Pharmaceuticals in industrial wastewater and their removal using photo-Fenton's oxidation

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Abstract

Active pharmaceutical ingredients are known contaminants of surface and ground water. In some cases these are persistent organic chemicals which are only partially eliminated during conventional wastewater treatment. They have been detected in the effluent of various European wastewater treatment plants. However, there is a growing body of evidence to suggest that discharges from pharmaceutical plants themselves can contribute to the levels of pharmaceuticals in the environment. This project is based on both the detection and removal of pharmaceuticals from industrial sources. A SPE-LC-MS/MS method for the detection of famotidine, tamsulosin hydrochloride and solifenacin succinate in wastewater at a pharmaceutical production facility was developed and validated. The results of a six month sampling programme showed substantial concentrations of the analytes in both influent and effluent. Famotidine was detected at an average concentration of 1.6 mg/L and 2.6mg/L, tamsulosin hydrochloride at 5 µg/L and 4 µg/L and solifenacin succinate at 39 µg/L and 28 µg/L in influent and effluent respectively. Photo-Fenton's oxidation conditions were optimised for the removal of each of the three APIs from aqueous solutions. Intermediates and final products of the degradation have been identified via LC-MS.

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Abbreviations

AC	- Activated carbon
AOP	- Advanced oxidation process
API	- Active pharmaceutical ingredient
AS	- Activated sludge
BDD	- Boron doped diamond
BFR	- Biofilm reactor
BOD	- Biological oxygen demand
COD	- Chemical oxygen demand
CPC	- Compound parabolic collector
CSTR	- Continuously stirred tank reactor
CV	- Coefficient of variation
DAD	- Diode array detector
DEET	- N,N-Diethyl-meta-toluamide
DI	- Direct infusion
DO	- Dissolved oxygen
DSSR	- Double skin sheet reactor
EDC	- Endocrine disrupting compounds
EIC	- Extracted ion chromatogram
EPA	- Environmental Protection Agency
ESI	- Electrospray ionisation
GAC	- Granular activated carbon
GC	- Gas chromatography
HPLC	- High performance liquid chromatography
HRT	- Hydraulic retention time
HUASR	- Hybrid up-flow anaerobic sludge reactor
LC	- Liquid chromatography
LOD	- Limit of detection
LOQ	- Limit of quantitation
MBR	- Membrane batch reactor
MF	- Microfiltration
MP	- Mobile phase
MPG	- Methyl-phenylglycine
MRM	- Multiple reaction monitoring
MS	- Mass spectrometry
MS/MS	- Tandem mass spectrometry
n	- Sample size
nd	- Not detected
NF	- Nanofiltration
OLR	- Organic loading rate
PAC	- Powdered activated carbon
PFP	- Pentyflorophenyl propyl
pK_a	- Acid dissociation constant
PLE	- Pressurised liquid extraction
PTR	- Parabolic trough reactor
R	- Regression coefficient
R&D	- Research and development
RO	- Reverse osmosis

RP	- Reverse phase
RSD	- Relative standard deviation
RT	- Retention time
SBR	- Sequence batch reactor
SMA	- Specific methanogenic activity
SPE	- Solid phase extraction
SS	- Suspended solids
SXC	- Strata-X-C
TCEP	- Tris(2-carboxyethyl)phosphine
TOC	- Total organic carbon
TFFBR	- Thin film fixed bed reactor
UASBR	- Upflow anaerobic sludge blanket reactor
UASR	- Upflow anaerobic sludge reactor
UF	- Ultrafiltration
UPLC	- Ultra performance liquid chromatography
WW	- Wastewater
WWTP	- Wastewater treatment plant

Publications, posters and oral presentations

Publications

"Treatment options for wastewater from pharmaceutical companies: a review" A-M. Deegan, K. Urell, B. Shaik, M. Oelgemoeller, J. Tobin, K. Nolan, A. Morrissey, International Journal of Environmental Science and Technology, accepted for publication.

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Posters

"Assessment of pharmaceutical residue levels in industrial wastewaters and their removal using photocatalysis" A-M. Deegan, J. Tobin, A. Morrissey, M. Oelgemöller, Biannual Meeting, Questor Centre, Queens University, Belfast (Northern Ireland), 18-19 November 2008.

"A SPE-LC-MS/MS method for the detection of three active pharmaceutical ingredients in industrial wastewaters and their removal using photocatalysis" A-M. Deegan, K. Nolan, M. Oelgemöller, A. Morrissey, J. Tobin, 1st European Congress on Photochemistry, Journées Européenne de la Photocatalyse, Bordeaux (France), 21-22 September 2009.

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"Assessment of pharmaceutical residue levels in industrial wastewaters and their removal using photocatalysis" A-M. Deegan, J. Tobin, A. Morrissey, M. Oelgemöller. Questor Centre Biannual Meeting, Queens University, Belfast (Northern Ireland), May 2008.

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"Assessment of pharmaceutical residue levels in industrial wastewaters and their removal using photo-Fenton's oxidation" A-M. Deegan, J. Tobin, A. Morrissey, M. Oelgemöller. Questor Centre Biannual Meeting, Queens University, Belfast (Northern Ireland), 2009.

1. Introduction

1.1 Project overview

Pharmaceuticals enter the aquatic environment at trace concentrations through their continuous release from numerous sources including run-off from land, hospital and municipal wastewater (Figure 1.1). The effects of individual APIs as well as synergistic effects of combinations of APIs in drinking water raise concern over possible human health effects (Fatta et al., 2007; Webb et al., 2003). APIs have been detected in surface and wastewaters (Suarez et al., 2009; Tabak and Bunch, 1970; Watkinson et al., 2007). This has been associated with damage to aquatic biota as well as bioaccumulation in terrestrial biota (Larsson et al., 1999; Oaks et al., 2004). The contribution of pharmaceutical production wastewaters to the environmental loading of APIs represents a significant knowledge gap.

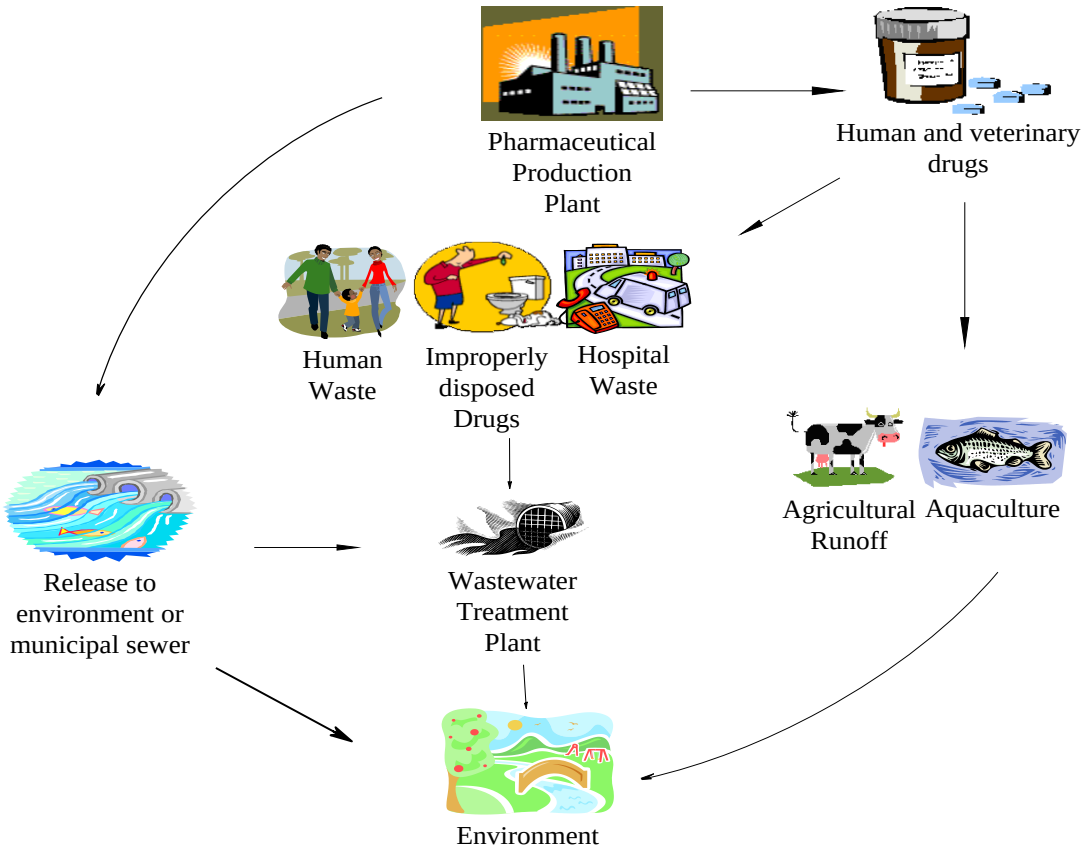


Figure 1.1: Routes of pharmaceutical entry into the environment.

This project involves both the detection and removal of pharmaceutical residues from production wastewater at a chemical synthesis manufacturing

facility based in Mullhudart, Co. Dublin. The project was divided into three work packages as outlined below.

Work package 1: To develop an SPE-LC-MS/MS method for the monitoring of three pharmaceuticals, namely famotidine, tamsulosin hydrochloride and solifenacin succinate in industrial wastewater.

Step 1.1: Development and optimisation of HPLC method.
Step 1.2: Development and optimisation of SPE method.
Step 1.3: Development and optimisation of MS method.
Step 1.4: Integrate developed methods into a single method suitable for the analysis of the drugs in chemical synthesis wastewater.

Work package 2: A six month sampling programme for the monitoring of the three APIs.

Step 2.1: Monitor influent and effluent concentrations of the APIs at the wastewater treatment plant for 6 months.
Step 2.2: Examine the data obtained to establish the concentrations of pharmaceuticals lost to the municipal sewers as well as determining the efficiency of the wastewater treatment facility.

Work package 3: Develop and optimise a method for the removal of the three pharmaceuticals from the production facility wastewater using photo-Fenton's oxidation.

Step 3.1: Optimisation of Fe(II) and H_2O_2 concentrations.
Step 3.2: Monitor removal rates and kinetic evaluation using HPLC.
Step 3.3: Carry out an intermediate study to identify the degradation pathways using LC-MS.

1.2 Limitations of project

Monitoring of the discharge and effects of pharmaceuticals on the environment represents a small but important portion of the topic of micro-pollutants in the environment. This project is limited to the monitoring and removal using photo-Fenton's oxidation of three pharmaceuticals in wastewater with regards to the actual concentrations of pharmaceuticals released from pharmaceutical production facilities. The scope for further investigation in this area is large and for example other analytes of various environmental significance including EDCs should be investigated. Other types of production facilities such as fermentation, R&D facilities and facilities that recover active ingredients from natural resources may contribute to a significantly different environmental loading than a chemical synthesis facility. Even between similar facilities the environmental loading may vary.

Investigation of the biotoxicity of the analytes under investigation in this project and of the degradation products are beyond the scope of this project. However, the importance of this information in regards the photo-Fenton's oxidation should not be under-estimated. It is possible that products formed during photo-oxidation/catalysis may be more toxic than the parent compound.

As discussed in the literature review there is a large number of treatment methods available for the removal of pharmaceuticals and for the treatment of pharmaceutical wastewater generally. This project is limited to photo-Fenton's oxidation.

Further possible avenues of investigation beyond the scope of this project include legislative investigations and risk assessments of pharmaceuticals to the environment.

1.3 The analytes under investigation

There were three pharmaceuticals in production at the Astellas Manufacturing facility in west Dublin during this project namely famotidine, tamsulosin hydrochloride and solifenacin succinate.

1.3.1 Famotidine (CAS No. 76824-35-6)

Famotidine is a H_2-Receptor antagonist commonly used in the reduction of stomach acid production (Figure 1.2). A number of studies have investigated the presence of the API in municipal wastewater and surface waters (Gros et al., 2006; Gros et al., 2010; Muñoz et al., 2009). Famotidine was not detected in river water samples but was detected in municipal wastewater samples. .

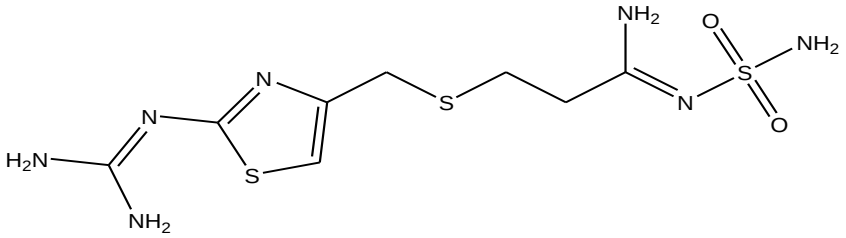


Figure 1.2: Famotidine structure

1.3.2 Tamsulosin hydrochloride (CAS No. 106133-20-4)

Tamsulosin hydrochloride is a A1a-selective alpha blocker used in the treatment of benign prostatic hyperplasia (Figure 1.3). To the best of the authors knowledge no studies have taken place monitoring the concentrations of tamsulosin in effluents or environmental waters.

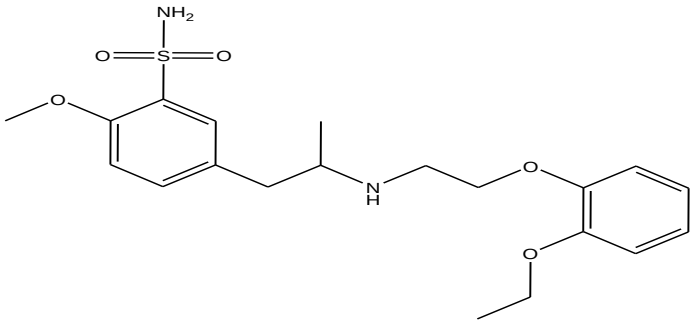


Figure 1.3: Tamsulosin hydrochloride structure

1.3.3 Solifenacin succinate (CAS No. 242478-38-2)

Solifenacin Succinate is a urinary antispasmodic used in the treatment of overactive bladder (Figure 1.4). To the best of the authors knowledge no studies have taken place monitoring the concentrations of tamsulosin in effluents or environmental waters.

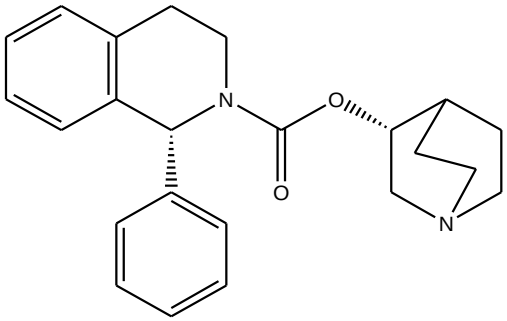


Figure 1.4: Solifenacin succinate structure

1.3 Thesis layout

This thesis is divided into a literature review, materials and methods, results and discussion, conclusions and opportunities for further investigation.

Chapter 2: Literature review

The literature review discusses the options for the treatment of pharmaceutical production facility wastewater focusing on the removal of APIs. The review includes a comprehensive list of tables showing removal of APIs using various treatment methods.

Chapter 3: Materials and methods

The materials and methods section focuses on the methods used for the detection of the APIs. Separate validated SPE-HPLC and SPE-HPLC-MS/MS methods are outlined for the detection of the three APIs. The methods used in the optimisation of the photo-Fenton's oxidation of the three APIs and methods for the monitoring of intermediates are also included.

Chapter 4: Results and discussion

The concentrations of the three APIs in influent and effluent from the wastewater treatment facility from November 2009 to April 2010 are presented in the results and discussion. The method validation for both the SPE-HPLC method in distilled water and SPE-LC-MS/MS method in influent and effluent samples is included. Optimised photo-Fenton's oxidation conditions, kinetic results and degradation pathways for the three APIs are discussed.

Chapter 5: Conclusions and further research opportunities

Conclusions are drawn on the developed methods, results of the six month sampling programme and effectiveness of photo-Fenton's oxidation for the removal of the three APIs. Further research directions to broaden our understanding on the contribution of industrial facilities to the environmental loading of pharmaceuticals and treatment at source are discussed.

Appendices

Appendix A: monthly summaries of results from the monitoring programme.

Appendix B: literature showing degradation of APIs via Fenton's oxidation.

Appendix C, D and E show the structures of the famotidine impurities, tamsulosin and solifenacin metabolites used to interpret photo-Fenton's intermediates.

2. Literature review

2.1 Introduction

The presence of pharmaceuticals and personal care products was first identified in surface and wastewaters in the United States and Europe in the 1960's and 1970's (Stumm-Zollinger and Fair, 1965; Tabak and Bunch, 1970). The issue attracted substantial interest after the occurrence of pharmaceuticals in river water was linked to feminisation of fish living downstream of WWTP outfalls (Larsson et al., 1999). Subsequently damage to aquatic and terrestrial biota has been confirmed. For example, the non-steroidal anti-inflammatory drug diclofenac has been directly correlated with the renal failure of vultures contributing to the >95% decline in their population in the Indian subcontinent since the 1990's (Oaks et al., 2004).

Sources of pharmaceutical contamination of the aquatic environment included pharmaceutical production plants, WWTPs, hospitals, agriculture, aquaculture, landfills and even graveyards (Khetan and Collins, 2007). The most investigated route of entry of pharmaceuticals into the environment was through municipal wastewater treatment plants. Herberer (2002) found that human excretion of unchanged or slightly transformed APIs conjugated to polar molecules such as glucoronides, entered the WWTP which may then be cleaved, releasing the original API into the environment. Consequently numerous studies have investigated the removal efficiencies of pharmaceuticals at municipal WWTPs. Activated sludge WWTPs have received particular attention (Jones et al., 2007; Watkinson et al., 2007). A limited number of studies also found pharmaceuticals in drinking water (Webb et al., 2003) and hospital wastewater (Suarez et al., 2009). Monitoring of the levels of APIs released from pharmaceutical production facilities was largely neglected and the importance of such releases has not been established (Larsson and Fick, 2009).

2.2 Review outline

This review begins with an overview of pharmaceutical industry wastewater. It examines the options for the treatment of pharmaceutical production facility wastewater. The review focuses not only on the ability of various treatment

methods to reduce the oxygen demand of the wastewater but the removal of various constituents, in particular APIs. Conventional and advanced treatment methods are examined including aerobic and anaerobic biological processes, activated carbon, ozonation and chlorination. Emerging technologies for micro-pollutant removal including advanced oxidation processes are outlined in the latter part of this review. The review also contains an extensive list of tables showing API levels using different treatment methods.

2.3 Overview of pharmaceutical industry wastewater

Pharmaceutical production facility wastewater was known to contain solvents, catalysts, additives, reactants, intermediates, raw materials and APIs (Sreekanth et al., 2009). The wastewater was typically high strength, with high COD, high BOD and high COD:BOD ratio. It could be toxic or odorous when released into the environment (Suman Raj and Anjaneyulu, 2005). The wastewater could also contain priority pollutants including cyanide (Suman Raj and Anjaneyulu, 2005). It was estimated that up to half the pharmaceutical wastewater produced worldwide was released without any treatment (Enick and Moore, 2007).

Pharmaceutical manufacturing processes included fermentation, chemical synthesis, isolation or recovery from natural resources or a combination of all three (EPA, 1997). Fermentation and chemical synthesis generated large volumes of wastewater, with a high organic load. The presence of toxic or recalcitrant substances in such wastewaters resulted in lower COD removal efficiencies (Chellipan et al., 2006). Recovery systems for the removal of solvents were applied. However solvents were still present in the wastewater. Wastewaters include wash water from cleaning equipment and floors, cooling waters, process water, municipal and storm water (EPA, 1997). Biological treatment of this water was common and economical (Kulik et al., 2008). However biological methods have shown to be insufficient for the removal of all potentially hazardous constituents of the wastewater (Clara et al., 2005; Joss et al., 2004; Joss et al., 2005; New et al., 2000; Suman Raj and Anjaneyulu, 2005). More advanced mechanisms for the treatment of the

wastewater was required. Recently MBR technology, ozonation and advanced oxidation processes have shown varying degrees of efficiency for the treatment of pharmaceutical wastewaters (Addamo et al., 2005; Alum et al., 2004; Andreozzi et al., 2005; Andreozzi et al., 2006; Doll and Frimmel, 2005 a, b and c; Helmig et al., 2007; Perez-Estrada et al., 2005 a; Ravina at al., 2002). Integrating various biological and more advanced treatment methods may provide the solution to the treatment of the wide variety of potentially hazardous substances present in pharmaceutical wastewater (Arana et al., 2002; Arslan-Alaton and Dogruel 2004; Helmig et al., 2007; Iketata et al., 2006).

Licensing agreements in the country in which the production facility was based governed the quality of the wastewater that was allowed to be released. In the US and Europe the majority of licences focused on BOD, COD, suspended solids and pH without any control on the release of specific APIs, intermediates, raw materials or other recalcitrant substances (Larsson and Fick, 2009). 'Ireland seems to be the only jurisdiction where specific regulatory licence limits have been established for APIs' (Helmig et al., 2007). While some attention has been focused on endocrine disrupting chemicals the presence of other APIs largely remain unmonitored.

2.4 Biological treatments

Biological treatment methods have traditionally been used for the management of pharmaceutical wastewater (Suman Raj and Anjaneyulu, 2005). Aerobic applications include activated sludge, membrane batch reactors and sequence batch reactors (Chang et al., 2008; Chen et al., 2008; Ileri et al., 2003; La Para et al., 2002; New et al., 2000; Noble, 2006; Suman Raj and Anjaneyulu, 2005). Anaerobic methods include anaerobic sludge reactors, anaerobic film reactors and anaerobic filters (Chellipan et al., 2006; Enright et al., 2005; Gangagai Rao et al., 2005; Nandy and Kaul, 2001; Oktem et al., 2007; Sreekanth et al., 2009).

Wastewater characteristics may suggest suitability or unsuitability of biological treatments for pharmaceutical industry wastewater. Solvents, APIs, intermediates and raw materials represent biologically recalcitrant substances which affect the efficiency of biological treatment systems (Helmig et. al., 2007; Oz et al., 2004). For example activated sludge treatment is unsuitable for wastewater with COD levels in excess of 4000 mg/L (Suman Raj and Anjaneyulu, 2005).

The biological processes presented in this review are well established for the treatment of municipal wastewater. However for the treatment of pharmaceutical wastewater the processes require modification due to its complex and toxic nature. It has been reported to contain solvents which may include methylene chloride, toluene, isopropyl alcohol, chloroform, chlorobenzene, chloromethane cyanide, phenol and benzene. These solvents have been mostly removed by solvent recovery systems but the wastewater may still have relatively high concentrations of these solvents (EPA, 1997). A fraction of solvents may be difficult to biodegrade and may adversely affect the performance of biological treatment systems (Akarsubasi et al., 2005).

In addition to high strength wastewater, solvents and recalcitrant substances there are approximately 3000 different APIs known to be manufactured worldwide (Helmig et al., 2007). These comprise a wide variety of structures, complexity and physiochemical properties. By design APIs are persistent with low biodegradability. APIs are often hydrophobic in nature which may cause them to concentrate in sludge and cause adverse effects on the anaerobic digestion process applied to sludges (Fountoulakis et al., 2008).

2.4.1 Activated sludge treatment

Activated sludge treatment is the agitation or aeration of wastewater in order to promote the growth of bacteria and microorganisms which digestion the organic components of the wastewater. Conventional activated sludge with a long hydraulic retention time (HRT) has historically been the method of choice for the treatment of pharmaceutical industry wastewater (El Gohary et al, 1995; Oz et al., 2004; Rosen et al., 1998). It has a lower capital cost than

more advanced treatment methods and a limited operational requirement; it is generally more environmentally friendly than harsher chemical methods such as chlorination (New et al., 2000). However high energy consumption, the production of large amounts of sludge (Sreekanth, 2009) and operational problems including colour, foaming and bulking in secondary clarifiers are associated with activated sludge plants (Oz et al., 2004).

Factors which affect the efficiency of activated sludge facilities for the treatment of pharmaceutical wastewater include HRT, temperature, pH, dissolved oxygen, organic load, the microbial community, the presence of toxic or recalcitrant substances and the batch operation of pharmaceutical production facilities (La Para et al., 2000; La Para et al., 2001a and b; La Para et al., 2002; New et al., 2000; Suman Raj and Anjaneyulu, 2005). These variables require modification for adaptation to pharmaceutical industry wastewater.

Temperature is a key factor in the efficiency of activated sludge facilities. It has an important role in selecting individual microbial species and overall microbial diversity in the activated sludge (La Para et al., 2000). Some pharmaceutical production facilities produce wastewater at high temperature which may affect the efficiency of activated sludge (AS) plants (La Para et al., 2000, La Para et al., 2001b). Therefore water from high temperature processes must be cooled prior to treatment by AS increasing time and cost of treatment.

Operation of AS plants at lower pH enhances the removal of acidic pharmaceuticals. Low pH enhances the removal of acidic pharmaceuticals due to adsorption of the API onto sludge particles where they are biologically degraded (Urase et al., 2005). The sludge must then be treated for the removal of APIs.

The variable nature of pharmaceutical wastewater due to batch processing and the intermittent production of wastewater may lead to shock loads, which negatively affect the stability of the microbial community and result in a

deterioration of bacterial flocs and loss of biomass to final effluent (La Para et al., 2002). In samples taken 15 days apart influent wastewater characteristics changed significantly while effluent quality remained high. Loading at high COD can reduce the efficiency of an activated sludge system. Above 4000 mg/L COD, the wastewater becomes unsuitable for AS treatment (Suman Raj and Anjaneyulu, 2005). Between 1500 and 4000 mg/L COD, AS produced consistently high COD removal using acclimised mixed consortia. In addition, AS is not effective in wastewater with COD levels above 4000 mg/L.

The impact of pharmaceuticals on AS process appeared to be negligible under usual operational conditions (Stamatelatou et al., 2003). However at higher concentrations, which may be expected in the wastewater of pharmaceutical manufacturing facilities, they may become inhibitory. Above 2 mg/L triclosan, significant reduction in nitrite consumption was noted (Dokianakis et al., 2004). There was a paucity of literature showing the removal of APIs from pharmaceutical manufacturing facilities. However from municipal facilities it has been shown that removal efficiency is dependent on the APIs present. Table 2.1 shows that AS was more efficient for the removal of some APIs in municipal facilities than others. β-Lactam and quinlone drugs in particular appeared to be susceptible to aerobic oxidation. In a WWTP in Brisbane Australia, β-Lactam antibiotics showed high biodegradability due to hydraulic cleavage of the β-lactam ring. Lincomycin and sulphonamides were least affected by AS treatment. Similar studies have also found that the efficiency of the process was dependent on the compound under investigation. Ibuprofen, naproxen, bezafibrate and estrogens (estrone, estradiol, and ethinylestradiol) showed a high degree of removal. Sulfamethoxazole, carbamazepine and diclofenac showed limited removal (Clara et al., 2005; Joss et al., 2004; Joss et al., 2005). Polar APIs such as diclofenac and carbamazepine were poorly adsorbed onto activated sludge particles. Polar APIs therefore were removed by biodegradation (Carballa et al., 2004). With the development of more metabolically resistant APIs this problem is likely to increase (Khetan and Collins, 2007).

