyclines	Oxytetracycline	80, [100°C, 12min, TS/Boiling]	Uno et al., 2006a	NA
Tetracyclines	Oxytetracycline	35, [200°C, 4min, TS/Baking]	Uno et al., 2006a	NA
Tetracyclines	Oxytetracycline	55, [180°C, 1min, TS/Frying]	Uno et al., 2006a	NA
Tetracyclines	Oxytetracycline	17, [100°C, 4min, TS/Boiling]	Uno et al., 2006a	NA
Tetracyclines	Oxytetracycline	21, [200°C, 4min, TS/Baking]	Uno et al., 2006a	NA
Tetracyclines	Oxytetracycline	22, [180°C, 1min, TS/Frying]	Uno et al., 2006a	NA
Tetracyclines	Chlortetracycline	8, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	5.559E-03
Tetracyclines	Chlortetracycline	9, [100°C, 15min, W/Boiling]	Hsieh et al., 2011	6.287E-03
Tetracyclines	Chlortetracycline	50, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	4.621E-02
Tetracyclines	Chlortetracycline	57, [121°C, 15min, W/Boiling]	Hsieh et al., 2011	5.626E-02
Nitrofuran	Furazolidone	NS, [210°C, 5min, TM/Frying]	Shitandi et al., 2008	NA
Others	Dimetridazole (DMZ)	NS, [100°C, 180min, W/Boiling]	Rose et al. 1998	0.000E+00
Others	Dimetridazole (DMZ)	17.6, [110°C, 180min, O/Frying]	Rose et al. 1998	1.075E-03
Others	Dimetridazole (DMZ)	60, [170°C, 180min, O/Frying]	Rose et al. 1998	5.091E-03

Others	Dimetridazole (RNZ)	58, [100°C, 180min, W/Boiling]	Rose et al. 1998	4.819E-03
Others	Dimetridazole (RNZ)	20, [110°C, 180min, O/Frying]	Rose et al. 1998	1.240E-03
Others	Dimetridazole (RNZ)	50, [170°C, 180min, O/Frying]	Rose et al. 1998	3.851E-03
Others	Levamisole	3, [100°C, 60min, W/Boiling]	Rose et al. 1995a	5.077E-04
Others	Levamisole	5.5, [100°C, 180min, W/Boiling]	Rose et al. 1995a	3.143E-04
Others	Levamisole	11, [100°C, 240min, W/Boiling]	Rose et al. 1995a	4.856E-04
Others	Levamisole	64, [260°C, 10min, O/Frying]	Rose et al. 1995a	1.022E-01
Others	Levamisole	99, [260°C, 30min, O/Frying]	Rose et al. 1995a	1.535E-01
Others	Levamisole	7.3, [Final 82 °C, 4.5+3.75min, TM/Microwave]	Rose et al. 1995a	NA
Others	Levamisole	6, [100°C, 40min, TM/Boiling]	Rose et al. 1995a	NA
Others	Levamisole	11.2, [NA, 18min, TM/Grilling]	Rose et al. 1995a	NA
Others	Levamisole	increased 13, [NA, 23.5min, TM/Frying]	Rose et al. 1995a	NA
Others	Levamisole	11, [Maximum internal temperature 57 °C, 4-6min, TM/Shallow Frying]	Cooper et al., 2011	NA
Others	Levamisole	42, [Maximum internal temperature 91.3 °C, 4-6min, TM/Shallow Frying]	Cooper et al., 2011	NA
Others	Oxfendazole	5, [100°C, 30min, W/Boiling]	Rose et al. 1997a	1.710E-03
Others	Oxfendazole	10, [100°C, 210min, W/Boiling]	Rose et al. 1997a	5.017E-04
Others	Oxfendazole	100, [150°C, 45min, O/Frying]	Rose et al. 1997a	NA
Others	Oxfendazole	100, [180°C, 10min, O/Frying]	Rose et al. 1997a	NA
Others	Oxfendazole	36, [NANAmin, TM/Microwave]	Rose et al. 1997a	NA
Others	Oxfendazole	42, [NA, TM/Braising]	Rose et al. 1997a	NA

Note: M=Milk, W=Water, O=Oil, TM=Tissue of meat, TS=Tissue of seafood, NS=Not significant, NA=Not available.

* The value was calculated based on D value (Equation 5).

** The value was reported in reviewed literatures.