Table 2.1: Removal of selected pharmaceuticals using activated sludge.

Pharmaceutical class	Pharmaceutical	Mean influent (ng/L)	Mean effluent (ng/L)	% Removal	Reference
Antibiotics	Amoxicillin	190	n.d.	100	Watkinson et al., 2007
		13mg/d/1000 pop	n.d.	100	Castiglioni et al., 2006
	Cefaclor	50	n.d.	100	Watkinson et al, 2007
	Cephalexin	4600	n.d.	100	Watkinson et al., 2007
	Ciprofloxacin	3800	640	83	Watkinson et al., 2007
		259mg/d/1000 pop	97mg/d/1000 pop	63	Castiglioni et al., 2006
	Clarithromycin	21mg/d/1000 pop	55mg/d/1000 pop[a]	0	Castiglioni et al., 2006
	Clindamycin	2	5	0	Watkinson et al., 2007
	Enrofloxacin	10	10	0	Watkinson et al., 2007
	Erythromycin	n.d.	5mg/d/1000 pop[a]	0	Castiglioni et al., 2006
	Lincomycin	3.4mg/d/1000 pop	5.4 mg/d/1000 pop[a]	0	Castiglioni et al., 2006
	Monensin	10	25 [a]	0	Watkinson et al., 2007
	Nalidixic acid	n.d.	55 [a]	0	Watkinson et al., 2007
	Norfloxacin	170	25	85	Watkinson et al., 2007
	Ofloxacin	360mg/d/1000 pop	233mg/d/1000 pop	35	Castiglioni et al., 2006
	Penicillin V	50	30	40	Watkinson et al., 2007
	Roxithromycin	WWTP1: 69	31	55	Clara et al., 2005
	Spiramycin	4.8mg/d/1000 pop	35mg/d/1000 pop	0	Castiglioni et al., 2006
	Sulfamethoxazole	n.d.	<166-<553	0	Lacey et al., 2008
		65mg/d/1000 pop	10mg/d/1000 pop	85	Castiglioni et al., 2006
		360	270	25	Watkinson et al., 2007
	Sulphathiazole	2	n.d.	100	Watkinson et al., 2007
	Trimethoprim	<171-< 57	<67-<360	0	Lacey et al., 2008
		340	50	85	Watkinson et al., 2007
Lipid regulators	Gemfibrozil	<26-<86	<32-<330	0	Lacey et al., 2008
	Bezafibrate	WWTP1:6840	692	90	Clara et al., 2005
		50mg/d/1000 pop	29mg/d/1000 pop	42	Castiglioni et al., 2006
	Clofibric acid	<222-<740	n.d.	100	Lacey et al., 2008
		96µg/m^3/d	84µg/m^3/d	13	Bernhard et al., 2006
				1-4 **	Zwiener and Frimmel, 2003
Antiepileptic's	Carbamazepine	1273µg/m^3/d	1190µg/m^3/d	7	Bernhard et al., 2006
		n.d.	<0.163–<0.881[a]	0	Lacey et al., 2008
		12 mg/d/1000 pop	28mg/d/1000 pop	0	Castiglioni et al., 2006
		28mg/d/1000 pop		100	

		704	952[a]	0	Clara et al., 2005
		WWTP 1: 280	290	0	Santos et al., 2007
		WWTP 2: 300	500	0	
		WWTP 3: 290	320	0	
		WWTP 4: 360	370	0	
Antiphlogistic's	Diclofenac	n.d.	<0.743-<2.478	0	Lacey et al., 2008
		3190	1680	47	Clara et al., 2005
		2133µg/m^3/d	1617µg/m^3/d	24	Bernhard et al., 2006
				1-4 **	Zwiener and Frimmel, 2003
	Ibuprofen	2448	20	99	Clara et al., 2005
		6810µg/m^3/d	212µg/m^3/d	97	Bernhard et al., 2006
		< 0.760-3.204	n.d.	100	Lacey et al., 2008
		122mg/d/1000 pop	28mg/d/1000 pop	77	Castiglioni et al., 2006
		WWTP 1: 280	290	0	Santos et al., 2007
		WWTP 2: 300	500	0	
		WWTP 3: 290	320	0	
		WWTP 4: 360	370	0	
				60**	Zwiener and Frimmel, 2003
	Ketoprofen	WWTP 1: 540	340	37	Santos et al., 2007
		WWTP 2: 300	210	30	
		WWTP 3: 460	360	22	
		WWTP 4: 1360	410	70	
		WWTP 1: 4040	2620	35	Santos et al., 2007
		WWTP 2:11140	1180-11830	0-89	
		WWTP 3: 5180	1960	62	
β-Blockers	Atenolol	494mg/d/1000 pop	281mg/d/1000 pop	43	Castiglioni et al., 2006

[a] Concentrations of APIs in effluent was greater than influent due to matrix and other effects (Herberer et al., 2002; Watkinson et al 2007) n.d.: not detected, all matrices are municipal wastewater, with the exception of those marked **, which are synthetic pharmaceutical wastewater.

2.4.2 Advances in aerobic treatment for pharmaceutical wastewater

Variations on AS including sequence batch reactors (SBRs) and membrane batch reactors (MBRs) were shown to have added advantages for the treatment of pharmaceutical wastewater. SBR is an activated sludge method of treatment in which separate tanks for aeration and sedimentation are not required and there is no sludge return. The fill, react, settle, draw and idle stages take place in one tank as opposed to sequential tanks. This type of process was ideal for use in small systems or when land is limited (Ileri et al., 2003). The four operational modes allow easy modifications of process conditions, enhancing the SBRs capacity for meeting effluent quality restrictions (Aguado et al., 2008).

MBRs combine activated sludge with membrane technology. There were two configurations of MBR, submerged or external mounted to a suspended growth bioreactor which allow liquid-solid separation to take place (Yang et al., 2006). Membranes are typically immersed in the aeration tank. This allows mixing, keeps solids in suspension and reduces fouling of the membrane. Solids are retained on one side of the filter removing the need for further treatment. Membrane processes are effective for the removal of bulk organics. They can replace traditional methods or operate in combination with conventional AS systems or as hybrid systems (Noble, 2006). Advantages of MBRs include shorter start-up times and they are suitable where space is limited (Yang et al., 2006). Variable wastewater composition and batch production may be treated using MBR (Chang et al., 2008). In conventional AS systems a sludge sedimentation tank is used to remove solids. In MBRs separation of solids is controlled by membranes.

The use of MBR in the treatment of pharmaceutical wastewater is still in its early development (Chang et al., 2008). Similarities exist between design parameters for municipal and industrial facilities however substantial changes in design, control and operational performances exist. For example, the running of MBRs at high sludge age in other words the length of time a

partical of suspended solids is retained in the process, results in higher microbial populations, leading to a lower food to microbial mass ratio and to more complete mineralisation (De Wever et al., 2007). It also allows for the growth and adaptation of microbes with greater physiological capabilities.

High COD and BOD removal have been demonstrated in pharmaceutical production facilities using MBR technology. A 10 m^3/d MBR operated at a pharmaceutical facility in Taiwan, for example, removed 95 and 99% of COD and BOD respectively and showed stable operation to different conditions (Chang et al., 2008). The plant consisted of an aeration tank and membrane bioreactor and was operated for 140 days. Initial wastewater concentrations were 800-11800 mg/L COD and 100-6350 mg/L BOD. The plant showed stable operation to different concentrations. However despite high COD/BOD removal MBRs still do not remove all APIs. Estrone, ethinyl estradiol and venlafaxine have proven difficult to degrade (Helmig et al., 2007). API removal by MBR is shown in Table 2.2.

Table 2.2: Removal of selected pharmaceuticals in a variety of waters following MBR treatment.

Pharmaceutical class	Pharmaceutical	Influent (ng/L)	Effluent (ng/L)	% Removal	Matrix	Reference
Antibiotics	Erythromycin	800	34	96	Primary effluent	Snyder et al., 2007
	Monensin	40	n.d.	100	Municipal WW	Watkinson et al., 2007
	Nalidixic acid	330	45	86	Municipal WW	Watkinson et al., 2007
	Roxithromycin	69	117	0	Municipal WW	Clara et al., 2005
	Sulfamethoxazole	23	<10	>56	Primary effluent	Snyder et al., 2007
Lipid regulators	Gemfibrozil	74	<10	>86	Secondary effluent	Snyder et al., 2007
	Bezafibrate	WWTP1:6840	1550	77	Municipal WW	Clara et al., 2005
	Clofibric acid	92µg/m^3/d	46µg/m^3/d	50	Municipal WW	Bernhard et al., 2006
Hormones	Androstenedione	<10	<10	0	Primary effluent	Snyder et al., 2007
Antiepileptics	Carbamazepine	189	<10	>95	Primary effluent	Snyder et al., 2007
		1287µg/m^3/d	1119µg/m^3/d	13	Municipal WW	Bernhard et al., 2006
		952	704	26	Municipal WW	Clara et al., 2005
	Dilantin	192	184	4	Primary effluent	Snyder et al., 2007
Antidepressant	Fluoxetine	44	<10	>77	Primary effluent	Snyder et al., 2007
Antiphlogistics	Diclofenac	16	<10	>38	Primary effluent	Snyder et al., 2007
		3190	2140	33	Municipal WW	Clara et al., 2005
	Ibuprofen	2448	20	99	Municipal WW	Clara et al., 2005
		6725 µg/m^3/d	92µg/m^3/d	99	Municipal WW	Bernhard et al., 2006
	Naproxen	70	<10	>86	Primary effluent	Snyder et al., 2007
Analgesics	Acetaminophen	<10	<10	0	Primary effluent	Snyder et al., 2007
	Hydrocodone	168	< 101	40	Primary effluent	Snyder et al., 2007

2.4.3 Anaerobic treatment

Anaerobic treatment methods consist of a number of processes in which microorganisms breakdown organic components of the wastewater in the absence of oxygen. The advantages of anaerobic treatment include its enhanced ability over aerobic processes to deal with high strength wastewater, low energy inputs, low sludge yield, low nutrient requirements, low operating cost, low space requirement and biogas recovery. Configurations of anaerobic reactors include upflow anaerobic reactors, anaerobic film reactors and upflow anaerobic filters (Chellipan et al., 2006; Chen, 1994; Gangagni Rao et al., 2005; Nandy, 2001; Oktem et al., 2007). Factors effecting treatment include HRT, temperature, pH, recalcitrant substances and biological community.

Anaerobes have been shown to be sensitive to certain compounds, since pharmaceuticals are designed to interfere with normal biological processes it is predicted that they would have some impact on methanogenesis or acetogenesis. Common drugs such as propranolol hydrochloride, diclofenac sodium and ofloxacin showed a reduction in methane production which correlated with the hydrophobicity of the drug. In a study diclofenac sodium and propranolol hydrochloride induced acute inhibitory effects only at high concentrations but at lower concentrations the inhibitory effect was negligible (Fountoulakis et al., 2008).

Upflow anaerobic stage reactors (UASR) have been shown to be efficient for the removal of pharmaceuticals even at high concentrations (Chellipan et al., 2006; Oktem et al., 2007) However wastewater composition and pharmaceuticals present may have a negative affect on the acetogenesis and methanogenesis (Oz, 2004; Fountoulakis, 2008). A UASR is a stage reactor in which each stage represents a separate compartment. Therefore by separating acetogenesis and methanogenesis, recalcitrant substances are in an environment more conducive to their degradation. Advantages of UASRs include no moving parts or mixing and stability to shock loading. UASRs have been suggested as a pre-treatment to activated sludge for trade effluent

(Chellipan et al., 2006). This is to reduce the API concentration prior to AS treatment and thus improve the treatability of the wastewater using AS. A UASR fed with real pharmaceutical wastewater containing the antibiotics tylosin and avilamycin showed a high degree of COD and drug removal (Chelliapan et al., 2006).

A hybrid upflow anaerobic sludge blanket reactor (UASBR) which combined a UASR and anaerobic filter technology from a chemical-synthesis based pharmaceutical factory showed significant removal at a much higher organic loading rate (OLR) (Oktem et al., 2007). A UASBR operating in thermophilic mode (55°C) showed a high COD removal (65-75%) and BOD removal (80-94%) even at high OLR of 9 kg COD/m^3/d (Sreekanth et al., 2009). Hazardous solvents, products, intermediates including phenol, 1,2-methoxy phenol, dibutyl phthalate, 1-bromo naphthalene, carbamazepine and antipyrine were present. With the exception of carbamazepine these hazardous compounds were removed. Table 2.3 shows removal of COD and APIs during anaerobic treatment of pharmaceutical wastewater.

Table 2.3: Results of anaerobic treatments.

Method	OLR (kg)	Temp. (°C)	HRT (d)	COD % removal	Specific methanogenic activity	API	API % removal	Ref.
UASR	1.86	37	4	70-75	-	Tylosin	75	Chellipan et al., 2006
HUASR*	9	55	1.7	65-75	320	Carbamazepine	Not degraded	Sreekanth et al., 2009
HUASR*	8	-	2	72	200	Bacampicillin Sultamicillin Tosylate	-	Oktem et al., 2007
CSTR*	6	35	2.5	71	166	Ampicillin, Amoxicillin Bacampicillin	-	Oz et al., 2003
Fixed film fixed bed reactor	10	35	6	76	-	Herbal	-	Nandy and Kaul, 2001
UFFR*	0.11-0.34	37	5-15	37-70	-	-	-	Moosvi and Madamwar, 2007
Fixed film reactor	10	35	1.7	60-70	-	Bulk drugs	-	Gangagni Rao et al., 2005

*(HUASR) Hybrid upflow anaerobic sludge reactor, (CSTR) Completely stirred tank reactor, (UFFR) Upflow fixed film reactor.

2.5 Physio-chemical treatment options

Physiochemical treatment options for the purpose of this review are divided into four main topics. The efficiency of these methods for the treatment of pharmaceutical wastewater varies significantly and is described below.

- membrane processes
- reverse osmosis
- activated carbon
- chlorination

2.5.1 Membrane filtration

For membrane filtration the degree of API, intermediate or raw material removal is directly related to the membrane characteristics such as molecular weight cut-off, pore size, surface charge, hydrophobicity/hydrophilicity and surface morphology. The molecular properties of the particular contaminants are also important such as molecular weight, molecular size, acid dissociation constant, hydrophobicity/hydrophilicity and the diffusion constant. The feed water composition, such as the pH, ionic strength, hardness and level of organic matter are also important in determining the efficiency of membrane filtration (Bellona et al., 2004).

Several membrane types and applications were evaluated for the removal of APIs at pilot- and/or full-scale, including microfiltration, ultrafiltration, nanofiltration, reverse osmosis, electrodialysis reversal, membrane bioreactors and combinations of membranes in series (Snyder et al., 2007). Microfiltration and ultrafiltration have little value for the removal of the vast majority of organic contaminants as pore sizes vary from 100–1000 times bigger than micropollutants so no direct physical retention takes place. When operated as MBRs they have shown some potential for removal. Retention is significantly increased above the levels of a secondary clarifier. Micro/ultra filtration had a high-energy requirement and a high cost. It was economically

viable where sensitive surface waters require advanced treatment or where space is limited (Larsen et al., 2004).

2.5.2 Reverse osmosis

Desalinisation of sea and brackish water forms the core of existing research into reverse osmosis (RO). While literature on the use of RO for pharmaceutical removal is limited, there are reports of pharmaceutical removal from municipal wastewater (Watkinson et al., 2007) and for water reuse (Drewes et al., 2003). In RO wastewater is allowed to pass through a membrane from an area of high pressure to an area of low pressure keeping the solute on the pressurised side. There is some debate as to the size exclusion range for reverse osmosis membranes. Removal is based more on the molecular length and width rather than the molecular mass (Watkinson et al., 2007).

RO in different configurations showed efficient removal of thirty-six personal care products and endocrine disrupting chemicals (Snyder et al., 2007). These findings are tabulated in Table 2.4. RO membranes removed the majority of compounds investigated to levels below the limit of detection. However, pentoxifylline, iopromide, DEET, meprobamate, TCEP, gemfibrozil, musk ketone and oxybenzone were detected in the permeate of a variety of the configurations. It was hypothesised that membrane breaching may be due to diffusion into and through the membrane. Short-circuiting of the membrane or failure of membrane support media may also have caused breaching of the membrane. A number of membranes in series may be the most successful method in the removal of trace contaminants (Snyder et al., 2007).

Table 2.4: Removal of selected pharmaceuticals following UF, MF or RO treatments.

Pharmaceutical class	Pharmaceutical	Treatment method	Influent (ng/L)	Effluent (ng/L)	% Removal	Matrix
Antibiotics	Oleandomycin	MF-RO	20	30	0	Municipal wastewater[a]
	Cefaclor	MF-RO	70	n.d.	100	Municipal wastewater[a]
	Cephalexin	MF-RO	55	n.d.	100	Municipal wastewater[a]
	Ciprofloxacin	MF-RO	110	n.d.	100	Municipal wastewater[a]
	Clindamycin	MF-RO	1	n.d.	100	Municipal wastewater[a]
	Enrofloxacin	MF-RO	40	10	75	Municipal wastewater[a]
	Erythromycin	Ultrafiltration	289	245	15	Secondary effluent[b]
	Chlortetracycline	MF-RO	10	n.d.	100	Municipal wastewater[b]
	Lincomycin	MF-RO	10	1	90	Municipal wastewater[b]
	Roxithromycin	MF-RO	140	15	89	Municipal wastewater[b]
	Salinomycin	MF-RO	5	n.d.	100	Municipal wastewater[b]
	Sulfamethoxazole	MF-RO	255	n.d.	100	Municipal wastewater[b]
		Ultrafiltration	66	63	5	Secondary effluent[a]
	Sulphasalazine	MF-RO	255	n.d.	100	Municipal wastewater[b]
	Trimethoprim	MF-RO	80	63	21	Municipal wastewater[b]
		Ultrafiltration	138	113	18	Secondary effluent[a]
	Tylosin	MF-RO	20	5	75	Municipal wastewater[a]
X-Ray contrast media	Iopromide	Ultrafiltration	75	79	0	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	265 278	<25 <25	>91 >91	Saline ground water[b]
		RO (virgin membrane) RO (fouled membrane)	165 125	<25 <72	>85 >42	Saline ground water[b]
Lipid regulators	Gemfibrozil	Ultrafiltration	82	89	0	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	230 234	<25 <25	>89 >89	Saline ground water[b]
Hormones and oral	Androstenedione	Ultrafiltration	77	22	71	Secondary effluent[b]

		RO (virgin membrane)	284	<25	>91	Saline ground water[b]
	Estradiol	Ultrafiltration	87	<1	>99	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	125 27	<25 <25	>80 >7	Saline ground water[b]
	Estrone	Ultrafiltration	78	<1	>99	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	125 51	<25 <25	>80 >51	Saline ground water[b]
	Ethinylestradiol	Ultrafiltration	98	<25	>74	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	167 83	<25 <25	>85 >70	Saline ground water[b]
	Progesterone	Ultrafiltration	64	1	98	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	285 250	<25 <25	>91 >90	Saline ground water[b]
	Testosterone	Ultrafiltration	81	23	72	Secondary effluent[b]
Antiepileptic's	Carbamazepine	Ultrafiltration	191	161	16	Secondary effluent[b]
	Dilantin	Ultrafiltration	130	98	25	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	259 239	<25 <25	>90 >90	Saline ground water[b]
Antidepressant	Fluoxetine	Ultrafiltration	45	14	69	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	263 564	<25 <25	>90 >96	Saline ground water[b]
Antiphlogistic's	Diclofenac	Ultrafiltration	38	37	3	Secondary effluent[b]
		RO (Virgin Membrane)	26	<25	>4	Saline ground water[b]
	Naproxen	Ultrafiltration	24	21	13	Secondary effluent[b]
		RO (virgin membrane) RO (fouled membrane)	118 91	<25 <25	>79 >73	Saline ground water[b]
Analgesics	Acetaminophen	Ultrafiltration	18	17	6	Secondary effluent[b]
	Hydrocodone	Ultrafiltration	105	90	14	Secondary effluent[b]

[b]Watkinson et al., 2007, [a] Snyder et al, 2007

2.5.3 Activated carbon

Activated carbon (AC) is an established conventional technology for the removal of both natural and synthetic organic contaminants (Annesini et al., 1987; Hrubec et al., 1983). AC is a form of carbon that has been processed to make it porous giving it a large surface area for adsorption. It is most commonly applied as a powdered feed or in granular form in packed bed filters. Granular activated carbon (GAC) can be used as a replacement for anthracite media in conventional filters, providing both adsorption and filtration. It can be applied following conventional AS treatment as an adsorption bed. Carbon regeneration and disposal are environmental considerations (Snyder et al., 2007).

Powdered activated carbon (PAC) and GAC at both laboratory scale and full-scale facilities showed high removal efficiencies (Snyder et al., 2007). PAC, which was tested at pilot scale, achieved greater than 90% removal for nineteen of twenty-six APIs tested including trimethoprim, carbamazepine and acetaminophen. Poorer results were seen where regular regeneration was not provided. GAC was tested at two full scale facilities. Facility one showed removal to below the limit of detection for all target pharmaceuticals. Facility two in contrast showed very limited removal. Facility one provides regular regeneration which accounts for the conflicting results. While GAC was also found to be highly effective in this study, hydrophilic contaminants have been noted to break through GAC more easily than hydrophobic contaminants. The steam-treatment of GAC was highlighted as significantly increasing its absorption capacity. The filtration step prior to the treatment of micropollutants by PAC is important (Hartig et al., 2001). The filtration step reduces the carbon demand of the wastewater due to less blocking of the micropores by high molecular weight compounds. Consequently PAC is only suitable for the treatment of pre-treated or wastewaters with a low organic loading.

2.5.4 Chlorination

Chlorination has been shown to be effective for the removal of pharmaceuticals including 17 α-ethinylestradiol and 17 β-estradiol (Alum et al., 2004) and sulfonamides (Qiang et al., 2006). Chlorine dioxide is also effective for the removal of sulfamethoxazole, roxithromycin, 17α-ethinylestradiol and diclofenac (Khetan and Collins, 2007). Chlorination and ozonation when compared for the removal of bisphenol A, 17β-estradiol, and 17α-ethinyl estradiol and by-product estrogenicity from distilled water showed comparable results with ozonation resulting in 75-99% removal (Alum et al., 2004). Residual chlorine and ozone was found to be low with >99% loss of the parent compound.

Acetaminophen, diclofenac, sulfamethoxazole and fluoroquinolone all become oxidised during chlorination. By-products of acetaminophen include the toxic by-products N-acetyl-p-benzoquinone imine and 1,4-benzoquinone. Both metoprolol and sulfamethoxazole form chloramines as one of their oxidation products (Pinkston and Sedlak, 2004). Chloramines are known carcinogens.

2.6 Oxidation reactions

The biological and physiochemical treatment methods described previously have shown limited success for the treatment of pharmaceutical wastewater. However the development of oxidation processes has shown varying higher degrees of success over the traditional treatment methods. Over the last twenty years various advanced oxidation processes have been developed (Carey, 1992). Oxidation reactions have primarily been used to supplement not replace conventional systems and to enhance the treatment of refractory organic pollutants (Balcioğlu and Ötker, 2003). This technology has been successfully applied to the treatment of pharmaceuticals (Khetan and Collins, 2007). Advanced oxidation processes (AOPs) are technologies that utilise hydroxyl and other radicals to oxidize compounds to various by-products and inert endproducts (Klavarioti et al., 2009). For AOPs to take place a chemical agent such as hydrogen peroxide, ozone, transition metals and metal oxides is required. In conjunction with this, an energy source such as ultraviolet-

visible radiation, electric current, gamma-radiation and ultrasound is necessary (Ikehata et al., 2006). The oxidation –reduction reactions are made more rapid by the addition of an energy source hence the term advanced oxidation processes. AOPs are based on the production of free radicals in particular the hydroxyl radical. AOPs frequently include ozonation coupled with hydrogen peroxide and ultra violet (UV) light. Fenton's and TiO_2 photocatalysis were also employed. Heterogeneous mixtures of ozone, hydrogen peroxide, Fenton's and titanium dioxide in light and dark have revealed a range of suitable treatment methods depending on the properties of the pharmaceutical and economic considerations (Carey, 1992; Ikehata et al., 2006).

Filtration or adsorption methods simply transfer the pollutants from the liquid to solid phase or concentrate in brine waste streams, which then required further treatment. AOPs however allow for the conversion of pollutants to less harmful and more biodegradable compounds (Ikehata et al., 2006). The ultimate aim of AOPs is the mineralisation of pollutants, with conversion to carbon dioxide, water, nitrogen and other minerals. AOPs may have changed a compound's polarity and the number of functional groups which affected the functionality of the pharmaceutical in the body. Original medicinal modes of action then ceased e.g. antibiotics which have been hydroxylated should not promote the formation of resistant strains (Ternes et al., 2003). However degradation compounds must be identified and monitored as they may be more toxic than the parent compounds (Vogna et al., 2002). Photocatalytic degradation studies using the analgesic drug, buspirone, have revealed that the intermediates produced reflected those found in biotransformation in animal models (Calza et al., 2004a). Methods that produced fewer intermediates must be developed to allow for effective modelling and application (Gaya and Abdullah, 2008).

Photoinitiated AOPs may be coupled with other biological, physical and chemical methods for mineralisation. Pretreatments such as micro or ultra filtration, reverse osmosis followed by an AOP have proved effective for the treatment of industrial wastewater (Ollis, 2003). AOPs may have enhanced

biodegradability as a pretreatment method to biological treatment (Cokgor et al., 2004; Oller et al., 2007). They may be used as a tertiary treatment, post activated sludge treatment. AOPs also handled fluctuating flow rates and compositions with less difficulty than microbes, as the same level of adaptation to the wastewater is not necessary (Ikehata et al., 2006).

Cost of both the chemical agent and the energy source has proved, in the past, a major block to implementation of AOPs on an industrial scale (Legrini et al., 1993). By using solar irradiation the capital cost of AOPs was substantially reduced (Trovo et al., 2008). Natural compounds as well as carbonate, bicarbonate and chloride ions may lead to a reduction in treatment efficiency as these compounds may act as antioxidants quenching the radical scavengers (Ikehata et al., 2006).

2.6.1 Ozonation

Ozone has been applied to the treatment of waters primarily due to its strong disinfection and sterilisation properties (Araña et al., 2002). Its application for the treatment of waters containing pharmaceutical residues is now a broad area of research (Alum et al., 2004; Andreozzi et al., 2005; Andreozzi et al., 2006; Balcioğlu and Ökter, 2003; Cokgor et al., 2004; Dantes et al., 2008; Ternes et al., 2003). Ozone may be implemented as the principle treatment method or to enhance the biodegradability and efficiency of subsequent treatment (Cokgor et al., 2004). Ozone production was an energy intensive process, making it costly to implement. An ozone treatment system may have increased the energy demand over a conventional wastewater treatment plant by 40-50% (Larsen et al., 2004).

A number of published works shows the breakdown of pharmaceuticals using ozone in water (Andreozzi et al., 2003a and b; Alum et al., 2004; Vogna et al., 2004a). A significant contribution to this work has been in the area of antibiotic removal (Andreozzi et al., 2005; Andreozzi et al., 2006; Balcioğlu and Ökter, 2003; Dantes et al., 2008; Ternes et al., 2003). A summary of results can be seen in Table 2.5. Although results in terms of degree of

removal and mineralisation of pharmaceuticals in water or synthetic industrial effluent were available, a significant gap in the literature exists regarding ozonation of pharmaceuticals in real pharmaceutical wastewater (Cokgor et al., 2004). Furthermore details of process optimisation and kinetics for the elimination of pharmaceuticals using ozone were limited (Arslan-Alaton and Caglayan, 2005).

Recent kinetics studies on pharmaceuticals including amoxicillin, lincomycin, clofibric acid, acetaminophen, bisphenol A, 17-estradiol, and 17-ethynyl estradiol showed ozone attack on aromatic rings and amino groups (Alum et al., 2004; Andreozzi et al., 2003 a and b; Andreozzi et al., 2005; Andreozzi et al., 2006; Arslan-Alaton and Caglayan, 2005). A kinetic study of the effect of ozone attack on the antibiotic amoxicillin showed direct attack on the phenolic ring leading to the formation of hydroxyl derivative intermediates, with no evidence of oxidation of the sulphur atom (Andreozzi et al., 2005). A kinetic analysis of the effect of 5-10 mg/L O_3 on four beta blockers, namely acebutolol, atenolol, metoprolol and propranolol from reverse osmosis permeate showed ozone attack on aromatic rings and amine groups (Benner et al., 2008). The reaction of the aromatic structure is independent of solution pH. However amine groups did not react directly with ozone and so the reactivity of amines strongly depended on the pK_a of the amine and the pH of the solution.

As with all oxidation processes degradation products must be analysed as they may be more toxic than the parent compound (Ikehata et al., 2006; Andreozzi et al., 2006). Microtox analysis showed a slight increase in acute toxicity in the first stage of ozonation of sulfamethoxazole (Dantes et al. 2008). It also must be considered that other compounds in the waste stream other than the target pharmaceutical may produce more harmful by-products as a consequence of the ozonation process. Brine from a RO process was an example of this. The RO concentrate contained 1200 µg/L bromide (Benner et al., 2008). Since ozonation of bromide results in the formation of bromate, a potential human carcinogen, levels of its production were monitored.

Table 2.5: Results on the treatment efficiency of ozonation for a variety of pharmaceuticals.

Pharmaceutical class	Pharmaceutical	Treatment efficiency	Matrix	Reference
Antibiotic	Clarithromycin	>76% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Erythromycin	>92% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Roxithromycin	>91% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Sulfamethoxazole	>92% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Trimethoprim	>85% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
Lipid regulator	Clofibric Acid	100% removal at 20 min O_3,	Distilled water	Andreozzi et al., 2003a
		50% removal at 5 mg/L O_3 >59% removal at 10-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Fenofibric	>62% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
Hormones and oral contraceptives	17α-ethinyl estradiol 17β-estradiol	>99% removal *Chlor/O_3	Distilled water	Alum et al., 2004
Antiepileptic	Carbamazepine	>98% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
β-blockers	Atenolol	61% removal at 5 mg/L O_3 >86% removal at 10-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Celiprolol	>82% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Metoprolol	78% removal at 5 mg/L O_3, > 93% removal at 10-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Propanol	> 72% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Sotalol	>96% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
Antiphlogistics	Diclofenac	>96% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
		100% removal after 10 min	Distilled water	Vogna et al., 2004a
	Ibuprofen	48% removal at 5 mg/L O_3 > 62% removal at 10-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Indomethacin	> 50% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
	Naproxen	> 50% removal at 5-15 mg/L O_3	Municipal wastewater	Ternes et al., 2003
Analgesic	Acetaminophen	Mineralisation of ca. 30% for O_3	Distilled water	Andreozzi et al., 2003b

2.6.2 Perozonation

Perozonation, a combination of hydrogen peroxide and ozone, has been successfully used to degrade penicillin formulation effluent (Arslan-Alaton et al., 2004; Balcioglu and Okter, 2003; Cokgor et al., 2004). The conjugate base of H_2O_2 at low concentrations increases the rate of decomposition of O_3 into hydroxyl radicals (Balcioğlu and Ökter, 2003). 30% removal of COD in penicillin formulation effluent was accomplished using ozonation alone (Arslan-Alaton et al., 2004). Removal efficiency was enhanced through the addition of H_2O_2, to a maximum of 76% in the presence of 2 mM H_2O_2. However it was found that a certain fraction of the resulting COD was non-biodegradable in the subsequent biotreatment. This inert fraction of the waste remained in the effluent. Only overall COD loading was monitored here and not actual penicillin levels or breakdown compounds. Thus, the true treatment efficiency of the method in terms of the penicillin removal is unclear. Other studies involving penicillin showed COD and aromaticity increased from 69% and 29% for ozone alone to 95% and 90% in the presence of 20 mM hydrogen peroxide (Balcioğlu and Ökter, 2003). The presence of UV increased the COD removal in penicillin formulation wastewater to almost 100%. For synthetic formulation effluent containing the antibiotics ceftriaxone and enrofloxacin only slight increases in efficiency were noted following the addition of hydrogen peroxide.

Combined UV, O_3 and H_2O_2 treatment was applied to municipal wastewater treatment plant effluent containing seventeen pharmaceuticals including antibiotics, β-blockers, antiepileptics, antiphlogistics and lipid lowering agents at a German Municipal WWTP (Ternes et al. 2003). The influent and effluent concentrations for a variety of pharmaceuticals using hydrogen peroxide are shown in Table 2.6.

Table 2.6: Removal efficiency for a variety of pharmaceuticals using hydrogen peroxide.

Pharmaceutical class	Pharmaceutical	Treatment method	Treatment efficiency	Matrix	Reference
Antibiotics	Ceftriaxone	H_2O_2/O_3	82% COD removal	Synthetic pharmaceutical wastewater	Balcioğlu and Ökter, 2003
	Enrofloxacin	H_2O_2/O_3	79% COD removal	Synthetic pharmaceutical wastewater	Balcioğlu and Ökter, 2003
	Penicillin	H_2O_2/O_3, H_2O_2/O_3/biological	COD removal: 83% H_2O_2	Antibiotic formulation effluent	Arslan-Alaton et al., 2004
		H_2O_2/O_3	71% COD removal	Synthetic pharmaceutical wastewater	Balcioğlu and Ökter, 2003
		H_2O_2/UV, Fe^{2+}/H_2O_2, Fe^{3+}/H_2O_2, Fe^{2+}/H_2O_2/UV Fe^{3+}/H_2O_2/UV	COD removal H_2O_2/UV 22% Fe^{2+}/H_2O_2/UV 56%, Fe^{3+}/H_2O_2/UV 66%, Fe^{2+}/H_2O_2 61%, Fe^{3+}/H_2O_2 46%	Antibiotic formulation effluent	Arslan-Alton and Dogruel, 2004
Lipid-regulator	Clofibric acid	H_2O_2/UV	90% Removal after 60 min with poor mineralisation	Distilled water	Andreozzi et al., 2003a
Hormones and oral contraceptives	Ethinylestradiol and estradiol	H_2O_2/UV	>95% removal UV/H_2O_2	Spiked synthetic drinking water and river water	Rosenfeldt and Linden, 2004
Antiepileptic	Carbamazepine	H_2O_2/UV	Removal after 4min TOC removal of 35%	Distilled water	Vogna et al., 2004b
Antiphlogistics	Diclofenac	H_2O_2	Removal after 100min at 50 mg/L API conc.	Distilled water	Pérez-Estrada et al., 2005b
		Fe^{3+}, H_2O_2	Removal after 60min Mineralisation: 100min of sunlight	Demineralised water	Pérez-Estrada et al., 2005a
		Fe^{3+}/H_2O_2/UV	Total mineralisation in <60min	Distilled water	Ravina et al., 2002
Analgesic	Acetaminophen	H_2O_2	Mineralisation of 40%	Distilled water	Andreozzi et al., 2003b

2.6.3 Direct photolysis

Photolysis occurs due to the direct breakdown of a compound by the absorption of light (Legrini et. al., 1993). Many pharmaceuticals are readily susceptible to photolytic transformation. APIs that do not absorb light above 290 nm are more resistant to direct photolysis (Khetan and Collins, 2007). Lamps employed in the removal of micropollutants focus mainly on low and medium pressure mercury lamps. Low pressure mercury lamps characteristically generate light at 254 nm while medium pressure lamps emit their energy at multiple wavelengths. Electrons in the outer orbital of the molecules present in the wastewater absorb photons, become unstable and may split or become reactive (Takashi et al., 2007).

Using an 110 W, 254 nm UV lamp at 313 K and 0.5 g/L, a 70% conversion of 0.25 L of 2-chloropyridine (typically found in effluent of pharmaceutical processing) was achieved in 20 minutes (Stapleton et al., 2006). Mefenamic acid was observed to undergo direct photolysis with a half-life of 33 hours under direct noon sunlight in mid-October at 45° latitude (Werner et al, 2005). Carbamazepine and clofibric acid have photodegradation half-life times of 100 days in winter at 50° N. Conversely sulfamethoxazole, diclofenac, ofloxacin and propranolol undergo faster degradation with half-lives of 2.4, 5.0, 10.6 and 16.8 days respectively.

A fundamental parameter that determines the rate of degradation for photolysis is the decadic molar adsorption coefficient which is a measure of the capacity of a compound to absorb photons. Ibuprofen, diphenhydramine, phenazone, and phenytoin have decadic molar adsorption coefficients of 256 $M^{-1}\,cm^{-1}$, 388 $M^{-1}\,cm^{-1}$, 8906 $M^{-1}\,cm^{-1}$ and 1260 $M^{-1}cm^{-1}$(Yuan et al., 2009). As indicated by the decadic molar extinction coefficients, 27.4% removal of 5µm initial concentration of ibuprofen, 26.3% of diphenhydramine, 95.8% and 87.8% degradation for phenazone and phenytoin. The experiment was carried out using 110 W low pressure lamp producing monochromatic UV light at 254 nm in a 500 mL quartz reactor. Only 6% removal of the antibiotic metronidazole with a low-pressure and 12% with a medium pressure mercury

lamp after 5 min exposure occured. Metronidazole has an absorption centred at about 310 nm since the low pressure lamp only emits light at 254 nm this reflects its low removal. UV bench scale collimated beam apparatus was used focused at a 70 x 50 mm crystallization dish (surface area 34.2 cm^2 with 100 mL of 6 µM metronidazole (Yuan et al., 2009).

2.6.4 TiO_2 photocatalysis

Photocatalysis is the acceleration of a photochemical transformation by the action of a catalyst (Chatterjee and Dasgupta, 2005; Dalrymple et al., 2007; Herrmann, 2005). A catalyst such as TiO_2 or iron(II) was required to accelerate light mediated breakdown of pharmaceuticals. Most photocatalysts are semiconductor metal oxides which characteristically possess a narrow band gap. Photons of light cause excitation of electrons in the photocatalyst. Electrons in the valence band which gain sufficient energy will change levels from the valence to the conduction band creating an electron-hole pair. The electron hole pair will migrate to the surface of the catalyst, where it either recombines or undergoes redox reactions with compounds absorbed onto the catalyst. Hydroxyl radicals are produced as a consequence of the interaction between electron holes and water or hydrogen peroxide. Superoxide radicals may also be formed as a result of the reaction between the electron hole and water. Radicals formed degrade impurities in the water relatively unselectively, reacting with impurities in the wastewater as well as the target pharmaceuticals (L'homme et al., 2008).

Since the degradation of chlorobiphenyls and biphenyls from aqueous media using TiO_2 photocatalysis was first reported (Carey et al., 1976) the literature on the removal of micro pollutants from aqueous media using TiO_2 has grown considerably (Addamo et al., 2005; Doll and Frimmel, 2005a, b and c; Pérez-Estrada et al., 2005a). Titania was the most widely investigated of the heterogeneous photocatalysts due to their cost effectiveness, inert nature and photostability (Gaya and Abdullah, 2008). Investigations into the removal of the pharmaceuticals using TiO_2, included but was not limited to work on antibiotics, lipid regulators, x-ray contrast media, antiepileptics and antiphlogistics (Addamo et al., 2005; Doll and Frimmel, 2005 a, b and c;

Perez-Estrada et al., 2005 a; Ravina at al., 2002). Results can be seen in Table 2.7. TiO_2 removal of organics as a tertiary treatment employing solar light and 2.3 g/L TiO_2, the TOC was reduced from 130 mg/L to 7 mg/L and total elimination of coliforms in less than 60 minutes (Araña et al. 2002).

TiO_2 photocatalysis with visible irradiation was effective for the detoxification of the pharmaceutical salbutamol in water (Sakkas et al., 2007). The most favourable values for drug abatement were 649 mg/L of TiO_2 at a pH of 7.4 irradiated with a 1500 W xenon lamp for 30 minutes which resulted in 93% degradation. A toxicological evaluation of the breakdown of salbutamol and its metabolites was undertaken. An initial toxicity of 4% increased to a maximum of 54% inhibition after 15 minutes. From 15 to 60 minutes of irradiation inhibition decreased to less than 1%. The major drawback of the study is that real wastewater was not employed. In real wastewater the matrix may interfere with the degradation of the salbutamol. For example, the presence of ions will reduce degradation rates as they absorb to the TiO_2 surface (Malato et al., 2002).

TiO_2 is available at a relatively modest price and would be recyclable in an industrial application when fixed on films or beads, reducing the quantities of TiO_2 required (Legrini et al., 1993). Furthermore solar studies have proved effective for a wide range of pharmaceuticals replacing the expense of generating UV light. There are difficulties in implementation on a commercial scale due to the number of operating parameters e.g. type and geometry of reactor, the photocatalyst, optimum energy use and wavelength of radiation.

Table 2.7: Treatment efficiencies for pharmaceuticals using the photocatalyst TiO_2.

Pharmaceutical class	Pharmaceutical	Treatment method	Glass	Light source	Treatment efficiency	Matrix	Reference
Antibiotics	Lincomycin	TiO_2/UV,	Pyrex	Med pressure Hg lamp	98% removal within 2h	Distilled water	Addamo et al., 2005
	Ranitidine	TiO_2/UV,	Pyrex	Med pressure Hg lamp	98% removal within 2h	Distilled water	Addamo et al., 2005
	Tetracycline	TiO_2/UV,	Quartz	Med pressure Hg lamp	98% removal within 2h	Distilled water	Addamo et al., 2005
X-ray contrast media	Iomeprol	Cross flow microfiltration TiO_2	Quartz	Low pressure Hg lamp	Degradation: Hombikat UV100: 6.9% Degussa P25 2.9%	Demineralised water	Doll and Frimmel, 2005 a
		TiO_2	Quartz	Short arc Xe lamp	Degradation: Hombikat UV100: 27.4% Degussa P25 15.6% Hombikat UV100: 93% Degussa P25 15%	Demineralised water bog-lake water (DOC: 6 mg/L)	Doll and Frimmel 2005 b
		TiO_2	Quartz	Short arc Xe lamp	Theoretically de-iodinated: Hombikat UV100: 57.7% Degussa P25 25.5%	Demineralised and bog-lake water	Doll and Frimmel, 2005 c
		TiO_2	Quartz	Short arc Xe lamp,	40 min degradation: 200 mg/L to 59 mg/L	Demineralised water	Doll and Frimmel, 2004
Antiepileptic	Carbamazepine	Cross flow microfiltration TiO_2,	Quartz	Low pressure Hg lamp	Degradation: Hombikat UV100: 7.2% Degussa P25: 3.8%	Demineralised water	Doll and Frimmel, 2005 a
Antiphlogistics	Diclofenac	TiO_2	Pyrex	Solar irradiation in CPC	100% removal 50 mg/L in 200min	Distilled water	Pérez-Estrada et al., 2005b

2.6.5 Fenton's reaction

Fenton's chemistry involves reactions of hydrogen peroxide in the presence of iron to generate hydroxyl radicals (Carey, 1992). Ultraviolet light enhances this generation by the photoreduction of Fe(III) to Fe(II). Since iron is abundant and non-toxic, Fenton's reaction is a viable option for wastewater treatment. Photo-Fenton's reaction has been used for the degradation of diclofenac (Pérez-Estrada et al., 2005b; Ravina et al., 2002). Compound parabolic collectors have also been used. Results for varying Fenton's reactions, lamps used and reactor configurations are shown in Table 2.8.

Fenton's (Fe^{2+}/ H_2O_2) and Fenton's-like (Fe^{3+}/H_2O_2) reactions were compared for both dark and photo-assisted reactions (Arslan-Alaton and Dogruel, 2004). After 40 minutes advanced oxidation by Fe^{2+}/H_2O_2 at pH3, penicillin was completely removed. Higher COD and TOC removals were obtained with dark Fenton's (Fe^{2+}/H_2O_2) at pH3 compared with dark Fenton's-like (Fe^{3+}/H_2O_2). Photo-assisted reactions using UV-C provided only slightly higher removal efficiencies. TOC removal was higher with photo-Fenton's reaction and COD removal was slightly higher with photo-Fenton's-like reactions.

Since Fenton's reactions operate at room temperature and normal pressure and highly complicated apparatus are not required, there should be a smooth transition from laboratory scale to large scale (Kavitha and Palanivelu, 2004). On the other hand, the strong dependence on the aqueous solution pH (optimum pH 2–4 for the production of $OH^{\cdot}$ radicals) and on the concentrations of hydrogen peroxide and ferric/ferrous ions and the disposal of the iron sludge are factors which need to be taken into consideration (Shemer et al., 2006). One possibility is the partial use of Fenton's reactions to produce a non-toxic and biodegradable intermediate which could then be treated in an inexpensive biological step to achieve complete mineralisation (Muñoz et al., 2006).

Table 2.8: Pharmaceutical treatment efficiencies using Fenton's reaction.

Pharmaceutical class	Pharmaceutical	Treatment method	Reactor configuration	Light source	Treatment efficiency	Matrix	Reference
Antibiotics	Penicillin	O_3/OH−, H_2O_2/UV-C, Fe^{2+}/H_2O_2, Fe^{3+}/H_2O_2, Fe^{2+}/H_2O_2/UV-C Fe^{3+}/H_2O_2/UV-C	2L capacity annular Plexiglas reactor	21W low pressure Hg lamp, quartz.	CODR: O_3/pH11 49%, H_2O_2 (30 mM)/UV/pH7 22%, Photo-Fenton's 56% Photo-Fenton's-like 66% Dark Fenton's 61%, Dark Fenton's-like 46%	Antibiotic formulation effluent	Arslan-Alton and Dogruel, 2004
Antiphlogistics	Diclofenac	Fe^{3+}, H_2O_2	CPC solar pilot plant Total volume: 35L, Illuminated volume: 22L Irradiated collector: 3.08m^2	Solar, Pyrex with 80% transmittivity between 320-400nm.	Degradation following 60min and mineralisation following 100min exposure to sunlight	Demineralised water	Pérez-Estrada et al., 2005a
		Fe^{3+}/H_2O_2/UV-C	Irradiated volume 900 mL. Total volume of the solution recycled in photo-reactor: 1400 mL.	400W low pressure Hg lamp.	Total mineralisation in <60min	Distilled water	Ravina et al., 2002
Antibiotic	Amoxicillin	FeO_x/H_2O_2/UV-A $Fe(NO_3)_3/H_2O_2$/UV-A	Upflow reactor with irradiated volume of 280 mL and a total volume of 800 mL. Recycled in reactor	15W black light fluorescent lamp	84% degradation after 1min. 62% degradation after 1min.	WWTP effluent	Trovo et al., 2008
Analgesic	Paracetamol	FeO_x/H_2O_2/UV-A $Fe(NO_3)_3/H_2O_2$/UV-A	Upflow reactor with irradiated volume of 280 mL and a total volume of 800 mL. Recycled in reactor	15W black light fluorescent lamp	98% degradation after 5min 53% degradation after 5min	WWTP effluent	Trovo et al., 2008
Anti-bacterial	Metronidazole	UV UV/H_2O_2 H_2O_2/Fe^{2+} $UV/H_2O_2/Fe^{2+}$	Bench scale UV collimated beam apparatus 70x50mm. Crystallisation dish containing 100 mL of solution with surface area of 34.2cm^2, open to the atmosphere.	Medium pressure Hg lamp 200-400nm wavelength.	12% degradation after 5min 64% degradation after 5min 76% degradation after 5min 94% degradation after 5min	Deionised water	Shemer et al., 2006
Lipid regulator	Bezafibrate	FeO_X/H_2O_2/UV-A $Fe(NO_3)_3/H_2O_2$/UV-A	Upflow reactor with irradiated volume of 280 mL and a total volume of 800 mL. Recycled in reactor	15W black light fluorescent lamp	98% degradation after 5min 89% degradation after 5min	WWTP effluent	Trovo et al., 2008

2.6.6 Photocatalytic reactors for water treatment

One of the major impediments to the commercialisation of photocatalytic water treatment was the high cost of generating artificial radiation. Therefore, solar photocatalytic reactors have received considerable interest. The design of the reactor is extremely important to ensure efficient conversion of the incident solar radiation to charge carriers (Bahnemann, 2004). There are four frequently used reactor configurations: parabolic trough reactor (PTR), thin film fixed bed reactor (TFFBR), compound parabolic collector (CPC) and double skin sheet reactor (DSSR) (Bahnemann, 2004).

PTRs concentrate sunlight into a focal line using parabolic mirrors. They have (using Degussa P25 particles (0.1%) as TiO_2 photocatalyst) reduced the concentration of trichloroethylene in contaminated water from 200 ng/L to 5 ng/L (Goswami, 1997). A TFFBR consists of a sloping plate coated with the photocatalyst and rinsed with the polluted water in a very thin film. The DSSR is a flat and transparent structured plexiglass® box. The polluted water and the photocatalyst can be pumped through channels in the box. A CPC is a combination of parabolic concentrators and flat static systems. Reactors can also be classified into concentrating and non-concentrating. Table 2.9 compares these types of reactors.

CPCs are low concentration collectors which are a good option for solar photocatalysis since they combine the better features of concentrating and non-concentrating collectors and none of the disadvantages. The photoreactor is tubular so that water can be pumped easily. CPCs use direct and diffuse solar radiation efficiently without solar tracking. The water did not heat up and there was no evaporation of volatile compounds (Malato et al., 2007).

Four different reactors – PTC, CPC, tubular collector and V shaped trough collector - were compared for their ability to degrade oxalic acid in an aqueous suspension of TiO_2 (Bandala et al, 2004). The performance of the four

detectors was similar in terms of energy accumulated however the tubular collectors produced the least degradation.

More recently, other reactors have been developed to overcome some of the problems associated with these reactors (Cernigoj et al., 2007; Danion et al., 2004; Puma and Yue, 2001; Zhang et al., 2004). TiO_2 was mostly applied in powder form and either it had to be separated at the end (which is time-consuming and costly) or it was immobilised on a rigid support as a thin film (which limited the contact between the reactants and catalyst and thus the reaction rate). A Carberry type photoreactor combines the compact and convenient parabolic collectors with glass tubes and turbulent flow with the immobilised catalyst. It was used to degrade 4-chlorophenol as a model organic compound. Its photocatalytic activity was 3.8 times higher than a configuration of two TiO_2 slides (which served as an approximation of a TFFBR) (Cernigoj et al., 2007). Other reactors are the optical fibre reactor (Danion et al., 2004), corrugated plate reactor (Zhang et al., 2004), fountain reactor with a parabolic profile (Puma and Yue, 2001), cylindrical photoreactor with TiO_2 immobilised on fibreglass cloth (Horikoshi et al., 2002), Taylor vortex reactor (Dutta and Ray, 2004), fluidised photoreactors (Lee et al., 2003), spinning disc reactor (Yatmaz et al., 2005) and labyrinth flow photoreactor with an immobilised TiO_2 bed (Mozia et al., 2005).

There has been very limited large-scale application of photocatalysis to wastewater treatment so far. One of these is located at the Plataforma Solar de Almería in Spain, a compound parabolic collector plant. However, the efficiency achieved at laboratory and pilot scale has not been achieved in these larger systems. One of the reasons for this is that small scale studies often fail to take into account the effect of other substances in the wastewaters. For example, the presence of natural organic matter and carbamazepine retards the photodegradation of clofibric acid (Doll and Frimmel, 2005b). Not all compounds were affected. In the treatment of wastewaters from a resins factory which contained phenol, phthalic acid, fumaric acid, maleic acid, glycols, xylene, toluene, methanol, butanol and phenylethylene amounting to 600 mg/L TOC, there was complete TOC

degradation within 44 minutes of 36.1 W/m^2 illumination of a dilute solution using 0.1 g/L of Degussa P25 and 7 mmol/L sodium peroxydisulfate as the additional oxidising agent (Malato et al., 1996).

CPCs have also been used to compare heterogeneous solar photocatalysis and solar photo-Fenton's reactions for the degradation of methyl-phenylglycine contaminated wastewaters (Muñoz et al, 2006). A life cycle assessment was done whereby the environmental impact was assessed by identifying and quantifying energy and materials usage and waste discharges impacts and evaluation of opportunities for environmental improvements over the whole life cycle. While both processes degraded 100% of the MPG from 500 mg/L, the environmental performance of solar photo-Fenton's coupled to biological treatment was 80-90% better than that of coupled heterogeneous photocatalysis to biological treatment. This was mainly due to the large CPC field (2150 m^2) and the electricity consumption of the TiO_2 microfiltration.

The TiO_2 band-gap only overlaps with 5% of the solar spectrum. Other catalysts may be found which correspond better and could improve the efficiency of photoreactors. Other possibilities were changing catalyst structure and composition, the addition of electron acceptors or doping and deposition with metal ions and oxides (Gryglik et al., 2004, Rios-Enriquez and Shahin, 2004).

Table 2.9: Comparison of reactor types (Bockelmann et al., 1995; Malato et al., 2007).

	Concentrating	Non-concentrating
Example	**PTR and CPC**	**TFFBR and DSSR.**
Advantages	Turbulent flow conditions which favour mass transfer and avoid catalyst sedimentation problems	Total global irradiation is usable.
	Two axis sun tracking system	High optical efficiency
	Closed reactor no vaporisation of volatile components	Low manufacturing costs.
	Smaller reactor tube area which is able to support higher pressures and a large amount of area per unit volume (Mehos and Turchi, 1992)	No additional H_2O_2 necessary since there is effective transfer of air into the water film.
		High quantum efficiency
		No heating needed
Disadvantages	Only direct irradiation can be used.	The volatile reactants can vaporise
	Low optical efficiency	The catalyst is not protected from pollution.
	Since sun-tracking is needed there are high investment costs.	A large catalyst area is needed when purifying large volumes of wastewater.
	The TiO_2 needs to be separated from the purified water.	There is low mass transfer due to the laminar flow conditions.
	Water over-heating can lead to leaks and corrosion.	Requires significantly more photoreactor area.
	Additional H_2O_2 may be needed.	

2.7 Electrochemical treatment options

Ultrasonic irradiation has been considered as a means of removing estrogenic compounds from contaminated water (Belgiorno et al., 2007; Suri et al., 2007). In ultrasonic irradiation sound waves are generated which create OH radicals as a result of the decomposition of water (Makino et al., 1982). Hormones, for example, estradiol, estrone and ethinylestradiol, were examined in single component batch and flow through reactors using 0.6, 2 and 4kW ultrasound sources (Suri et al., 2007). Results showed 80-90% reduction in the hormones within a 40-60 minute period and results are shown in Table 2.10. Further investigations in this area would be useful to determine the toxicity of breakdown products and to examine the feasibility of larger scale applications of the technology.

Electrochemistry is a method for the treatment of wastewater (Chen, 2004). The treatment of acetaminophen using anodic oxidation with a boron-doped

diamond electrode has been successful during small scale investigations (Brillas et al., 2005). This process allows complete mineralisation of the acetaminophen due to the generation of large concentrations of hydroxyl radicals by the electrode. The BDD electrode was efficient even at low concentrations. BDD has high thermal conductivity, wide band gap, high e^- and hole mobilities, high breakdown electric fields, hardness, optical transparency and chemical inertness (Chen, 2004). Future investigations are necessary for both of these technologies to determine the toxicity of breakdown products and to examine the feasibility of larger scale applications. Diamond anodes may produce $OH^{\cdot}$ radicals with high current efficiency. This was dependent on the mass transport of organic compounds to the anode not being a limiting factor (Kraft et al., 2003).

Table 2.10: Removal of a variety of hormones using ultrasound (Suri et al., 2007, matrix Milli-Q water).

Pharmaceutical class	Pharmaceutical	% removal in 60min (*35min)
Hormones and oral contraceptives	17α-dihydroequilin	99
	Equilin	64*
	17α-estradiol	98
	17β-estradiol	97
	Estrone	98
	Estriol	87
	17α-ethinylestradiol	91
	Norgestrel	95

2.8 Summary

This was a literature review of options for the treatment of pharmaceutical wastewater, focusing on the removal of pharmaceuticals. Biological, physical and chemical methods were reviewed. There was an extensive list of tables showing API reduction based on different treatment methods. The advantages and disadvantages of the technologies investigated in this review were included in Table 2.11.

Pharmaceutical production facility wastewater represented a challenge for both the management of the facility and legislators to ensure a high standard of effluent. Effluent should have low oxygen demand, low solids and should be free from other hazardous constituents such as APIs. Concerns have been

raised about ecosystems, antibiotic resistance and endocrine disrupters but the consequences of long term exposure to low concentrations of APIs were still not fully known. Where releases from pharmaceutical production facilities are regulated, little attention was paid to the API concentration of the wastewater. BOD, COD, pH and SS were normally the only wastewater characteristics that were routinely monitored. AS treatment is the most common and economical treatment method used for pharmaceutical wastewater. However, it was ineffective for the removal of a large number of APIs from wastewaters.

A significant amount of research in the area had focused on municipal wastewater, as data from municipal wastewater plants were relatively accessible. However, research into wastewaters from pharmaceutical manufacturing plants was more problematic due to difficulties in accessing information. Nevertheless, treatment technologies that work for municipal wastewaters should also be suitable with modification for industrial wastewaters.

AS was the traditional method of choice for the treatment of pharmaceutical wastewater. It has been only partially successful for the removal of the oxygen demand for the high COD wastewaters such as pharmaceutical wastewater and has proven to be only partly successful in effectively destroying pharmaceutical compounds. The efficiency of removal was usually dependent on properties of the pharmaceutical under investigation, such as polarity. AS is capable of partially removing estrogens (Joss et al, 2004) but not lipid regulators such as gemfibrozil and clofibric acid (Bernhard et al., 2006; Lacey et al., 2008; Snyder et al., 2007; Zwiener and Frimmel, 2003). Modification of AS methods may improve API removal efficiency such as operating at longer HRT or operating at low pH for the removal of acidic APIs. Shock loads disrupting the treatment efficiency of the plant were a major disadvantage of conventional AS systems. Biological treatment methods in general show considerable variation in the level of pharmaceutical degradation. For example, BFRs degraded some compounds but not clofibric acid and diclofenac (Zweiner and Frimmel, 2003). MBRs while only slightly more

efficient than AS and UASRs were efficient even at high concentrations for the removal of some APIs (Chelliapan et al., 2006). However MBRs were shown to be efficient for COD and BOD removal. Running MBRs at longer sludge ages may improve API removal.

Physical methods such as filtration, AC and RO were also discussed in this review. Micro and ultra filtration were not effective for the removal of pharmaceuticals as the pore size is 100 to 1000 times bigger than the pharmaceutical. RO has been shown to remove a number of pharmaceuticals to below the limit of detection however membrane breaching remained problematic (Synder et al., 2007). AC was an established method for the removal of micropollutants and had proven successful where regular regeneration of the carbon was provided. The main disadvantage of RO and AC methods was that the APIs was just removed from the wastewater but was not degraded. The chemical treatments of flocculation-settling/filtration did not eliminate endocrine disruptive compounds (Kulik et al., 2008) and chlorination removed some pharmaceuticals but many of the by-products were toxic (Pinkston and Sedlak, 2004).

AOPs offered a number of advantages over traditional methods. They degrade relatively unselectively contaminants in the wastewater ultimately leading to their mineralisation. They could be used as a pre-AS method to improve the biodegradable or as the main treatment method. Disadvantages included the need to monitor intermediates as they may be more toxic than the parent compound as well as the cost and removal of chemical agents. Ozonation, perozonation, direct photolysis, TiO_2 photocatalysis, solar photocatalysis, Fenton's reaction and ultrasonic irradiation have all been applied for the treatment of waters containing pharmaceuticals with varying degrees of success. Ozonation and Fenton's oxidation have in particular shown significantly enhanced removal rates for the more recalcitrant pharmaceuticals from wastewaters. Comparisons among these technologies are problematic, however, as the studies were carried out on different wastewater types. Research is required in this area to improve treatment

efficiencies, identify degradation compounds and to determine the cost and feasibility of full-scale applications.

The concentrations of APIs and other constituents of wastewaters from pharmaceutical industries represented a significant knowledge gap. The retrofitting or operational changes to existing wastewater treatment facilities, for the enhanced removal of APIs from industrial wastewaters remained an important avenue of research.

Table 2.11: Characteristics, advantages and limitations of treatment methods

Method	Characteristics	Advantages	Limitations
Biological			
AS	Aerated microbial digestion	Economical	Shock loads effect stability
	Treats bulk organic waste	Widely used	
SBR	Two or more AS tanks operated in sequence	Easy to modify process	Shock loads effect stability
BFR	Microbes fix to support material	Stable operation Low downtime	Blockage due to build-up of cells
MBR	Combine AS plant with membrane technology	Suited to limited space	Contaminants concentrate in brine
UASBR	Anaerobic stage reactor with separate acetogenesis and methanogenesis stages	Less sensitive to operation variables and shock loads No moving parts / mixing	
Physical			
Microfiltration/ Nanofiltration	Filtration	High quality effluent	Unsuccessful for removal of most APIs
		Suitable for limited space	Expensive/ energy intensive
RO	Membrane technology using pressure to force a liquid through the membrane	Multibarrier approach provides high quality effluent.	Membrane breeching, only retains contaminant
AC	Adsorbs and filters contaminants applied as powdered or granular feed	GAC: is easily recovered, steam treatment enhances adsorption	PAC: fed continually, only suitable for relatively clean waters; GAC requires regular regeneration
Chemical			
Chlorination	Addition of chlorine, commonly used to disinfect drinking water	Strong disinfection properties	Produces disinfection by-products that have been linked with cancer
Flocculation	Coagulating and flocculating agent is added to cause contaminants to settle	May remove small sized contaminants e.g. hormones	Expensive
O_3	Oxidation process, addition of O_3	Allows mineralisation of APIs	Energy intensive/ expensive
	Used as an alternative to chlorination	Strong disinfection properties, clean	
Perozonation	Oxidation process	More effective than ozone alone	Energy intensive/ expensive
	Mix of O_3 and H_2O_2	Allows for mineralisation of APIs	
Direct Photolysis	Direct breakdown of a compound by the absorption of light	Inexpensive; allows for mineralisation of APIs	Dependent on geographical region for direct sunlight
TiO_2	Creation of hydroxyl and super oxide radicals which react with impurities in the wastewater	Fixed to beads/film for removal/ recycling; mineralisation of APIs	Energy intensive/ expensive
Fenton's	Produce $OH^{\cdot}$ which react with APIs, coupling with UV enhances generation	Allows for mineralisation of APIs; Iron is abundant and non-toxic	Disposal of iron sludge; energy intensive/expensive

3. Materials and methods

3.1 Analytical method development and validation for the detection of three APIs in industrial wastewater

3.1.1 Chemicals and materials

Famotidine, tamsulosin hydrochloride and solifenacin succinate were donated by Astellas Ireland Pharmaceutical Limited. Water (mobile phase), acetonitrile and methanol were LC-MS grade and purchased from Fisher Scientific Ltd. Acetonitrile (0.1% ammonium acetate) and water (0.1% ammonium acetate) were LC-MS grade and purchased from Sigma-Aldrich Chemical Company. Dichlorodimethylsilane and toluene were reagent grade and purchased from Sigma-Aldrich Chemical Company. Formic acid (98% purity) and 25% ammonium hydroxide solution were analytical grade and purchased from Fluka. Two reverse phase Luna-PFP columns (column dimensions 150x4.6 mm and 5µm particle size, 150x2.0 mm and 5 µm particle size) and SPE (3 mL/200 mg) cartridges were purchased from Phenomenex. HPLC vials (APEX Scientific) and centrifuge vials (Fisher Scientific) were amber glass and silanised to prevent API degradation by light or adsorption onto glass. All solvents used in analysis were filtered through Pall nylon filters (0.2 µm pore size, 47 mm diameter) and degassed by sonication for 30 minutes prior to use. Whatman no. 3 glass fibre filters were used for sample filtration.

3.1.2 Glassware preparation

All glassware used was silanised by rinsing thoroughly with a 10% (v/v) solution of dichlorodimethylsilane in toluene followed by two toluene rinses and then two methanol rinses. This was to prevent any API adsorbing to the glassware (Lacey et al., 2008).

3.1.3 Sample preparation

Standards were prepared by dissolving 0.1 g of each API in 100 mL of methanol resulting in a 1 mg/mL stock standard and stored for up to 1 month at 4°C. A 100 µg/mL mixed working solution was prepared in mobile phase A. The working solution was used to prepare the standards for the SPE-HPLC

method. For the SPE-LC-MS/MS method stock standards were diluted to appropriate working solutions to the range of standards under investigation.

3.1.4 Sample collection

2 L wastewater samples were collected in plastic sampling bottles at the Astellas wastewater treatment facility at Mulhuddart, Co. Dublin and transferred on return to laboratory to silanised amber glass Winchester bottles. Samples used in method development and validation were collected on several dates between June and October 2009. Samples collected were typically between pH 7 and 9 and were low in solids. Samples were filtered through Whatman glassfibre filters to remove any particulates and adjusted to pH 3. Two 2 L weekly influent and effluent samples were collected between November 2009 and April 2010 to fulfil the requirements of the monitoring programme.

3.1.5 Method development

A primary aim of this project was to develop a combined analytical method for the monitoring of low levels of the three APIs in Astellas pharmaceutical wastewater. The method consisted of a concentration, chromatographic separation and identification steps. The method development process required the separate optimisation of the three analytical processes involved, namely solid phase extraction, high pressure liquid chromatography and mass spectrometry. Each of the three streams were optimised separately and combined. An SPE-HPLC method was validated using ultra-pure water but was deemed unsuitable for the monitoring of the APIs without the specificity of the mass spectrometry step. Validation of the SPE-LC-MS/MS method was conducted to confirm suitability of the method in real wastewater as described in section 3.1.6.2. A six month sampling programme was conducted to determine both the efficiency of the treatment plant and the concentrations of pharmaceuticals released by the production facility to the municipal sewer.

3.1.5.1 SPE method development

3.1.5.1.1 SPE cartridge selection

Four SPE cartridges, Strata-X, Strata-X-C, Strata-X-CW and Strata-X-AW were investigated to determine the optimum cartridge for the recovery of the three APIs. The methods used for the extraction of the three APIs from ultra-pure water are shown in Table 3.1. A 12 position vacuum manifold purchased from phenomenex was used in all SPE experiments. 100 mL of ultra-pure water was spiked with 5 mg/L of each API. Pre and post-extraction spiked samples were compared to determine the percentage recovery. The optimum cartridge determined by this experiment underwent validation in the combined SPE-HPLC and SPE-LC-MS/MS method.

Table 3.1: Individual methods used for determining the optimum SPE cartridge for the concentration of the three APIs adapted from Phenomenex Strata products general guidelines.

	Strata-X	**Strata-X-C**	**Strata-X-CW**	**Strata-X-AW**
1.Condition	Methanol	Methanol	Methanol	Methanol
2.Equilibrate	Water	Water	Water	Water
3.Load	pH 3 adjusted	pH 3 adjusted	pH 4.5 adjusted	pH 3 adjusted
4.Wash 1	5% methanol	0.1% phosphoric acid	25 mM ammonium acetate	Water
5.Dry	3 min	3 min	-	-
6.Wash 2	-	Methanol	Methanol	Methanol
7.Dry	-	-	3 min	-
8.Elute	50:50 methanol: acetonitrile	5% ammonium hydroxide in methanol	2% formic acid in 20:80 methanol: acetonitrile	2% ammonium hydroxide in methanol

3.1.5.1.2 Validated SPE method

The Strata-X-C cartridge was used in the validated method. The steps in the method are shown in Table 3.2. The SPE method is the manufacturers

recommended method (Technical data sheet, 2008) and it produced effective results. Therefore no further optimisation was deemed necessary.

Table 3.2: SPE method used including volumes and the purpose of the steps.

Step	Description	Volume	Purpose
1	Sample pre-treatment: Filtered and pH 3 adjusted	2 L	Remove particulates and acidify
2	Condition: methanol	6 mL	To wet the cartridge
3	Equilibrate: water	6 mL	Wash away methanol prepare for sample loading
4	Sample loading	25 mL	Load sample
5	Wash 1: 0.1% phosphoric acid	6 mL	Remove acidic/neutral interferences
	Dry under vacuum	3 min	Prepare for methanol wash
	Wash 2: methanol	6 mL	Remove hydrophobic interferences
6	Elute: 5% ammonium hydroxide solution	6 mL	Basic solution to release analytes

3.1.5.2 HPLC method development

The primary aim of the HPLC method development was to develop a single optimised method that was suitable for transfer to the mass spectrometer for the three pharmaceuticals produced by Astellas Ireland Pharmaceutical Limited at their chemical synthesis plant in Mulhuddart, Co. Dublin. Wavelength, column, mobile phase, gradient and injection volume were the parameters to be optimised. The HPLC system used in the method development process was an Agilent 1100 Series high-performance LC (HPLC) system with a fixed wavelength UV detector.

3.1.5.2.1 Wavelength optimisation

Optimum wavelength was determined for famotidine, tamsulosin hydrochloride and solifenacin succinate by UV scanning from 190 to 400 nm on a Beckman DU 520 general purpose UV/VIS spectrophotometer with a path length of 1 cm. APIs were dissolved in HPLC grade water at a concentration of 10 mg/L. A quartz cuvette purchased from Fisher Scientific

Ltd. was used. 205 nm was optimum for famotidine and tamsulosin while solifenacin had an optimum wavelength of 215 nm.

3.1.5.2.2 Stationary phase optimisation

Famotidine has proven difficult to retain under reverse phase conditions on a C-18 column due to its polar nature (Zhong et al., 2001). Therefore the PFP column which has enhanced mechanisms of retention over a C-18 column was adopted. In contrast tamsulosin hydrochloride and solifenacin succinate are non-polar.

3.1.5.2.3 Mobile phase optimisation

Initial method development used acetonitrile and water with a 0.1% ammonium acetate buffer. Three single isocratic methods for each of the three APIs were first developed, followed by the development of a single gradient method. However, while good response and peak shape were attained using this mobile phase, the acetonitrile was expensive and problems with security of supply arose. It was therefore decided to move to a methanol based mobile phase.

The relationship between pH and pK_a is important in relation to mobile phase and sample pH in HPLC. To ensure good peak shape all analyte molecules should be protonated or deprotonated. If the analyte molecules are present in more than one form this can result in peak broadening. A low pH (at least 2 pH units below the pK_a of the analyte) ensured that all molecules will be in the protonated form (McMaster, 2007). The pH which is a log measurement of the number of hydrogen ions present in a solution was adjusted to pH 3.

$$pH = -\log[H+]$$

The pk_a (acid dissociation constant) of each of the three APIs are 6.8, 8.4 and 8.5 for famotidine, tamsulosin and solifenacin respectively (Degim et al., 2001; Maniscalco et al., 2006). Defined as the negative log of the dissociation constant, pK_a, is a measure of strength of an acid or base.

$pK_a = -\log(K_a)$

It determines if a molecule or atom group in a molecule will be protonated or deprotonated in solution at a certain pH using the Henderson–Hasselbalch equation.

$pH = pKa + \log([A^-]/[HA])$

Mobile phase was adjusted using formic acid to pH 3. Peak shape was good so no buffer was used.

3.1.5.3 Mass spectrometry method development

A Brüker Daltonics Esquire-LC ion trap mass spectrometer with an electrospray ionisation interface at atmospheric pressure was used for analysis. 1 mg/L standards of each of the three analytes were directly infused into the mass spectrometer to determine molecular and fragment ions for the three analytes in positive and negative mode. Formic acid in positive mode and ammonium hydroxide in negative mode were added to determine optimum sample composition. The electrospray ionisation conditions including capillary, end plate, skims, capillary exit offsets, octopoles, trap drive, lenses and fragmentation amplitudes were individually optimised using software on the mass spectrometer. Nebuliser conditions including dry gas pressure, dry gas flow and temperature were optimised manually. Following optimisation of mass spectrometry conditions LC gradient conditions were reoptimised using a low flowrate of 0.3 mL/min and narrow bore LC column (150x2.0 mm, 5 µm pore size).

3.1.6 Method validation

3.1.6.1 SPE-HPLC validation

Validation of the SPE-HPLC method for water sample analysis was completed in ultra-pure water. Accuracy, precision, linearity, limit of detection, limit of quantitation and selectivity were examined. SPE recovery was determined by spiking ultra-pure water with mixed standard and extracting with Strata-X-C cartridges in triplicate. The detected concentrations were compared with standard solutions. Standard ranges are shown in Table 3.3.

Table 3.3: Standard ranges used for SPE-HPLC validation.

API	Standard range (µg/L)
Famotidine	5.0-60
Tamsulosin hydrochloride	2.5-60
Solifenacin succinate	10-60

3.1.6.2 SPE-LC-MS/MS validation

Influent and effluent samples were used in the validation of the SPE-LC-MS/MS method. Precision, intermediate precision, linearity, LOD, LOQ and % ion suppression were examined. SPE recovery was determined by spiking influent and effluent with mixed standard and extracting with Strata-X-C cartridges in triplicate. Pre-extraction standard addition was compared with post-extraction standard addition to real wastewater samples. Standard addition was used for the quantitation of the level of the APIs in the wastewater as it accounts for signal suppression or enhancement caused by the matrix. Standard ranges used are shown in Table 3.4.

Table 3.4: Standard ranges used in standard addition for SPE-LC-MS/MS validation.

API	Influent (µg/L)	Effluent (µg/L)
Famotidine	400-5000	400-5000
Tamsulosin hydrochloride	1-15	0.8-18
Solifenacin succinate	6-50	6-16

3.1.7 Validated method conditions

3.1.7.1 Validated SPE-HPLC conditions

The solid phase extraction procedure is outlined in Table 3.2. Each step used two cartridge volumes (6 mL) of solution. Different loading volumes and reconstitution volumes were used for the SPE-HPLC and SPE-LC-MS/MS methods. For the SPE-HPLC method the loading volumes used was 100 mL and reconstituted in 500 µL. Following elution and collection of samples in 20 mL vials the sample was dried on a Genovac MiVac centrifuge dryer and reconstituted in mobile phase A for analysis.

Gradient conditions and wavelength are shown in Tables 3.5. The HPLC column used was a Luna-PFP reverse phase column (dimension 150x4.6 mm and 5 µm particle size). Analysis was carried out at ambient temperature. Mobile phase A consisted of 10:90 (v/v) methanol: water with 0.1% formic acid and mobile phase B was 90:10 (v/v) methanol: water with 0.1% formic acid. The injection volume used was 50 µL and flowrate was 1 mL/min.

3.1.7.2 Validated SPE-LC-MS/MS conditions

The SPE method used was as described above however the sample loading volume used was 25 mL and reconstituted in 2.5 mL. The HPLC system consisted of an Agilent 1100 Series high-performance LC (HPLC) system with a diode array detector (D.A.D.). A Brüker Daltonics Esquire~LC ion trap MS with an electrospray ionisation interface at atmospheric pressure was used for mass spectral analysis. The HPLC column used was a Luna-PFP reverse phase column (dimension 150x2.0 mm and 5 µm particle size). Analysis was carried out at ambient temperature. Mobile phase A consisted of 10:90(v/v) methanol: water with 0.1% formic acid and mobile phase B was 90:10(v/v) methanol: water with 0.1% formic acid. The injection volume used was 20 µL and flowrate was 0.3 mL/min. Gradient conditions are shown in Table 3.5. ESI and nebuliser conditions are shown in Table 3.6. Wavelengths monitored by D.A.D. analyser were 205 nm, 210 nm, 215 nm, 254 nm and 270 nm.

Table 3.5: Validated gradient and wavelength conditions.

Gradient					Wavelength switching	
	SPE-HPLC		SPE-LC-MS/MS			
Time	Mobile phase		Mobile phase		Time	Wavelength
(min)	A (%)	B (%)	A (%)	B (%)	(min)	(nm)
0	90	10	95	5	0	205
3	90	10	95	5	5.5	254
4	55	45	45	55	8.5	205
15	55	45	45	55	16.0	254
16	40	60	25	75	20.5	215

Table 3.6: SPE-LC-MS/MS validated method optimised ESI and nebuliser conditions.

Parameter	Optimised condition
Dry gas pressure	50 PSI
Dry gas flow	8 L/min
Temperature	325 °C
Capillary	4500 V
End plate	913.11 V
Skim 1	30.3 V
Cap exit offset	66.39 V
Octopole	3.95 V
Octopole delta	1.53 V
Trap drive	52.95
Skim 2	9.84 V
Oct. R.F	226.23 Vpp
Lens 1	-2.48
Lens 2	-57.54
Frag. amp.	1.73 V

3.1.8 COD analysis

COD measurement was conducted using a standard HACH method and the results were provided by Astellas. No correlation was observed between COD and analyte concentration for any of the three APIs.

3.2 Photo-Fenton's optimisation for the removal of APIs from water

3.2.1 Chemicals and materials

Famotidine, tamsulosin hydrochloride and solifenacin succinate were donated by Astellas Ireland Pharmaceutical Limited. Reaction solutions were prepared with water obtained from a Millipore Milli-Q system. Water (mobile phase), acetonitrile and methanol were LC-MS grade and were purchased from Fisher Scientific Ltd. Fe(II) sulfate heptahydrate (99.5% purity) and formic acid (98% purity) were analytical grade and purchased from Fluka. Hydrogen peroxide (30% purity) was purchased from Sigma-Aldrich. Nylon syringe filters (2 µm) and two reverse phase Luna-PFP column dimensions (150x4.6 mm and 5 µm particle size, 150x2.0 mm and 5 µm particle size) were purchased from Phenomenex. HPLC vials (APEX Scientific) were amber glass and silanised to prevent degradation by light or adsorption onto glass. All solvents used in HPLC analysis were filtered through Pall nylon filters (0.2 µm pore size, 47 mm diameter) and degassed by sonication for 30 minutes prior to use.

3.2.2 Reactor configuration

The reactor configuration used in all experiments is shown in Figure 3.1. The light source consisted of a 400 W Defender portable halogen work light with a 400 W halogen bulb purchased from B&Q Ireland Ltd (R7 230-240V). The reactor consisted of a borosilicate glass immersion well (model 7857), 290 mm in length and 45 mm internal diameter. The reaction solution was maintained at 21 ˚C.

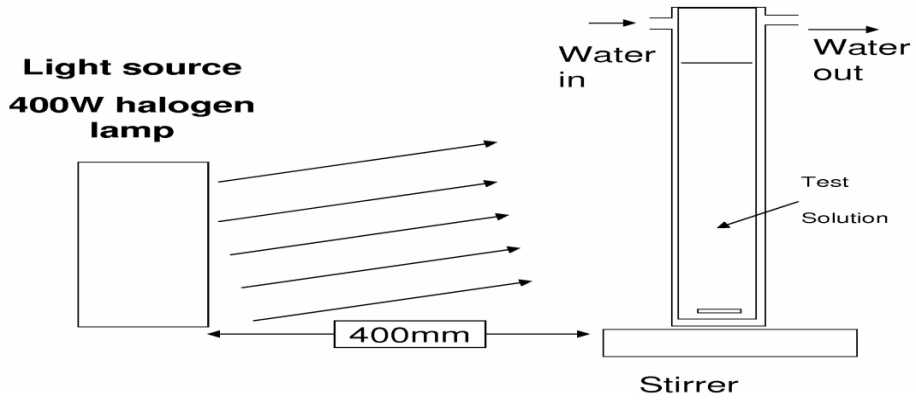


Figure 3.1: Reaction set-up for photo-Fenton's experiments.

3.2.3 Experimental procedures

The halogen lamp was turned on 30 minutes before the beginning of the experiment to ensure steady output of light. 100 mL solutions of Fe(II) and API were made up separately and mixed immediately before the experiment began to avoid any complexing of the API and Fe(II) (Méndez-Arriaga et al., 2010). The reaction solution (200 mL) was adjusted to pH3 using 5M HCL solution. The 0 min sample was taken prior to the addition of hydrogen peroxide. The solution was stirred throughout the 2 hour experiment and the temperature maintained at 21 °C. Samples were taken at 0, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100 and 120 minutes. All samples taken were filtered through 0.2 µm nylon syringe filters and analysed by HPLC. Methanol and sodium sulphite were investigated as possible quenching agents. No suitable quenching agent was identified, therefore samples were not quenched. As a result the reaction time was extended to include the time up to the HPLC analysis. This was only applied for famotidine, degradation between the time of sampling and analysis was not noted with tamsulosin and solifenacin. HPLC runs were started 35 min into the experiment. HPLC methods were all isocratic with 6 min runtime, 50 µL injection volume, 1 mL/min flowrate and PFP column. All mobile phases were methanol: water with 0.1% formic acid. Percentage organic was 15% for famotidine, 46% for tamsulosin and 70% for solifenacin.

3.2.4 Optimisation of famotidine removal by photo-Fenton's oxidation

0.012 mM iron(II) and 20 mM H_2O_2 were the concentrations used in initial experiments to determine the effect of different reactants on the degradation of famotidine at 0.1 mM. Light, H_2O_2/light, H_2O_2, Fe(II)/light, Fe(II) and dark Fenton's processes were compared. Concentration ranges used were based on those previously reported (Mèndez-Arriaga et al., 2010). Photo-Fenton's process was optimised by first increasing Fe(II) concentrations between (0.012-0.120 mM Fe(II)) then decreasing H_2O_2 concentrations (5-20 mM H_2O_2) which were in excess. Fe(II) concentrations were further increased to maintain optimum degradation (0.120-0.180 mM Fe(II)). Experimental results

were fitted to both first and second order rate equations. Good straight line fits were obtained using only first order rate equations.

3.2.5 Optimisation of tamsulosin hydrochloride and solifenacin succinate removal by photo-Fenton's oxidation

Optimisation of tamsulosin hydrochloride and solifenacin succinate conditions began at the optimum reactant concentrations for the removal of famotidine at 0.12 mM Fe(II), 5 mM H_2O_2. Complete removal was noted after the first sample was taken. Fe(II) concentrations between 0.03-0.09 mM Fe(II) were investigated for both analytes. Experimental results were analysed kinetically using first order rate equations. The effect of the different reactants at optimised conditions was also investigated. Light, H_2O_2/light, H_2O_2, Fe(II)/light, Fe(II) and dark Fenton's processes were compared.

3.2.6 Determination of intermediates by LC-MS

The reaction solution (200 mL) was adjusted to pH 3 using 5 M HCL solution. The 0 min sample was taken prior to the addition of hydrogen peroxide. The solution was stirred throughout the 7 hour experiment and the temperature maintained at 21°C. Samples were taken at 0, 15, 30, 45, 60, 90, 120, 240, 300, 315, 330, 345, 360, 390 and 420 minutes. After 300 minutes there was a further addition of H_2O_2. All samples taken were filtered through 0.2 µm nylon syringe filters and analysed immediately by LC-MS. LC-MS methods were all isocratic with 10 min runtime, 20 µL injection volume, 0.3 mL/min and PFP column. All mobile phases were methanol: water with 0.1% formic acid. Percentage organic was 15% for famotidine, 46% tamsulosin and 70% solifenacin. Optimum nebuliser and ESI conditions for individual APIs were used as described in section 4.1.4. The mass spectrometer had a lower mass to charge cut-off limit of 80m/z.

4. Results and discussion

4.1 Analytical method development

Identifying the requirements of the analytical method was the first step in its development (outlined in section 4.1.1). The subsequent development process had three separate streams: the development of the SPE method (concentration step), HPLC method (separation step) and MS method (identification step). Following separate optimisation of the three streams the methods were amalgamated and final optimisation completed (Figure 4.1). The method was then validated in influent and effluent samples and applied to a six month sampling programme. The results and discussion of the development process of the SPE-LC-MS/MS method are set out and discussed in the following sections.

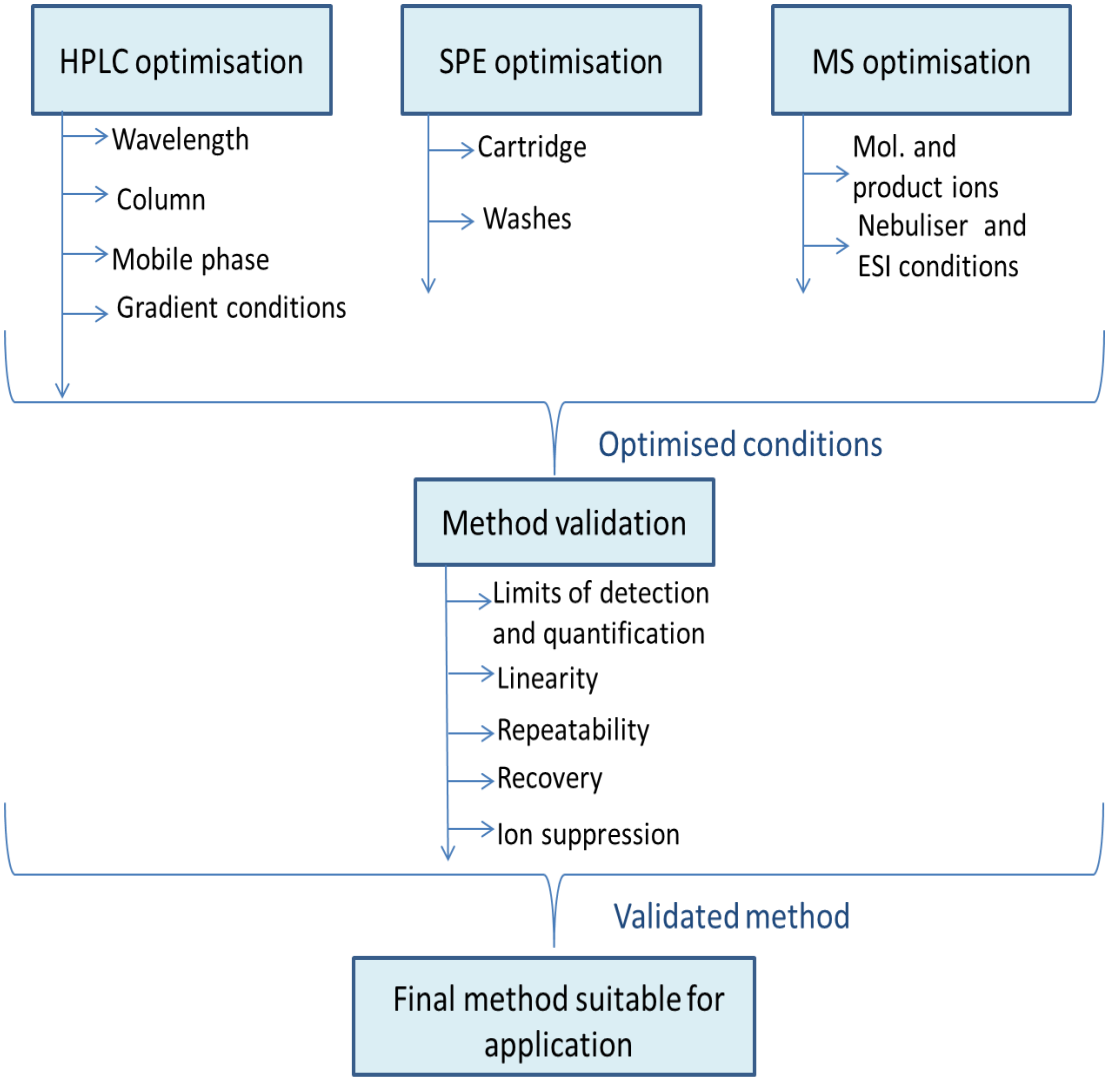


Figure 4.1: Method development process.

4.1.1 Method requirements, considerations and applications

1. One method was required for efficient and routine analysis of all three APIs in production at Astellas Ireland Pharmaceutical Limited manufacturing facility at Mullhuddart, Co. Dublin. While individual methods for the three pharmaceuticals are available (Table 4.1), no method combining any of the three APIs is available in the literature.

2. The method must be simple, rapid, reproducible and inexpensive for routine analysis.

3. Solvents, mobile phase additives and columns used in SPE and HPLC must be transferable to LC-MS should the MS be needed to enhance selectivity and sensitivity in real wastewater analysis.

4. The method must be validated to ensure the quality of the results.

Table 4.1: Available methods for the analysis of the three APIs under investigation showing type of LC, MP compositions, RT and the method of detection used.

API	Type of LC	Column	MP	RT	Detection	Reference
Famotidine	Ion exchange	Cation Exchange	Isocratic 20% acetonitrile: 80 % sodium acetate buffer (0.1M) with glacial acetic acid (0.1M pH5)	5.1min	UV, 230nm	Ashiru et al., 2007
Famotidine	UPLC	C-18	Gradient (5-90% B) A) 5 mM NH_4Ac/acetic acid (pH 4.8) (B) 2:1 acetonitrile:methanol	1.9min	+ve mode MS 338>>259,189m/z	Petrovic et al., 2006
Famotidine	Reverse phase	C-18	Gradient (5-100% A) A) acetonitrile B) water 0.1% formic acid	Not cited	+ve mode MS 338>>259,189m/z	Jelić et al., 2009
Tamsulosin	Reverse phase	C-8	Gradient A) water (0.1%) formic acid B) water: acetonitrile: formic acid (50:50:0.1%).	4.2min	+ve mode MS 409>>271,228m/z	Keski-Rahkonen et al., 2007
Solifenacin	Reverse phase	C-8	Isocratic 2 mM ammonium formate (pH 3.0, with formic acid) in water: acetonitrile (15:85, v/v).	1.8min	+ve mode MS 363.3 >> 110.2m/z	Mistri et al., 2008

4.1.2 Plant description

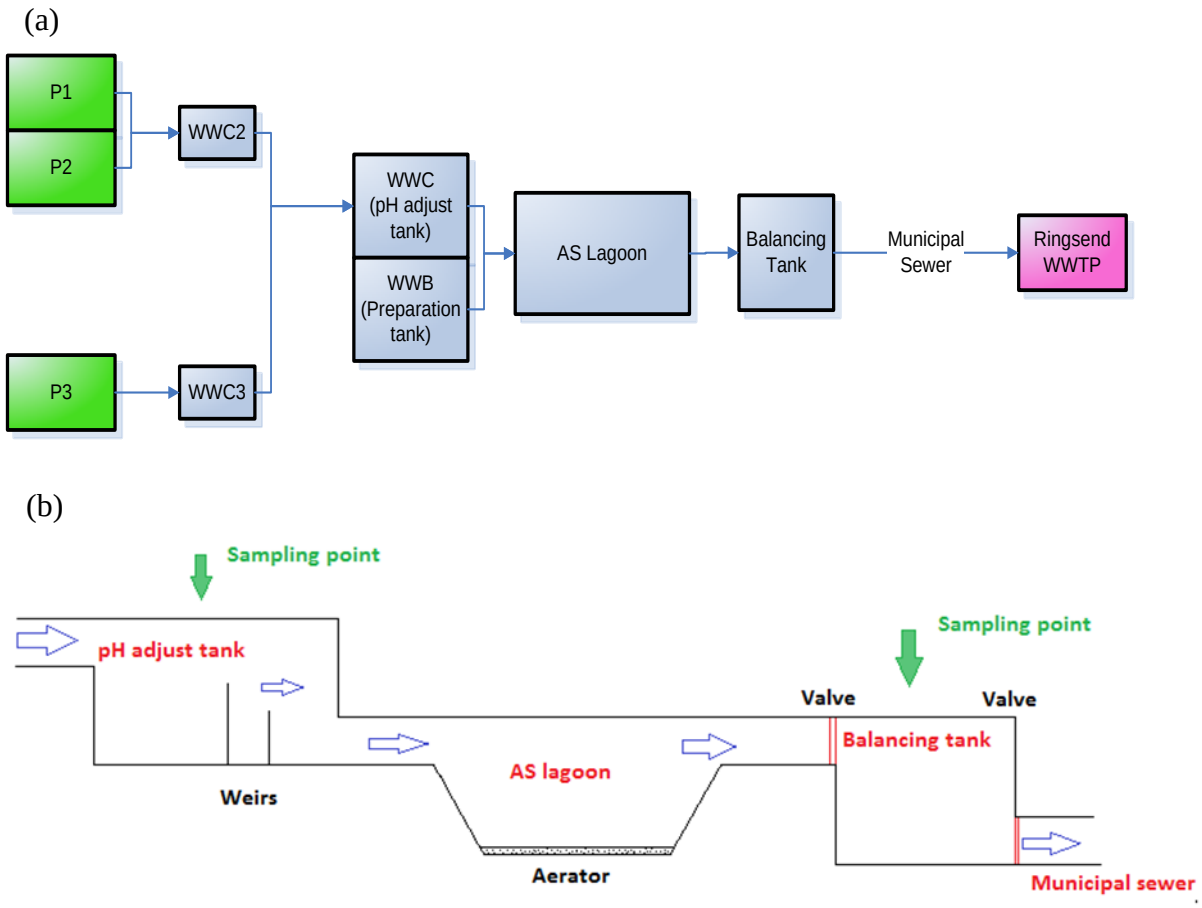


Figure 4.2: Wastewater flows (a) from production through wastewater system and (b) through wastewater treatment plant showing sampling points

A description of flows of wastewater from production to municipal wastewater treatment is shown in Figure 4.2. In process plants P1 and P2 famotidine and solifenacin succinate are produced. These two processes are produced by two independent plants housed in the one building which enter one local wastewater pit (WWC2). Tamsulosin hydrochloride is produced in a smaller multipurpose production facility P3 for the manufacture of low dosage high strength pharmaceutical materials. The wastewater from this process enters a second local wastewater pit (WWC3). Wastewaters from all three production facilities as well as from the incinerator enter the wastewater treatment facility through the pH adjust tank. In the pH adjust tank the wastewater is adjusted to between pH 6 and 9 by the addition of sulphuric acid or caustic soda. The water trickles through a series of weirs from the pH adjust tank to activated

sludge lagoons. A second feed is added at night through WWB. This contains nutrients to feed the microbial populations in the lagoons. Water enters from the pH adjust tank on a continual basis. Following activated sludge treatment in the lagoons water is discharged to a balancing tank, which is released each afternoon to the municipal sewer, which ultimately brings the wastewater to Ringsend WWTP in Co. Dublin for final municipal treatment. Monitoring of wastewater quality including COD and microbial analysis is carried out onsite. This ensures the wastewater treatment plant is operating to specifications and a healthy mixed consortium of microorganisms is present.

The two sampling points for this project are at the pH adjust (influent to wastewater treatment) and balancing tank (effluent from wastewater treatment). Samples were collected in plastic bottles onsite in accordance with the plants safety regulations and transferred on return to laboratory silanised amber glass Winchester bottles. The length of time samples were left in plastic bottles was kept at a minimum to avoid adherence of the APIs to the bottle wall.

4.1.2 Sample pre-treatment

Solid phase extraction is used for both sample clean-up and concentration of samples. Due to a gap in the literature in relation to actual concentrations of pharmaceuticals in industry wastewater, the target concentration ranges were largely unknown. However, from environmental levels and levels measured at sewage treatment facilities, it was thought that the likely concentration would be in the µg/L or ng/L range (Gomez et al., 2010; Lopez-Rolden et al., 2010). Furthermore, the reaction vessels are generally first solvent-washed then water washed to remove the solvent. The water washes form the bulk of the liquid entering the wastewater treatment plant. It was therefore expected that levels of analyte in the wastewater would be low and so it was anticipated that a sample concentration step would be necessary.

Solid phase extraction and liquid-liquid extraction are the main processes used in the extraction and clean-up of samples. While liquid-liquid extraction

is the more traditional method, solid phase extraction was chosen as the method of choice for the following reasons:

- No emulsions are formed
- Better and more reproducible recoveries
- Cleaner extracts are achievable
- Interferences and matrix components can be removed more selectively
- Lower quantities of solvents are required
- Lower volumes of waste solvents are produced (Simpson, 2000)

Using a sorbent based on polarity was unlikely to be suitable as famotidine is polar while tamsulosin hydrochloride and solifenacin succinate are non-polar molecules. All three drugs are mildly basic therefore a strong cation exchange resin is theoretically the most suitable choice for the extraction of all three analytes from a single mixture. The decision pathway for selecting the cation exchange cartridge is highlighted in Figure 4.3.

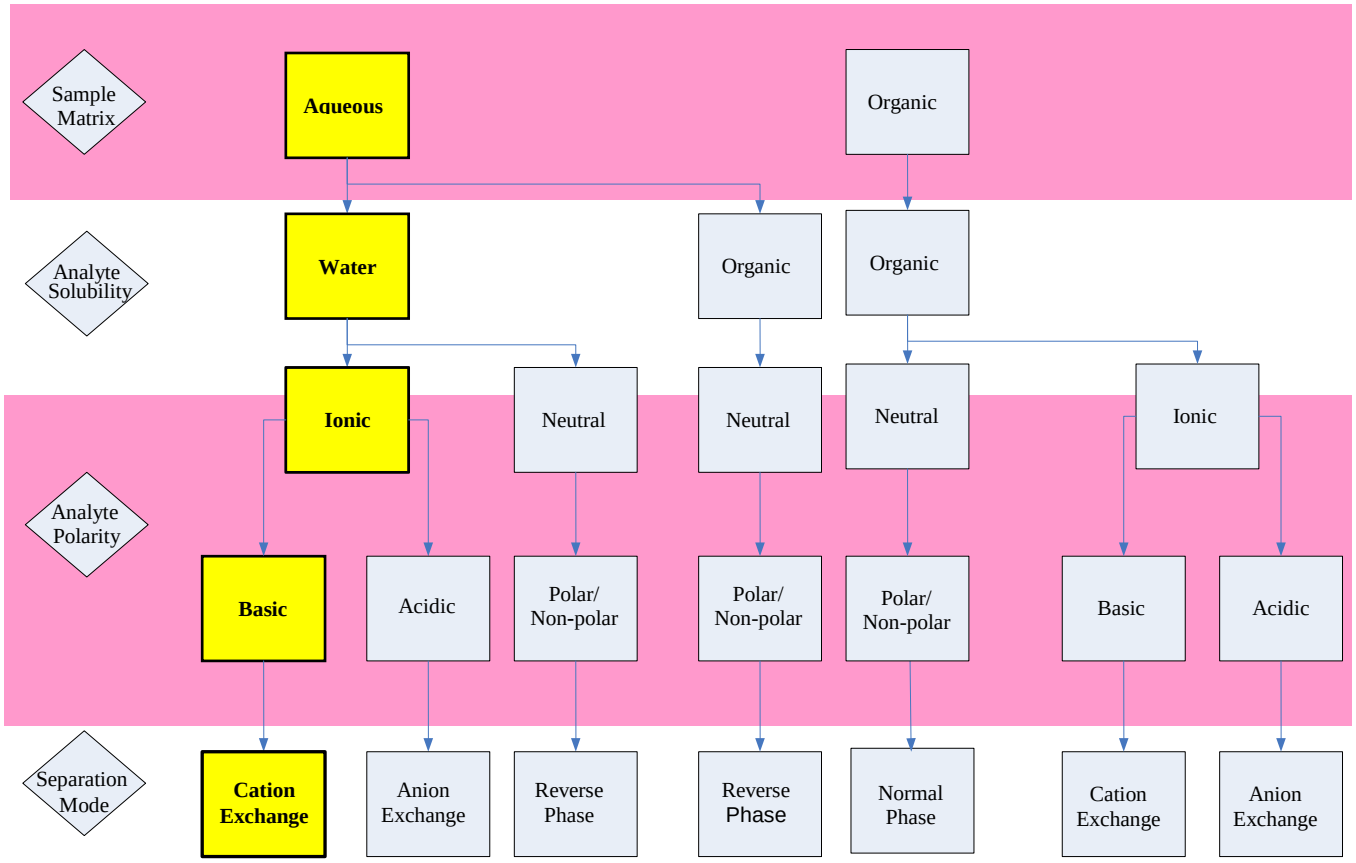


Figure 4.3: SPE selection chart modified from Phenomenex, Inc., UK.

To confirm this experimentally four different cartridges were investigated, based both on polarity and ion exchange to determine the optimum cartridge for the extraction of all three analytes from a single mixture. Strata-X (polar non-polar interactions), Strata-X-C (mixed mode, strong–cation exchange resin), Strata-X-CW (mixed mode, weak–cation exchange resin) and Strata-X-AW (a mixed mode, weak anion exchange) cartridges were investigated using the method recommended by the manufacturer (described in section 3.1.5.1). Famotidine was poorly recovered from all cartridges investigated with the exception of the Strata-X-C (Figure 4.4). Tamsulosin hydrochloride was recovered well on each cartridge except the anion exchange resin (X-AW). Strata-X showed the best retention of the highly non-polar, solifenacin followed closely by the X-C and X-AW, while the X-CW cartridge showed no retention of the drug. Therefore, the cation exchange resin Strata-X-C was selected as optimum for the combined analysis of all three APIs.

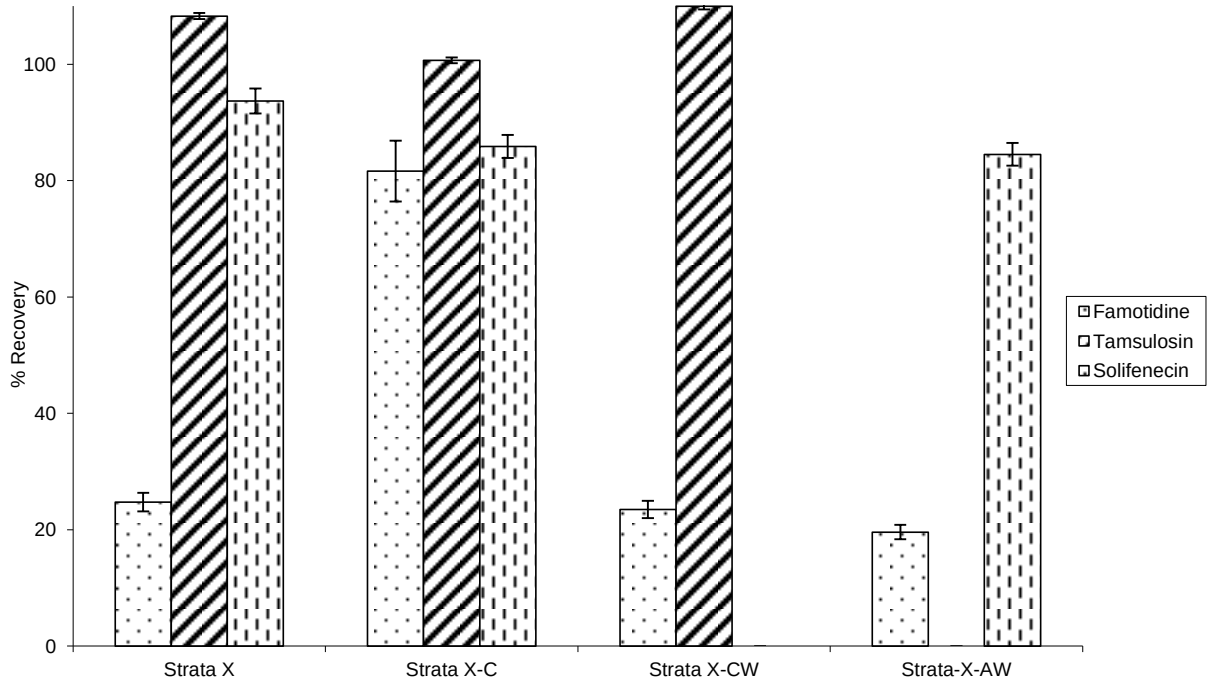


Figure 4.4 Recovery of three APIs on different cartridge types, Strata-X (polar – non-polar interactions), X-C (strong cation exchange), X-CW (weak cation exchange) and X-AW (weak anion exchange).

The Strata-X-C cartridge is a mixed mode cartridge which functions on the basis of both ion exchange and polarity. The polymeric backbone provides polar, non-polar interactions. The samples were acidified following collection to pH3 (sampling procedure is outlined in section 3.1.4) and so in acidic solutions basic wastewater constituents were expected to bond with the negatively charged functional group of the Strata-X-C resin (Figure 4.5).

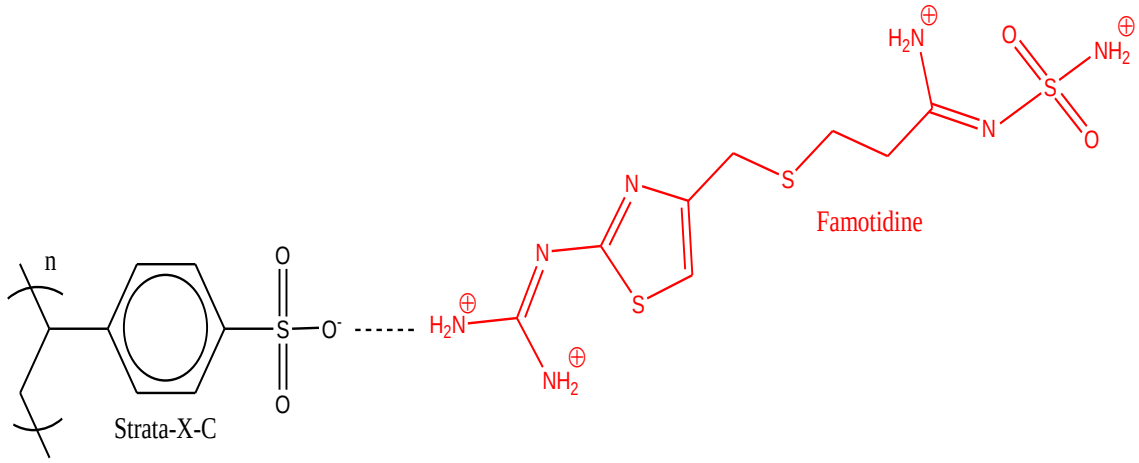


Figure 4.5: Strata-X-C reverse phase and cation exchange resin sample interaction with famotidine.

4.1.3 HPLC method development

Due to the complex nature of the wastewater a separation method is necessary. Both HPLC and GC are widely used for the monitoring of micropollutants and each has advantages and disadvantages. Lower standard deviations are associated with LC-ESI-MS over GC-MS; however, GC-MS methods generally have a lower LOD (Fatta et al., 2007). The analytes under investigation are non-volatile and would require derivatisation for GC analysis. This contributes to the sample preparation time. Therefore HPLC was chosen as the separation method of choice.

4.1.3.1 Wavelength optimisation

HPLC optimisation began with the determination of the optimum wavelength for the monitoring of the three APIs. The UV-Vis spectra of the three APIs

each at a concentration of 10 mg/L in HPLC grade water are shown in Figure 4.6. Famotidine (280nm) and tamsulosin hydrochloride (270nm) show significant peaks at higher wavelengths. Solifenacin succinate shows limited absorbance at higher wavelengths. Higher absorbances were observed for all three analytes at lower wavelengths, famotidine (205nm), tamsulosin (205nm) and solifenacin (215nm). While the higher wavelengths could be used to improve selectivity for famotidine and tamsulosin this would also result in decreased sensitivity.

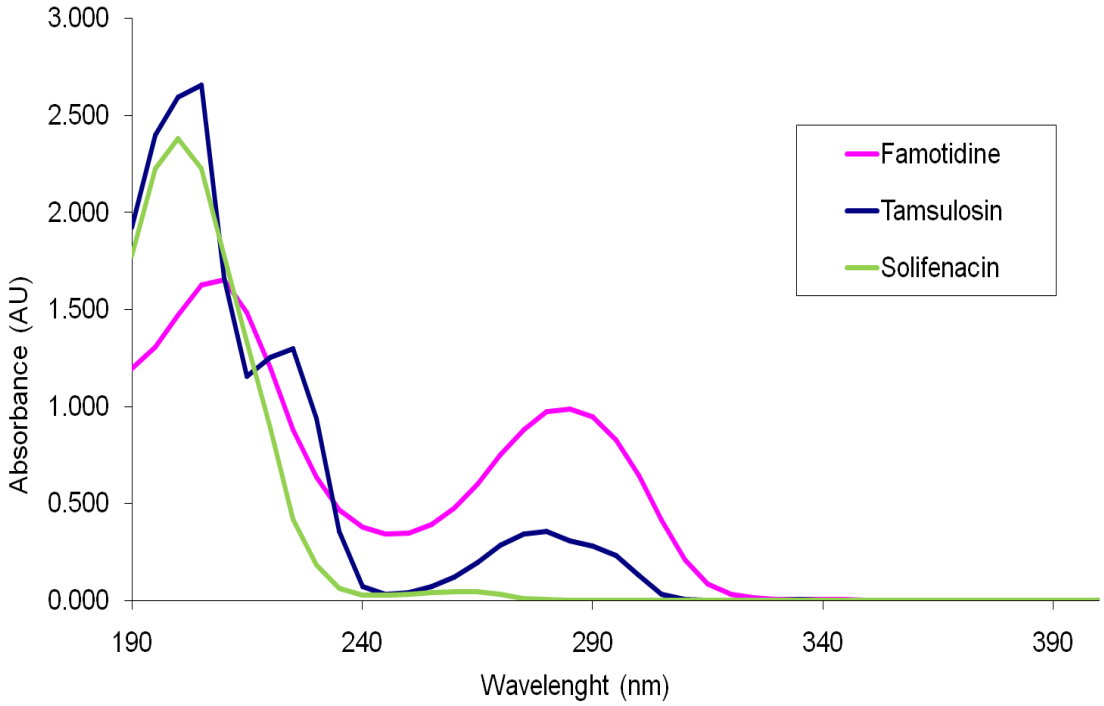


Figure 4.6: UV spectra of three APIs at neutral pH.

The molar extinction coefficient for each of the three APIs was 19570.3, 31991.0, 16072.3 $cm^{-1}M^{-1}$ for famotidine, tamsulosin and solifenacin respectively. The molar extinction coefficient was calculated by dividing the UV absorbance (cm^{-1}) at the chosen wavelength by the molar concentration (M). This coefficient is an expression of the UV energy absorption and a low absorption might imply low degradation of the drug as a result of UV light (Kim et al. 2009).

4.1.3.2 Stationary phase optimisation

The polarities of the three APIs vary greatly: famotidine is a highly hydrophilic compound and tamsulosin and solifenacin are hydrophobic. Reverse phase methods using C-18 columns have been developed for tamsulosin and solifenacin (Keski-Rahkonen et al. 2007; Mistri et al. 2008). While there are some methods that use a C-18 column for the retention of famotidine they typically have complicated mobile phases with additives that are unsuitable for MS and/or had very short retention times. In general, famotidine is difficult to retain under reverse phase conditions using a C-18 column (Zhong et al. 2001). What is required is a phase that offers both the hydrophobic characteristics of a C-18 and the ability to act as a hydrogen bond acceptor. The PFP phase meets both of these requirements. The PFP column (Figure 4.7) has the hydrophobic characteristics of a C-18 column for the reverse phase retention of tamsulosin and solifenacin and also has hydrogen bonding acceptor properties that are needed for optimised interaction with famotidine.

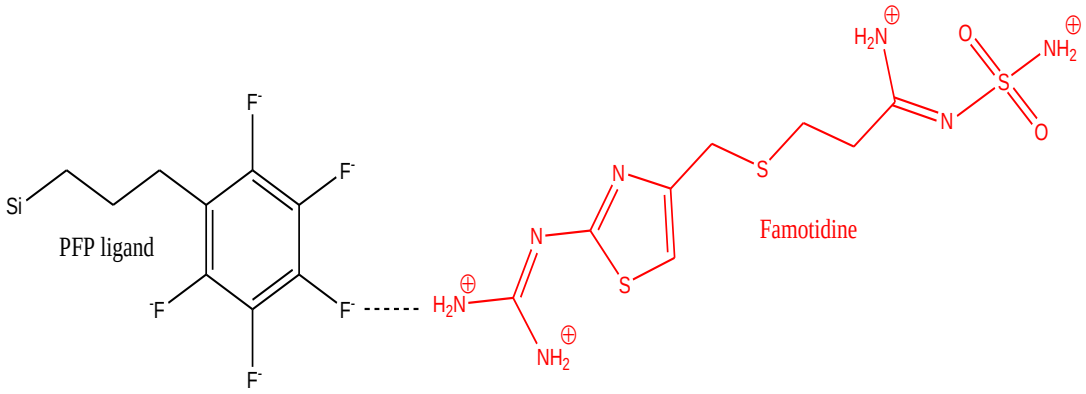


Figure 4.7: Example of interaction between Pentyfluorophenyl propyl ligand attached to silica and famotidine. The PFP phase has enhanced mechanisms of bonding over C-18 stationary phase. Its main mechanisms of interaction are hydrogen bonding, dipole-dipole, aromatic and hydrophobic interactions. The PFP column was chosen due to the difficulty in retaining famotidine on the C-18 column.

4.1.3.3 Mobile phase optimisation

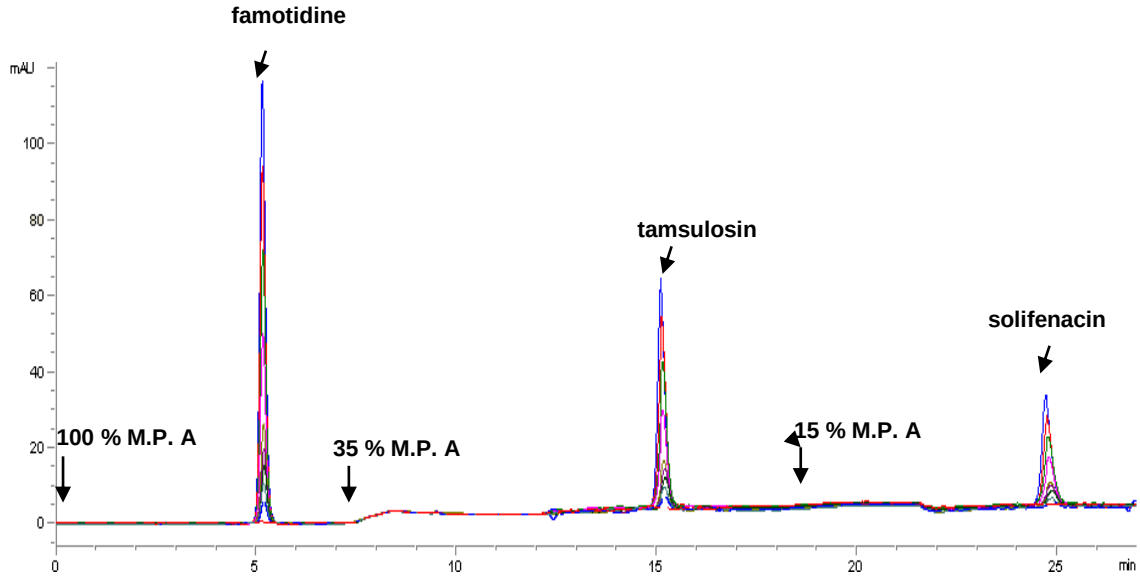
Mobile phase optimisation began with development of individual isocratic methods for the separate analysis of the three APIs. This showed the % organic required for the elution of the three pharmaceuticals. These methods were then combined to determine the optimum gradient conditions for the elution of the three APIs in a single combined method.

Independent isocratic methods were first developed for all three APIs using acetonitrile with a 0.1% ammonium acetate buffer on a PFP column (Table 4.2). This determined the approximate organic percentage of the mobile phase required for the analytes to elute. Standard curves were produced for each of the three analytes and gave high R values at low concentrations (0.2-5 mg/L).

Table 4.2: Individual methods for the analysis of the three APIs.

Analyte	% ACN	Retention time (min)	Wavelength (min)	R
Famotidine	10	5.5	205	0.9973
Tamsulosin	35	6.5	205	0.9989
Solifenacin	65	7.9	215	0.9961

Due to a large difference in mobile phase composition used between the three methods, a single isocratic method is not appropriate for the analysis of all three APIs due to long analysis times and peak broadening. Therefore, a gradient method was developed to analyse for all three APIs at the same time. One problem that arose in developing the gradient method was that the low wavelengths at which the three analytes absorb caused significant baseline drift. As a result, wavelength switching had to be employed to account for the drift. In other words, the wavelength was increased to 254 nm at steps in the gradient. Initial method development was conducted using 10 % to 65 % acetonitrile with a 0.1 % ammonium acetate buffer (Figure 4.8).



Mobile phase (M.P.) A: 10 % ACN (0.1% A.A.)
Mobile phase (M.P.) B: 65 % ACN (0.1 % A.A.)

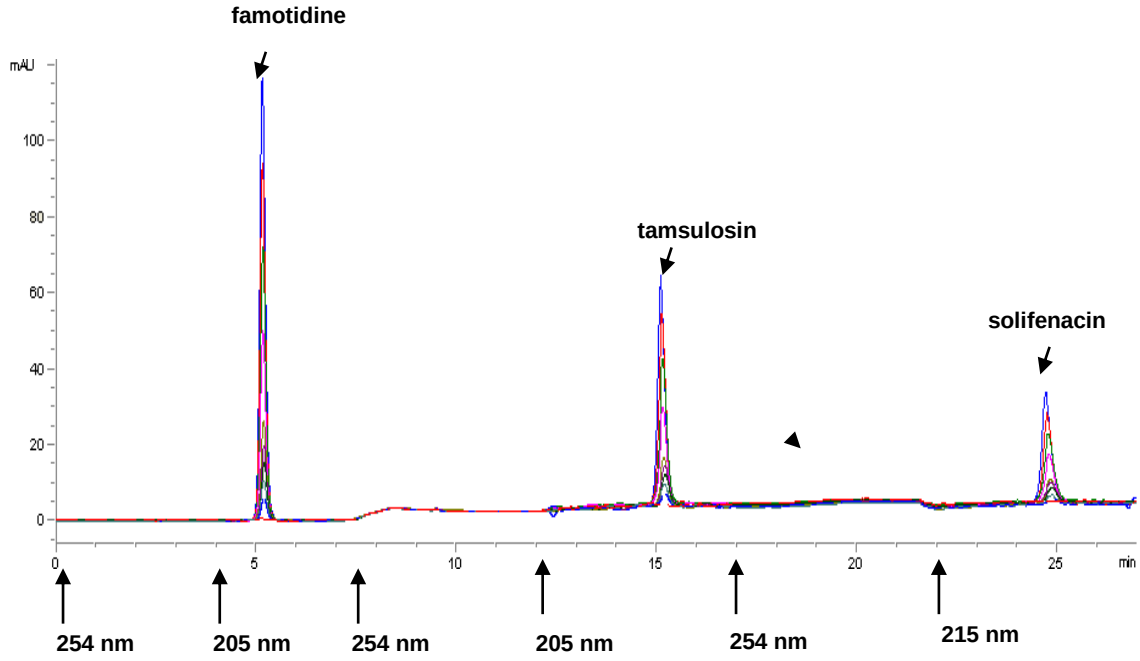


Figure 4.8: 0-5 mg/L overlay of famotidine, tamsulosin hydrochloride and solifenacin succinate. The top chromatogram shows the effect of changes in mobile phase composition on the chromatogram. The bottom chromatogram shows arrows indicating the points at which the wavelength was changed.

Although good R values (Figure 4.9), responses and peak shapes were achieved, problems arose with the availability and price of acetonitrile (Sigma Aldrich price: Acetonitrile 0.1 % Ammonium acetate (€77.67 /2.5L) during the method development phase and in Autumn 2008 a switch was made to a methanol-based mobile phase (10 % to 90 %). Formic acid (0.1 %) was added to the methanol/water mobile phase to adjust it to pH 3 which resulted in enhanced response and peak shape. Acidification is also recommended for reproducible retention times (Petrovic et al., 2005). It was not necessary to add a buffer since peak shape was deemed acceptable. Standard injection volume of 50 µg/L and flowrate of 1 mL/min were used. No improvement in peak shape was noted with changes in these parameters. Slight reoptimisation of gradients was necessary on changing to low flowrate (0.3 mL/min), lower injection volume (20 µL) and narrow bore column for MS analysis. Figure 4.10 shows a chromatogram using low flow-rate, lower injection volume and narrow bore column.

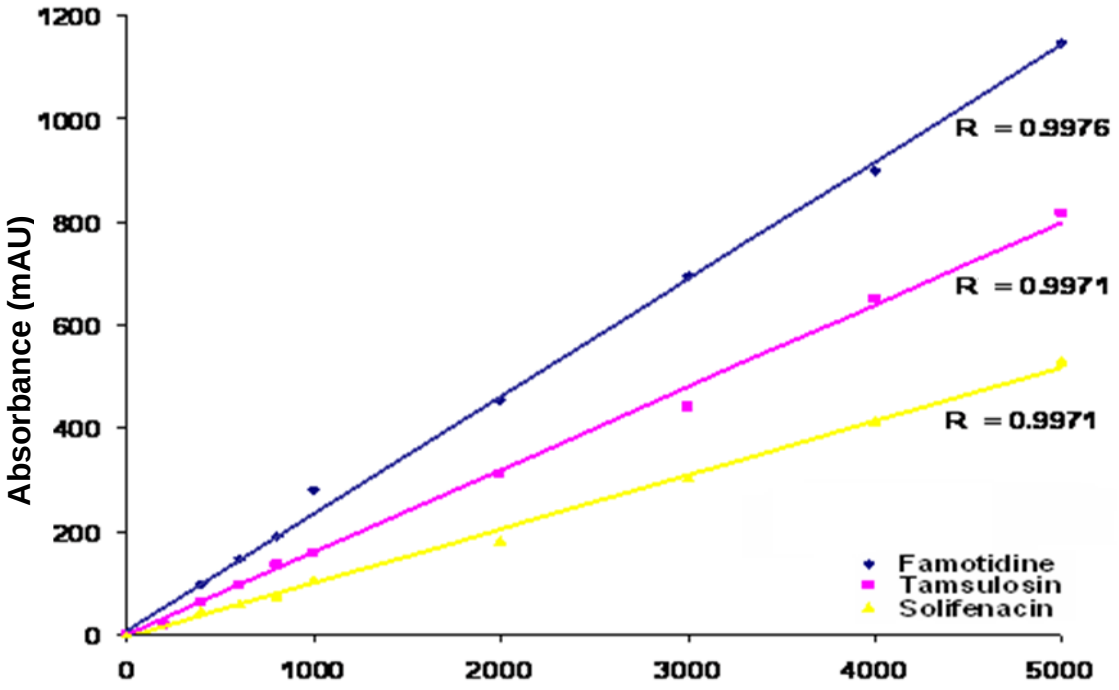


Figure 4.9: 0-5 mg/L standard curve of combined famotidine, tamsulosin hydrochloride and solifenacin succinate method.

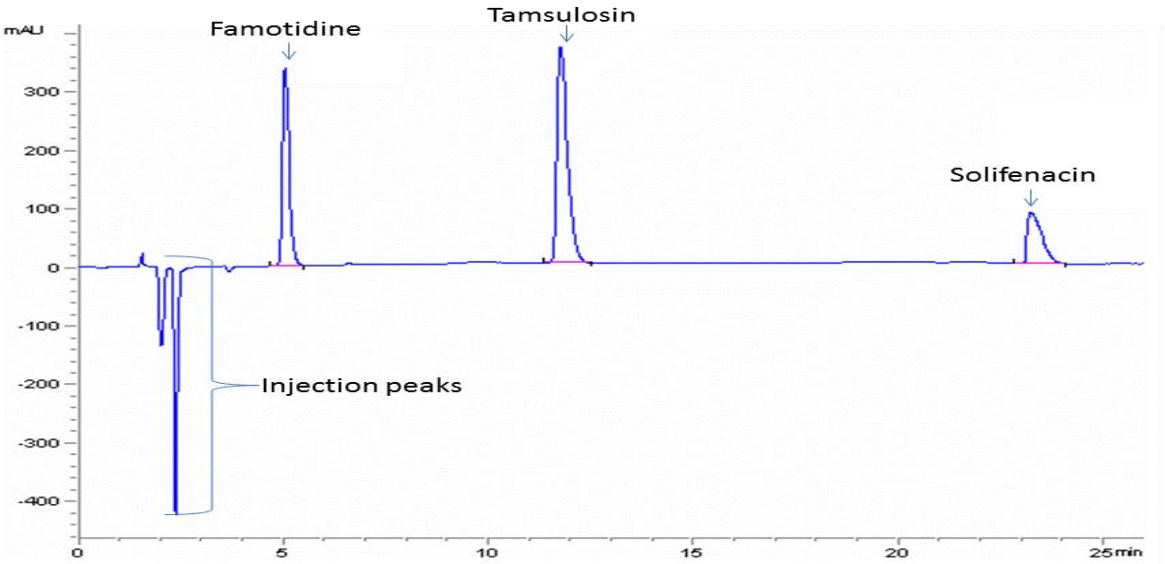


Figure 4.10: Chromatogram of 5 mg/L standard using switched wavelength on HPLC. Methanol mobile phase with 0.1% formic acid, a narrow bore column, 0.3 mL/min flowrate and 20 µL injection.

4.1.4 Mass spectrometry optimisation

Mass spectrometry is an analytical technique that measures the mass-to-charge ratio of charged particles. In LC-MS/MS, a HPLC is used as the separation technique the sample is then pumped into a mass spectrometer. There the sample is vaporised, ionized and accelerated through a series of electrical and vacuum gradients to the ion trap. In the trap the ions are then separated on the basis of mass to charge. Target ions are then fragmented, separated a second time and sent to the detector. A mass spectrum is produced showing relative abundance of ions as a function of the mass to charge ratio.

LC-MS offers enhanced sensitivity and specificity over HPLC alone. Due to the presence of other co-eluting matrix components UV detection was not specific enough for the quantitation of analytes and therefore mass spectrometry detection was used. Tandem MS was used both for compound identification and quantitation. The MS was operated in positive mode. The MH^+ ions were isolated in the ion-trap for subsequent fragmentation. Under multiple reaction monitoring (MRM) mode breakdown of each of the molecular ions was monitored. Two MRM transitions were monitored to confirm the

identity of each API. Molecular and product ions were determined under tandem mass spectrometry conditions in positive and negative modes and are shown in Table 4.3 and Figure 4.11. Positive mode was optimum for all three APIs. While monitored ions were determined by direct infusion of standards, other LC-ESI-MS/MS methods in the literature also monitored similar MRM transitions: molecular ion 338 m/z to product ions 259 and 189 m/z for famotidine (Jelic et al., 2009; Petrovic et al., 2006), molecular ion 409 m/z to product ions 271, 228 and 148 m/z for tamsulosin (Keshi- Rahkonen et al., 2007) and molecular ion 363 m/z to 110 m/z for solifenacin (Mistri et al., 2008).

The positive mode showed higher sensitivity than the negative mode for all three analytes. Formic acid (to enhance ionisation in positive mode) and ammonium hydroxide (to enhance ionisation in negative mode) were added to each standard to determine optimum sample composition for ionisation. Formic acid was found to have negligible signal enhancement for all three analytes in positive mode. Ammonium hydroxide also showed negligible signal enhancement in negative mode. Mass spectrometry parameters were optimised by direct infusion of standards for each analyte individually and are also shown in Table 4.3. ESI parameters were optimised automatically using Brüker Daltonics software. Nebuliser conditions were optimised manually by changing a parameter and waiting for the signal response to adapt.

Table 4.3: Nebuliser and electrospray ionisation conditions, also molecular and product ions. Average ESI conditions did not show as high a response as the tamsulosin optimum for each of the APIs. Molecular and product ions as indicated by direct infusion of standards in positive mode.

	API	Famotidine	Tamsulosin	Solifenacin	Optimised condition
	Mol. Weight (g/mol)	337	408	362	-
	Mol. Ion (m/z)	$338(M+H)^+$	$409(M+H)^+$	$363(M+H)^+$	-
	Product ions (m/z)	$259(M+2H)^+$ $189(M+2H)^+$	$228(M)^+$ $271(M)^+$	$110(M)^+$ $236(M)^+$	-
ESI	Capillary (V)	4500	4500	4500	4500
	End plate (V)	775.4	913.1	740.0	913.1
	Skim 1 (V)	15.0	30.3	38.7	30.3
	Cap exit offset (V)	50.0	66.4	77.9	66.4
	Octopole (V)	3.6	4.0	3.5	4.0
	Octopole delta (V)	2.2	1.5	2.6	1.5
	Trap drive	55.0	53.0	49.7	53.0
	Skim 2 (V)	7.4	9.8	13.0	9.8
	Oct. R.F. (Vpp)	197.5	226.2	209.8	226.2
	Lens 1	-5.0	-2.5	-3.1	-2.5
	Lens 2	-60.0	-57.5	-56.4	-57.5
	Frag. Amp. (V)	1.8	1.7	1.8	1.7
Nebulizer	Dry gas pressure (PSI)	-	-	-	50PSI
	Dry gas flow (L/min)	-	-	-	$8Lmin^{-1}$
	Temperature (˚C)	-	-	-	325ºC

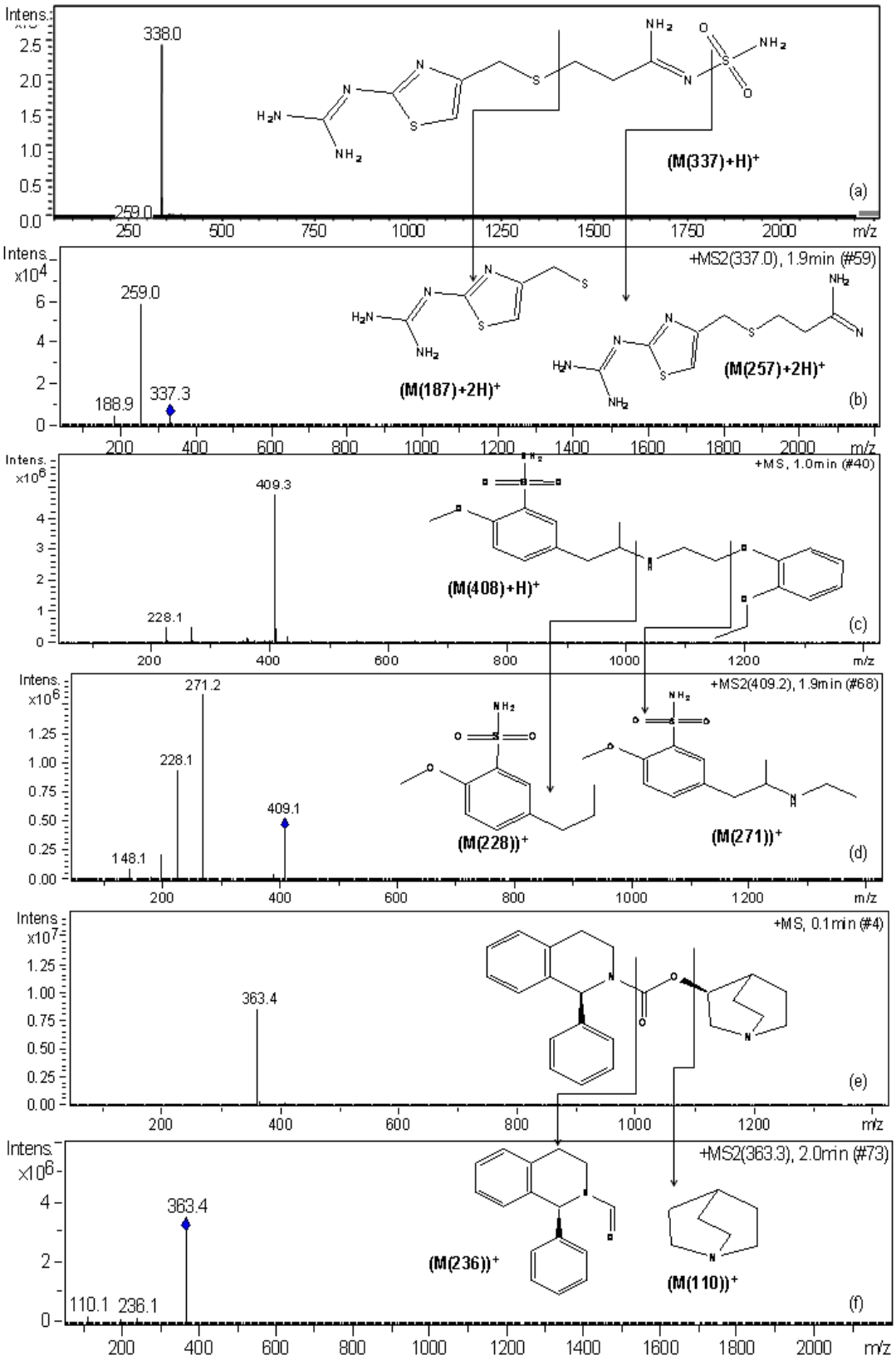


Figure 4.11: Molecular and product ions for (a) famotidine molecular ion 338m/z [MS], (b) famotidine product ion 259, 189m/z [MS/MS], (c) tamsulosin molecular ion 409m/z [MS], (d) tamsulosin product ion 271, 228, 148m/z [MS/MS], (e) solifenacin molecular ion 363m/z [MS], (f) solifenacin product ion 236, 110m/z [MS/MS].

4.1.5 Detection of APIs in real wastewater

Initial SPE-LC-MS/MS experiments with real wastewater samples demonstrated qualitatively the presence of the pharmaceuticals in all wastewater samples (Figures 4.12, 4.13 and 4.14). Samples shown were collected on the 27th and 28th of October 2009. A higher response of the APIs in effluent compared to the influent highlighted the need to monitor ionisation effects caused by matrix components as the presence of other compounds in the matrix may lead to signal suppression or enhancement (Taylor, 2005).

Signals of famotidine and tamsulosin were greater than ten times the noise level and therefore above the LOQ and solifenacin was detected above the LOD at more than three times the noise level (Fajgelj and Ambrus, 2000). Solifenacin had been out of production since summer 2009. It was therefore anticipated that when production resumed that concentrations would increase. The SPE concentration of 10X used was therefore considered suitable for method validation.

There are three main methods used for quantitation. They are internal standards, external standards and standard addition. External standards are the simplest method but do not account for any matrix effects. Standard addition or internal standards can be used to account for different ionisation between samples. Standard addition involves spiking the sample with known quantities of the analyte of interest. In comparison internal standard uses a similar but not the same compound as the analyte of interest. The ratio of internal standard signal to analyte signal as a function of analyte concentration is used for quantitation. The main disadvantage with the internal standard method is the difficulty in finding compounds that match closely enough the analyte of interest but are still readily distinguishable by the instrument. Therefore standard addition was used as the method of choice for validation of the SPE-LC-MS/MS method and the sampling programme.

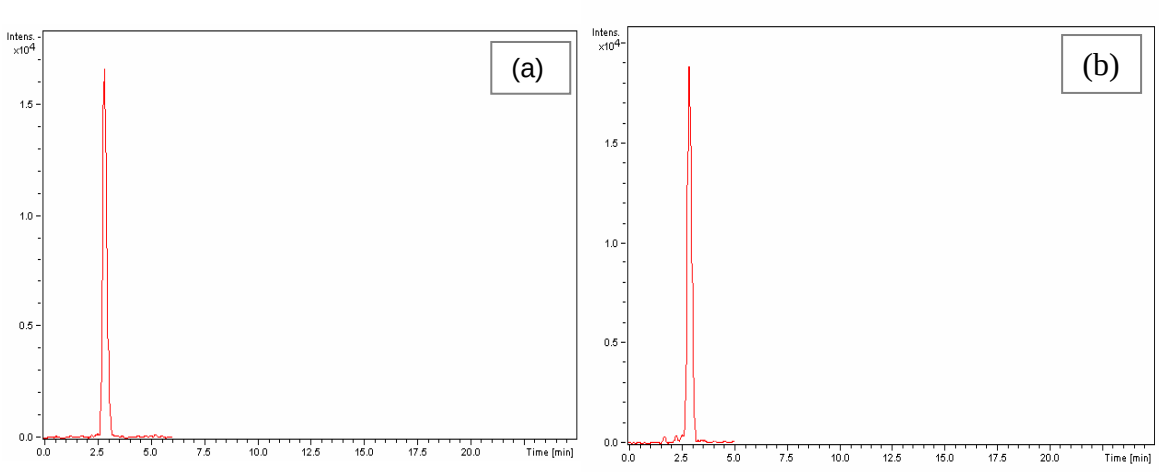


Figure 4.12: (a) influent and (b) effluent samples showing extracted ion chromatogram for famotidine [ms/ms].

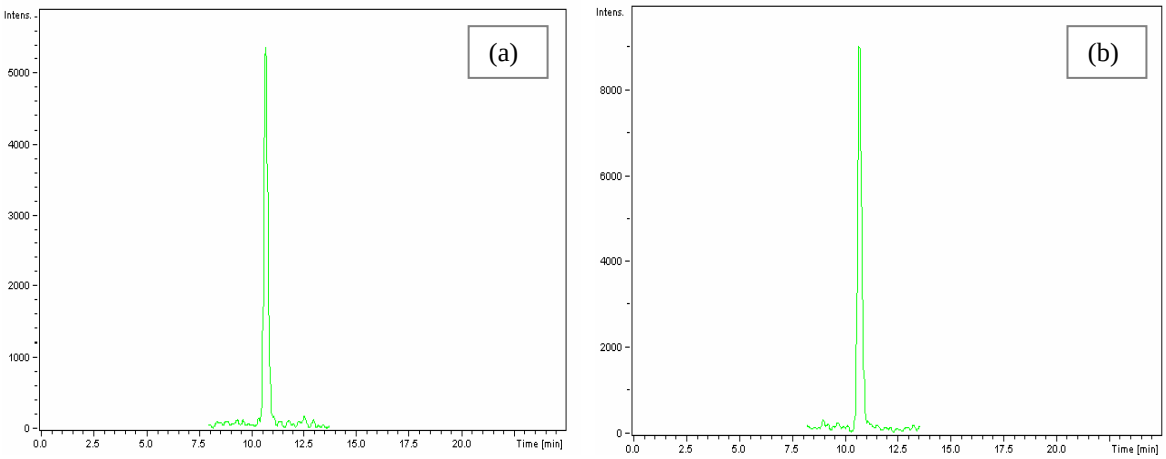


Figure 4.13: (a) influent and (b) effluent samples showing extracted ion chromatogram for tamsulosin hydrochloride [ms/ms].

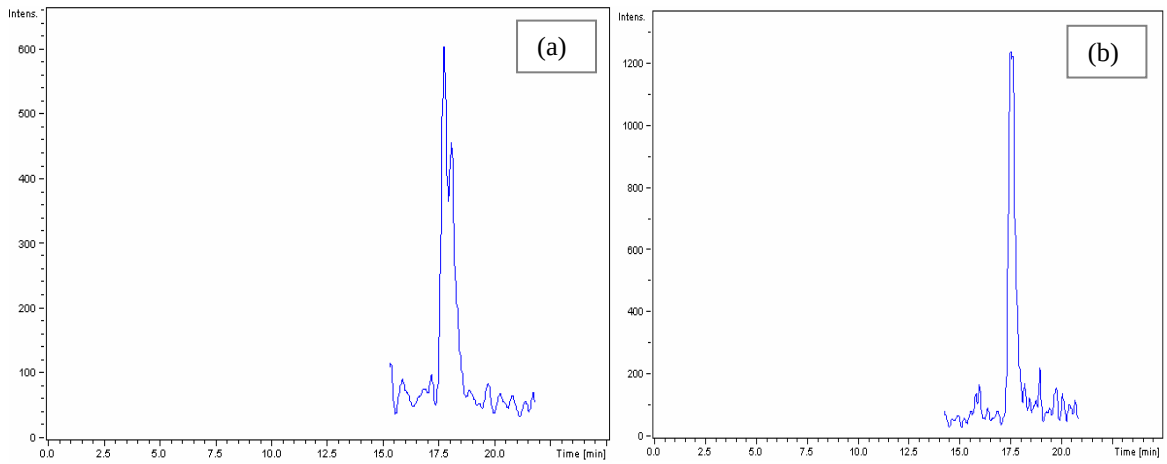


Figure 4.14: (a) influent and (b) effluent samples showing extracted ion chromatogram for solifenacin succinate [ms/ms].

4.1.6 Validation of SPE-HPLC method

SPE-HPLC methods are available and validated for the analysis of certain pharmaceuticals in complex matrices (Babic et al., 2006). It was anticipated throughout the development process that a confirmation step such as MS would be necessary. However, a SPE-HPLC method without the MS step offers advantages:

1. The ease of availability of HPLC instruments over LC-MS
2. Time saved on MS method development
3. No suppression or enhancement of ionisation by matrix components

The SPE-HPLC method was validated using spiked ultra-pure water. Selectivity, linearity, LOD, LOQ, precision and recovery were determined. All SPE-HPLC validation data are shown in Table 4.4. All validation was conducted using standards that underwent SPE. Concentrations shown are representative of SPE of 100 mL of spiked ultra-pure water. Comparison of chromatograms for spiked and unspiked ultra-pure water was used to determine the selectivity of the analytical method. Unlike LC-MS, HPLC alone cannot provide compound confirmation. Therefore compound retention time and the absence of false positives from the comparison of spiked vs. unspiked water were used for confirming compound selectivity (Figures 4.15 and 4.16).

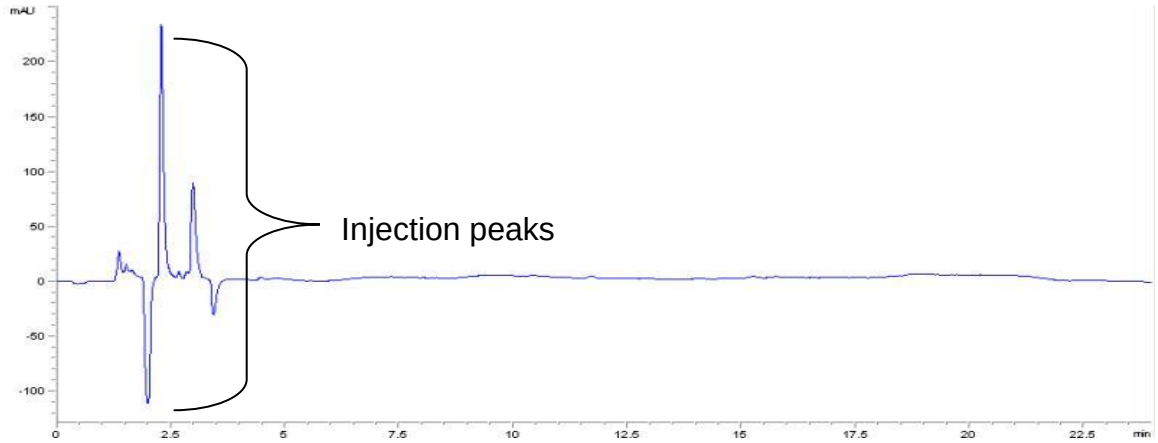


Figure 4.12: Chromatogram of unspiked water for the analysis of famotidine, tamsulosin hydrochloride and solifenacin succinate showing series of injection peaks.

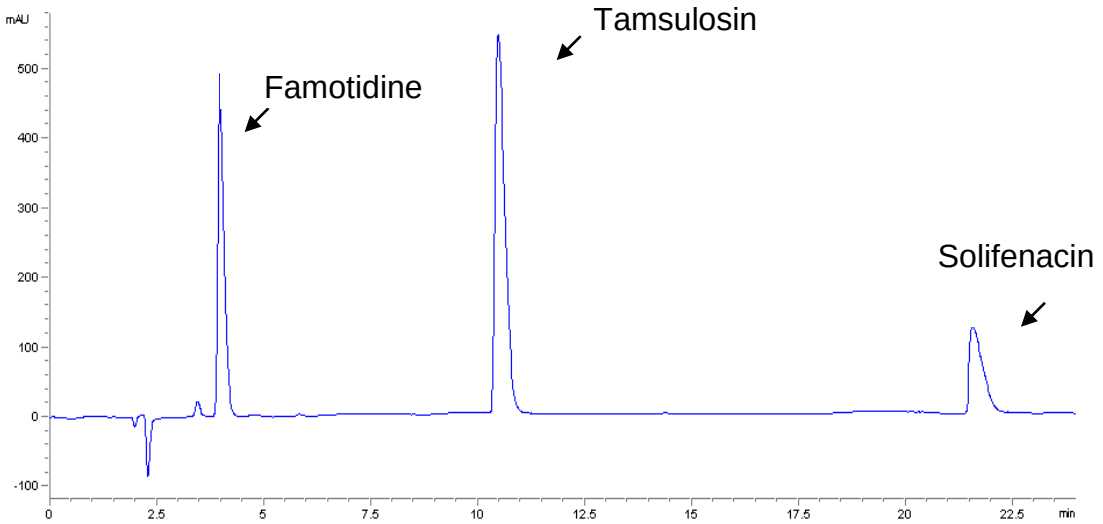


Figure 4.13: Chromatogram of water spiked to 60 µg/L with famotidine, tamsulosin hydrochloride and solifenacin succinate.

Linearity of response was determined for famotidine, tamsulosin hydrochloride and solifenacin succinate using at least five concentrations. Each standard was injected three times. Standard curves were prepared by plotting concentration of analyte in the spiked water *vs.* peak area units. Blanks were also prepared for quality control. Coefficients of correlation were all >0.995 confirming the linearity of the method. R values correspond with similar methods in the literature (Malintan and Mohd, 2006).

Recoveries were determined at low, medium and high concentrations (n=3). Ultra-pure water was spiked to a concentration of 20, 40 and 60 µg/L. The concentration recovered from the Strata-X-C cartridge was compared to the initial spiking concentration. Recovery ranged from 87.4 ± 0.8% to 90.1 ± 2.9% for famotidine, 95.2 ± 1.4% to 100.7 ± 4.1% for tamsulosin hydrochloride and 88.2 ± 4.3 to 99.2 ± 4.2% recovery for solifenacin succinate.

For the determination of the LOD and LOQ, standards were serially diluted until the signal-to-noise ratio reached a value of 3:1 for the LOD and an LOQ of 10:1 (Lacey et al., 2008). Precision is defined as the degree to which repeated measurements under unchanged conditions show the same results and accuracy defined as the closeness of repeat measurements to their true value were determined at low (20 µg/L), medium (40 µg/L) and high (60 µg/L) quality control levels at three replicates per concentration (n=3) (Hospodsky et

al., 2010). The values for the intra-day precision were 19.2 ± 0.5 µg/L to 60.9 ± 0.8 µg/L for low and high levels, while inter day variability values were 18.0 ± 5.8 µg/L to 60.8 ± 1.2 µg/L.

Following validation the method was applied to real wastewater samples. Both spiked and unspiked influent samples were analysed (Figure 4.17). However, the method was not applied to the six month monitoring of the wastewater. A very large famotidine peak in the early part of the chromatogram where other matrix components elute is seen in the influent samples. It is possible that other components of the wastewater may be co-eluting therefore a confirmation step is necessary.

Further optimisation of the method may result in greater specificity such as optimisation of the SPE washes for higher selectivity in the removal of interferences. However washes were concentrated with a 100% methanol wash for removal of polar interferences and an acid wash for the removal of acidic interferences. Interferences may be intermediates or impurities in the production process which may have similar structures. Optimisation of washes is time consuming and the same washes may remove both the analyte of interest and the interference. Further optimisation of the SPE-HPLC method was therefore not continued.

A similar method for the quantitation of pharmaceuticals in industrial wastewater used UV spectra at the retention time (RT) of the APIs as a confirmation step. This method had a similar LOQ from 1.5-100 µg/L. A number of the peaks shown in the real wastewater samples were not confirmed when compared to UV spectra. This highlighted the requirement for a confirmation step even for relatively clean chromatograms and in areas of the chromatogram where matrix components are usually less problematic (Babic et al., 2006).

Table 4.4: SPE-HPLC validation parameters including range, LOD, LOQ, linearity, line equation, SPE recovery, precision and accuracy for each API. All validation parameters shown were evaluated in ultra-pure water.

	Concentration (µg/L)	Famotidine	Tamsulosin	Solifenacin
Range of calibration curve (µg/L)		5.0-60	2.5-60	10-60
LOD (µg/L)		2.0	1.0	2.5
LOQ (µg/L)		5.0	2.5	10
Slope of calibration curve (m)		83.1±0.3	142.3±0.4	44.5±0.3
Y-axis intercept (c) (mAU)		-68.7±6.1	-86.4±5.1	-51.0±7.5
Coefficient of correlation (R)		0.9970±0.0003	0.9969±0.0004	0.9950±0.0007
SPE Recovery Mean concentration ± %CV	20	87.9±8.0	95.6±0.5	92.3±1.3
	40	87.4±0.8	95.2±1.4	88.2±4.3
	60	90.1±2.9	100.7±4.1	99.2±4.2
Inter-day Mean concentration ± %CV (% Bias)	20	18.0±6.8(10.0)	19.5±0.2(2.3)	19.6±1.9(1.9)
	40	35.9±6.3(10.2)	38.3±1.0(4.3)	38.7±3.8(3.2)
	60	57.1±6.1(4.9)	60.3±0.3(-0.5)	60.8±1.2(-1.3)
Intra-day Mean concentration ± %CV (% Bias)	20	19.2±0.5(3.9)	19.7±0.5(1.7)	19.4±0.8(2.9)
	40	37.7±1.4(5.8)	38.1±0.3(4.7)	37.8 ± 0.8(5.4)
	60	60.5±0.4 (-0.8)	60.9±0.8 (-1.5)	61.4 ± 0.5 (-2.4)

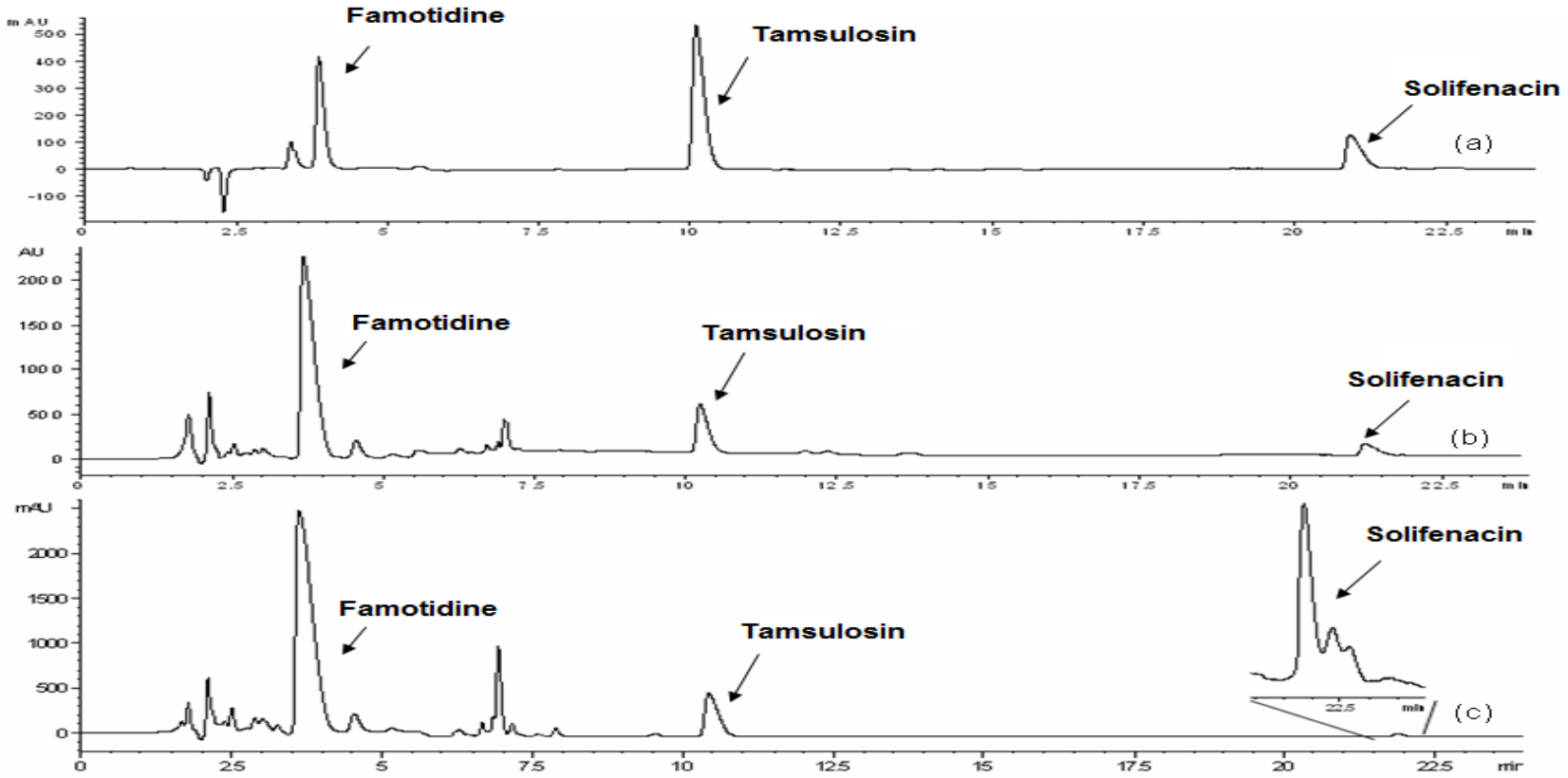


Figure 4.14: Application of SPE-HPLC method (a) 20 µg/L spiked ultra-pure water showing famotidine, tamsulosin and solifenacin (b) 20 µg/L spiked real wastewater sample (c) unspiked influent sample. The early elution of famotidine with other contaminants may lead to interfering peaks. Co-elution of other constituents in the water may lead to the large peak seen at the same retention time as famotidine in the samples. Without the confirmation offered by mass spectrometry this peak cannot be identified as famotidine with a high degree of certainty.

4.1.7 Validation of SPE-LC-MS/MS method

Mass spectrometry is commonly used for the analysis of low concentrations of target analytes in waters (Gros et al., 2006; Lòpez-Rolden et al., 2010; Petrovic et al., 2006).

The method was validated in both influent and effluent sample matrices and all validation data are presented in Table 4.5. Linearity was determined using regression analysis between the peak area ratios and concentration. Correlations of R>0.9 were obtained for all APIs. In the case of famotidine R>0.99 was recorded for standard addition in both influent and effluent samples. However, due to the presence of much higher concentrations of the API in the wastewater a higher range of standards was used.

The LOD and LOQ are very low for both tamsulosin and solifenacin in influent and effluent. The low ionisation of famotidine has led to a much higher LOD and LOQ in both matrices. The precision of the overall method was determined from six injections of a low level spiked standard and intermediate precision by injection of six replicates of a low-level spiked sample. Precision and intermediate precision were less than 15% in all cases. While 15% is a high variance, it is typical when using standard addition for SPE-LC-MS/MS methods for micro-pollutants in complex matrices (Hernando et al., 2004; Lacey et al., 2008).

To quantify the level of ion suppression or enhancement, in both influent and effluent samples, a sample was extracted and then spiked with a standard solution of each analyte. Spiked extracted wastewater samples were compared to extracted standards in distilled water. Unspiked wastewater samples were also analysed to determine the level of target compounds present in the sample prior to spiking. Any difference in the signal obtained from the spiked wastewater sample from that of the spiked distilled water was due to matrix components. High signal suppression in the influent was contrasted by enhancement of signal in the effluent for all analytes is likely to be due to the presence of different matrix components.

Recoveries were determined by comparing pre-extraction and post-extraction spiked influent and effluent samples. Recoveries were greater than 91% for all three APIs in both influent and effluent. Studies of SPE methods are not common in the literature for the recovery of the three APIs under investigation. Acceptable recoveries have been reported from 50-96% for these APIs using a variety of cartridges (Gros et al., 2006; Chambers et al., 2008).

Table 4.5: SPE-LC-MS/MS validation results in influent and effluent wastewaters.

		Famotidine	Tamsulosin	Solifenacin
Influent	Range of calibration curve	0.4-5.0 mg/L	1-15 µg/L	6-50 µg/L
	Slope of calibration curve (m)	$1x10^5$	$2x10^7$	$4x10^6$
	Y-axis intercept (c) (Intens.)	-12394	-14796	-11496
	LOD (µg/L)	100	0.4	2.0
	LOQ (µg/L)	400	1.0	6.0
	% Precision (%CV) n=6	2.5	14.8	11.9
	% Intermediate precision (%CV) n=6	7.8	11.0	8.7
	Coefficient of correlation (R)	0.9940	0.9775	0.9707
	% SPE recovery	114.6	103.2	91.2
	% Ion suppression	41.8	23.1	51.3
Effluent	Range of calibration curve	0.4-5.0 mg/L	0.8-18 µg/L	6.0-16 µg/L
	Slope of calibration curve (m)	$1x10^5$	$2x10^7$	$2x10^6$
	Y-axis intercept (c) (Intens.)	30457	8360.9	3522.3
	LOD (µg/L)	110	0.3	4.0
	LOQ (µg/L)	400	0.8	6.0
	% Precision (%CV) n=6	2.0	11.0	10.9
	% Intermediate precision (%CV) n=6	1.7	6.8	10.2
	Coefficient of correlation (R)	0.9912	0.9815	0.9301
	% SPE recovery	116.2	96.3	114.8
	% Ion suppression	-49.3	-49.1	-72.4

4.2 Results of the sampling programme

4.2.1 Occurrence of the APIs in wastewater

Influent and effluent samples were collected on a weekly basis from the onsite wastewater treatment plant from November 2009 to April 2010 (26 weeks). All three analytes under investigation were detected in both influent and effluent at different times during the study.

Famotidine and tamsulosin hydrochloride were detected in 85 and 88% of influent samples and 100 and 96% of effluent samples respectively. Solifenacin succinate was detected less frequently at 65% in both influent and effluent samples. Solifenacin succinate was out of production from summer 2009 until January of 2010 which likely accounts for the less frequent detection of the pharmaceutical. The very low LOQ of tamsulosin hydrochloride meant that despite its low level production it was almost always detected in the wastewater at trace concentrations.

Of the three drugs famotidine was present at the highest concentrations (average 1.6 mg/L in influent, 2.6 mg/L in effluent). On average it was detected at two orders of magnitude higher than solifenacin (0.039 mg/L in influent, 0.028 mg/L in effluent) and three orders of magnitude higher than tamsulosin (0.005 mg/L in influent, 0.004 mg/L in effluent). Famotidine is produced in the highest amounts of the three pharmaceuticals at 385 Kg of famotidine per batch which represents a yield of approximately 65%. Each batch takes approximately 10 days and often two batches are in production simultaneously. During the monitoring period with the exception of the Christmas shutdown period (22nd December to 6th January) the famotidine process averaged two batches every two weeks.

A summary of the occurrence of the three APIs over the six month monitoring period from November 2009 to April 2010 is shown in Table 4.6. Student paired t-test showing difference between influent and effluent samples with 95% confidence limit. For both famotidine and solifenacin the influent and effluent are significantly different. In the case of tamsulosin, the influent and

effluent are not significantly different Appendix C shows the monthly summaries of results.

Table 4.6: Occurrence of APIs in wastewater over 6 month period from November 2009 to April 2010. Minimum, maximum, median and average concentrations are shown for 26 weekly influent and effluent samples.

API	WW*	n>LOD	%Freq	Min (mg/L)	Max (mg/L)	Median (mg/L)	Average (mg/L)	t-test	Sig (2-tailed)
Famotidine	I	22	85	<LOQ (0.4)	5.8	1.5	1.6	-3.347	0.003
	E	26	100	0.7	7.3	2.0	2.6		
Tamsulosin	I	23	88	<LOQ (0.001)	0.019	0.003	0.005	0.104	0.918
	E	25	96	<LOQ (0.0008)	0.032	0.003	0.004		
Solifenacin	I	17	65	<LOQ (0.006)	0.174	0.027	0.039	2.283	0.031
	E	17	65	0.007	0.079	0.018	0.028		

*Wastewater (WW), influent (I), effluent (E)

The concentrations detected for famotidine in particular were much higher than originally expected. However, they are broadly in line with the concentrations detected in two studies reporting concentrations of pharmaceuticals in effluents from large industrial complexes in Korea and India. Larsson et al., 2007 reported concentrations of pharmaceuticals in effluent from a common effluent treatment plant in India serving approximately 90 bulk drug manufacturers. The antibiotic ciprofloxacin was detected at concentrations up to 31 mg/L and the H_2-receptor antagonist ranitidine at concentrations as high as 0.16 mg/L. The Korean study focused on APIs in the effluent from both individual manufacturing facilities and pharmaceutical complexes and reported levels of up to 43.9 mg/L of the antibiotic lincomycin and 0.15 mg/L of the antiepileptic carbamazepine (Sim et. al., 2011). The concentrations of famotidine in this work (max detected 7.3 mg/L) are broadly the same order of magnitude as those in the Korean and Indian studies.

Pharmaceuticals have been detected in effluents from municipal and hospital wastewaters and surface waters typically in ng/L to low ug/L ranges. Table 4.7 shows that industrial wastewater contains higher concentrations of pharmaceuticals than municipal and hospital wastewater in the majority of cases. It has been generally accepted that the main route of entry of pharmaceuticals into the environment is through human ingestion and excretion and subsequent release through municipal facilities (Khetan and Collins, 2007). This work together with the Indian and Korean cases suggests that concentrations of APIs released by the pharmaceutical industry may be contributing a higher environmental loading than previously acknowledged.

Table 4.7: Typical concentrations of pharmaceuticals released into the environment from different sources (µg/L).

Wastewater	Hospital		Industrial		Municipal		
	Min.	Max.	Min.	Max.	Min.	Max.	Ref.
Caffeine	12.1	45.7	2.26	262	1.94	75.7	[1]
	12.3	42.0	ND	ND	17.1	113.2	[2]
Carbamazepine	0.018	6.08	0.035	19.1	0.095	21.6	[1]
	n.d.	1.7	ND	ND	n.d.	1.1	[2]
Diclofenac	0.028	6.88	160	203	0.094	0.523	[1]
	n.d.	6.3	ND	ND	n.d.	3.9	[2]
Ibuprofen	7.0	8.9	ND	ND	2.8	25.4	[2]
Trimethoprim	0.028	7.26	0.056	162	0.036	1.51	[1]
	5	2.9	ND	ND	0.18	1.4	[3]
Ciprofloxacin	0.460	5.03	0.528	34.6	0.124	0.246	[1]
	0.8	2	ND	ND	0.2	1	[3]
	ND	ND	28,000	31,000	ND	ND	[4]

ND not determined [1] Sim et al., 2011[2] Kosma et al., 2010, [3] Brown et al., 2006, [4] Larsson et al., 2007

4.2.2 Trends in API concentration over sampling period

Trends in famotidine influent and effluent concentrations over the analysis period are shown in Figure 4.18. After a pre-Christmas ramp-up in production, levels of famotidine in the influent rose to a maximum concentration of 5.8 mg/L. According to a previous study this would correspond to approximately 1.65 Kg or 0.4% of the overall yield of famotidine (Cullen, 2009). During the

Christmas shutdown (23rd December- 6th of January 2010) levels of famotidine in the influent fell to a minimum of 0 mg/L on the 12th of January. Concentrations of famotidine in the effluent fell at a slower rate to a low on the 26th of January of 1.2 mg/L. This was two weeks later than the low point in the influent and over a month after the shutdown period began.

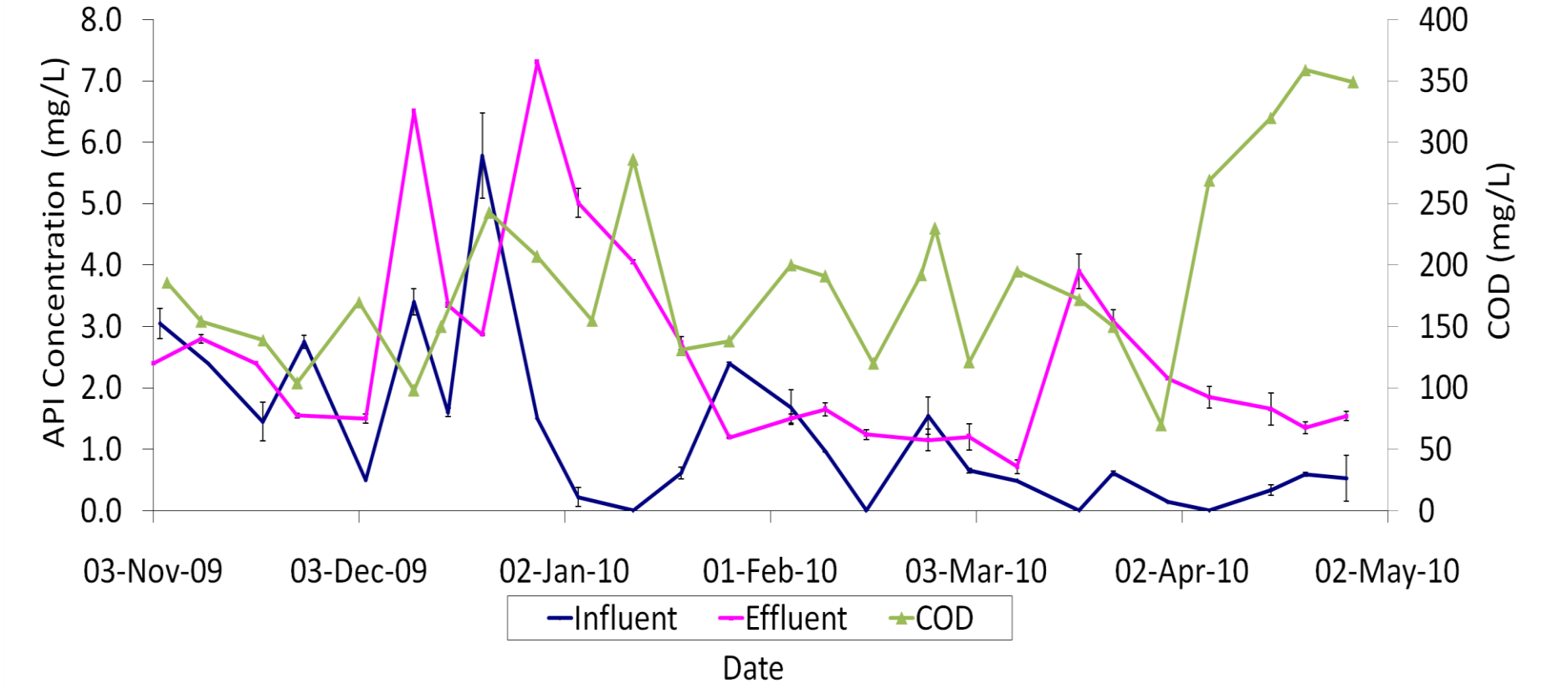


Figure 4.15: Influent and effluent concentrations of famotidine from November 2009 to April 2010

Tamsulosin hydrochloride is produced in a small multipurpose unit for the low level production of potent APIs. Approximately 15Kg of tamsulosin is produced per batch (approximately every 2 weeks). A low concentration of tamsulosin was detected throughout the monitoring period in both influent and effluent at a mean value of 0.003 mg/L (Figure 4.19). Maximum values of tamsulosin were 0.019 mg/L on the 26th of January 2010 in influent and 0.032 mg/L in the effluent on the 18th of March 2010. On the 11th of January 2010 the number of carbon filtration units in the final step of the process was halved from two to one which saw a yield increase of 0.6Kg per batch. However it corresponds with a large increase in tamsulosin in the influent seen at the end of each tamsulosin production cycle. Concentrations of tamsulosin in the effluent remained at a more steady concentration than the cyclic two week pattern seen in the influent.

Solifenacin succinate was out of production from summer 2009 until the 12th of January 2010. Solifenacin reached a max concentration of 0.174 mg/L in the influent and 0.079 mg/L in the effluent (Figure 4.20). Astellas have stated that eight batches of solifenacin were made from the 12th of January to the 22nd of April at a yield of 200Kg of solifenacin succinate per batch. A new solifenacin process was introduced on the 22nd of April (towards the end of this analysis period). The new process increases the yield per batch by 150Kg.

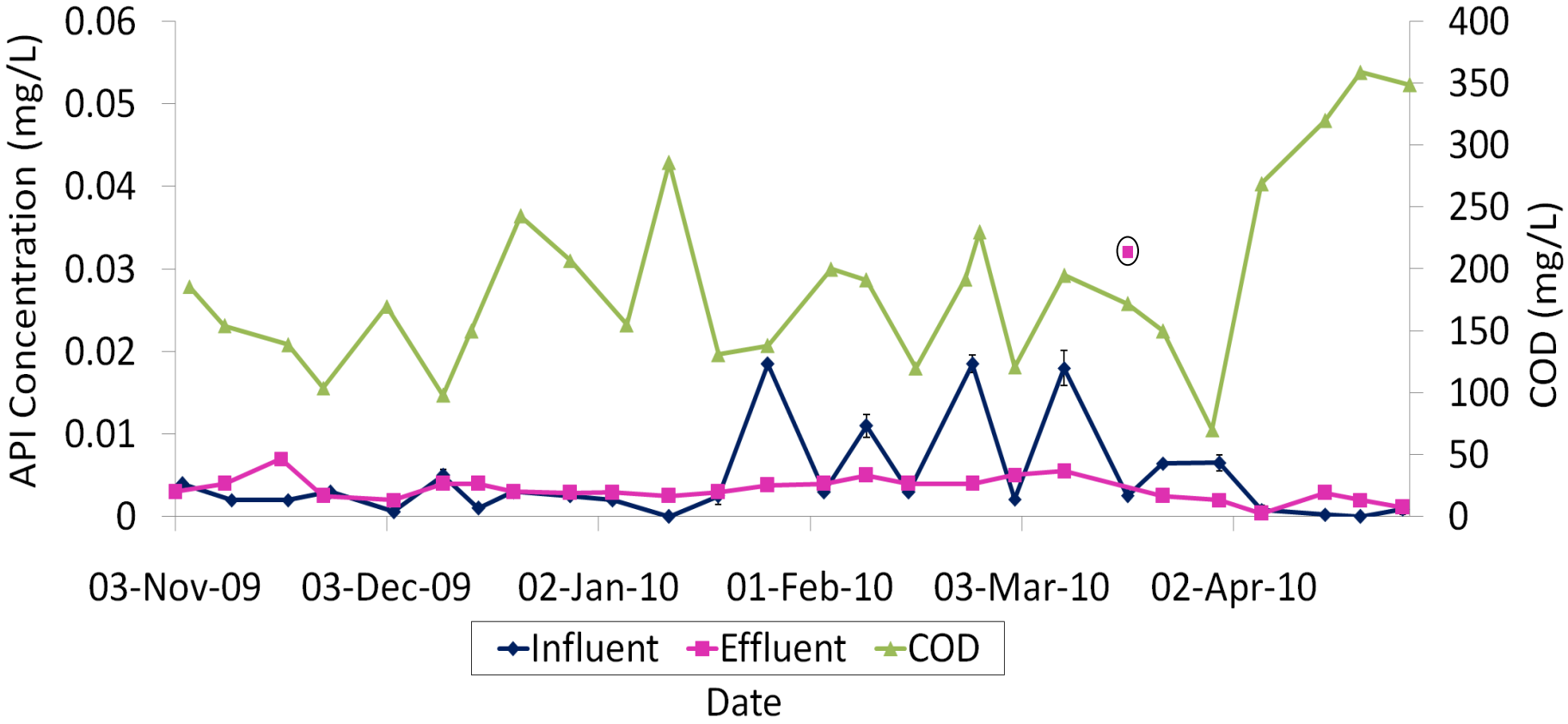


Figure 4.16: Influent and effluent concentrations of tamsulosin hydrochloride from November 2009 to April 2010

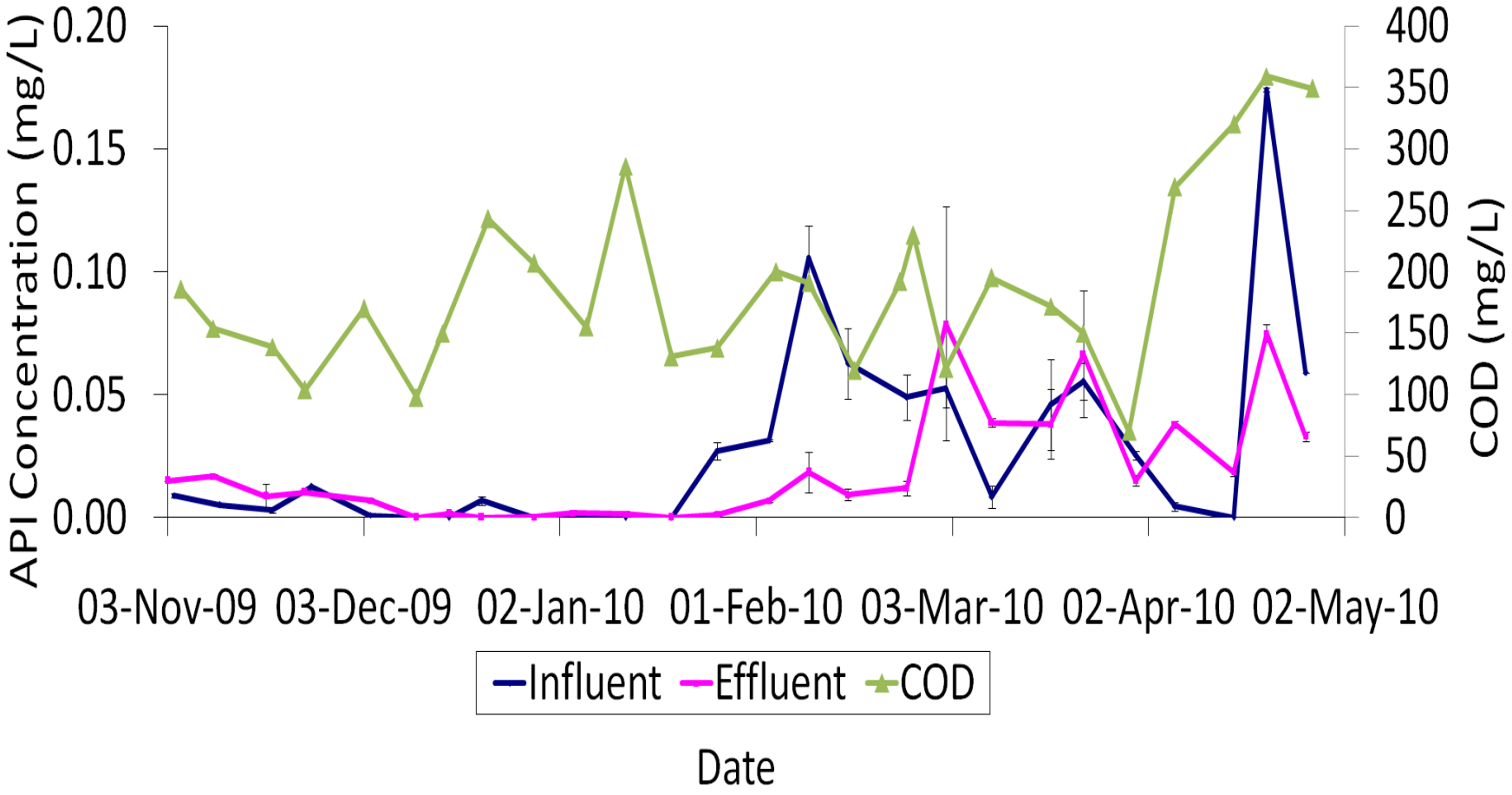


Figure 4.17: Influent and effluent concentrations of solifenacin succinate from November 2009 to April 2010.

4.2.3 Treatment efficiency of onsite treatment plant

The concentrations of the three APIs in influent and effluent appear to be broadly similar. This indicates that the treatment facility (described in Appendix B) is ineffective for the removal of the APIs. The wastewater treatment plant consists of a basic activated sludge tank which is batch fed at night to keep the activated sludge consortium alive although there is no sludge settlement tank or tertiary treatment and no sludge is ever removed from the plant indicating that it may be washed out with the effluent.

4.2.3.1 Efficiency of plant for removal of famotidine

The concentrations of famotidine in the effluent exceeded the concentration in the influent 77% of the time. Influent concentrations had a median value of 1.5 mg/L as compared to 2.0 mg/L in the effluent. The activated sludge in the lagoons is batch fed at night with a feed that is made up in wash water from a step in the famotidine production cycle. According to Astellas concentrations of famotidine in this filtrate were significant, with approximately 20 kg of famotidine dissolved in the 1700 litre filtrate. It is likely that this is the source of the higher concentrations of famotidine in the effluent. Since July 2010, this filtrate is no longer used to make up the feed and the new feed is made up with filtrate that contains about 0.5 kg of famotidine. Generally, high concentrations of the API in the effluent suggest that the treatment facility is ineffective for its removal. Famotidine has been effectively treated at municipal treatment facilities when operated at a longer HRT (up to 14 days) (Clara et al., 2005). Polar drugs like famotidine are generally removed in AS systems by biodegradation (Carballa et al., 2004). Of the three APIs investigated famotidine appears to be the most metabolically resistant. It is released from the body relatively unchanged and has no major metabolites. Metabolically resistant pharmaceuticals tend to be more resistant to AS treatment (Khetan and Collins, 2007).

4.2.3.2 Efficiency of plant for removal of tamsulosin hydrochloride

Tamsulosin hydrochloride remained in the wastewater throughout the monitoring periods at trace concentrations. The median influent and effluent concentrations over the six month period were the same at 0.003 mg/L. The average concentration of tamsulosin in the influent was only marginally higher than the effluent at 0.005 mg/L in comparison to 0.004 mg/L. These results indicate that there was no removal of tamsulosin hydrochloride by the treatment facility.

4.2.3.3 Efficiency of plant for removal of solifenacin succinate

The average concentration of solifenacin was 0.039 mg/L in the influent and 0.028 mg/L in the effluent. This corresponds to an average removal of 28% from November 2009 to April 2010. During the period from November to January solifenacin was out of production and concentrations dropped sharply in the influent but remained high in the effluent. When examining the period when solifenacin was in production, the percentage removal rises to 40%. Polarity of the API appears to be an important factor when considering removal efficiencies from AS plants. Polar drugs adsorb less onto the activated sludge particles. Solifenacin appears to have the highest removal efficiency but is also the most non-polar of the three APIs (Carballa et al., 2004).

4.2.4 Pharmaceutical wastewater regulations

In many parts of the world pharmaceutical wastewater is released into the environment without any treatment (Enick and Moore, 2007). At Astellas Ireland Pharmaceutical Limited the wastewater is treated at onsite wastewater treatment plant and then released to municipal sewer where it is treated again at a municipal sewage treatment plant. Wastewater from pharmaceutical facilities in Europe is subject to regulation through their IPPC licences. These waste licences tend to focus on COD, BOD, nitrates, phosphates and metals. There is little regulation on the releases of APIs. ‘Ireland seems to be the only jurisdiction where regulatory limits have been placed on the release of APIs from industrial facilities’ (Helmig et al., 2007). However, to the authors knowledge Wyeth

Pharmaceutical Ltd. in Newbridge, Co. Kildare is the only production facility with such limits (Table 4.8). There is no such limit on the APIs released by Astellas (IPPC licence, 2006). The levels of famotidine detected in the Astellas wastewater are higher than the discharge limits for all the APIs at the Newbridge facility. However famotidine does not have the same environmental significance as the antibiotics and EDCs produced at the Wyeth facility. Any possible future requirement may not have as low a limit. This highlights the need to assign environmental risk data to drugs to determine if limiting discharge of certain pharmaceuticals to the environment is necessary.

Table 4.8: Discharge limits Wyeth, Newbridge (Molyneaux, 2009).

API	mg/L
Lederle (antibiotic)	1
Tranquilisers	0.5
Oral contraceptives	0.02
Hormone replacement therapy	0.02

4.2.5 Treatment options for the reduction of API concentration in wastewater

Treatment options for the removal of APIs from industrial wastewater are discussed extensively in Chapter 2. The simplest solution for the treatment of APIs is the extension of HRT for the enhanced removal of more metabolically resistant APIs such as famotidine. Retrofitting of existing treatment plants for the enhanced removal of pharmaceuticals is an economic solution. MBRs followed by ozonation have been successfully applied for retrofitting of pharmaceutical facilities (Helmig et al., 2007). Removal of pharmaceuticals from wastewater using photo-Fenton's oxidation has been widely successful in bench scale studies (Ay and Kargi, 2010; Mèndez-Arriaga, 2010; Shemer et al., 2006; Xu et al., 2009). However no reference to photo-Fenton's degradation of the three APIs in this project is available in the literature. A study of the application of Fenton's reaction for the removal of the APIs is presented and discussed in the following sections.

4.3 Fenton's oxidation

4.3.1 Introduction to Fenton's oxidation

Fenton's reaction has been used successfully for the removal of pharmaceuticals from wastewater (Arslan-Alaton and Dogruel, 2004; Pérez-Estrada et al., 2005b; Ravina et al., 2002). Fenton's reagent consists of hydrogen peroxide, iron(II) and light. Iron salts and hydrogen peroxide are inexpensive and readily available. Disadvantages of Fenton's reaction include the need to remove the iron salts following treatment as well as the requirement for pH neutralisation. The main reactions are outlined below many other side reactions occur the exact schematic of these reaction remains a source of some debate.

Hydrogen peroxide alone is a strong oxidant, but is ineffective for the degradation of concentrated refractory compounds. It can be activated using iron salts in the dark Fenton's reaction (Alegría et al., 2003),

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

UV light also reacts with H_2O_2 to create hydroxyl radicals

$$H_2O_2[+UV] \rightarrow 2OH^{\cdot}$$

Important factors in the optimisation of Fenton's conditions include pH, temperature, and concentration of hydrogen peroxide, iron salts and pollutant. The photo-Fentons oxidation process was optimised in terms of complete removal of the parent compound after the first sample was taken using the minimum concentrations of Fe(II) and H_2O_2. An acidic environment is necessary for the decomposition of H_2O_2 due to the abundance of free H^+. The reaction is slowed down at very low pH values (<2.0) due to the formation of complex iron species and formation of the oxonium ion $[H_3O_2]^+$. At higher pH (pH > 4) the generation of hydroxyl radicals gets slower because of the formation of ferric-

hydroxo complexes (Tekin et al., 2006). Generally, the pH value is considered optimum at approximately 3.

$$2Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + 2H_2O$$

The ratio of iron to hydrogen peroxide is important. If the concentration of Fe^{2+} exceeds hydrogen peroxide concentration then chemical coagulation will occur, resulting in the settling out of iron salts and pollutants rather than chemical oxidation (Neyens and Baeyens, 2003)

$$[Fe^{2+}] > [H_2O_2] \text{.....chemical coagulation}$$
$$[Fe^{2+}] < [H_2O_2] \text{.....chemical oxidation}$$

By applying light to the Fenton's process additional hydroxyl radicals are generated and ferric ions are reduced into ferrous. Iron salts absorb light up to approximately 400nm (Figure 4.21), therefore solar light may be used, removing the need for UV lamp (Pérez et al., 2002). The photo-Fenton's process equation is as follows:

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + OH\cdot + H^+$$

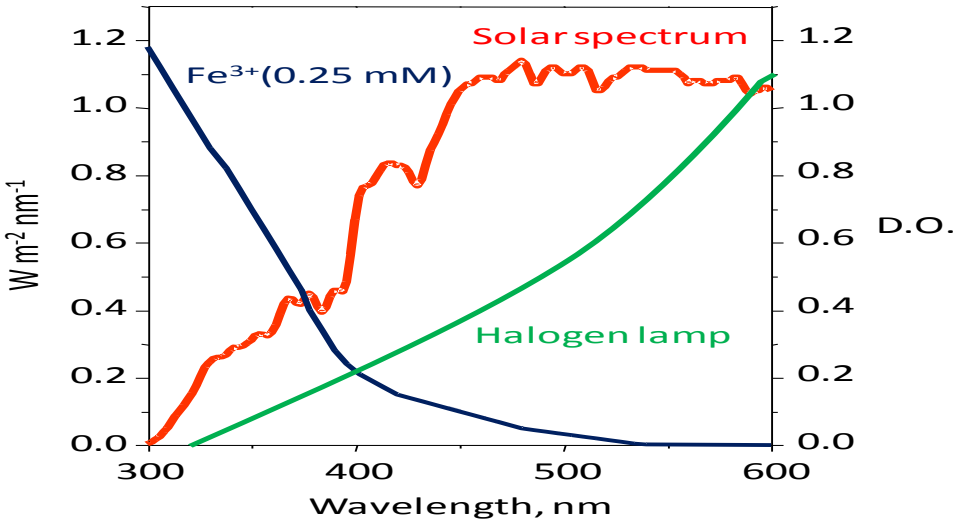


Figure 4.18: Absorbance spectrum of Fe(III) in comparison with solar spectrum and halogen lamp spectrum adapted from Ibáñez et al., 2009.

4.3.2 Kinetic evaluation

The degradation of each of the three pharmaceuticals predominantly exhibits a mono-exponential trend and so pseudo-first order kinetics can be applied. The pseudo-first order rate equation is:

$$\ln(\frac{C_0}{C}) = k_{app}t$$

where, k_{app} is the apparent pseudo-first order rate constant, C_0 is the initial concentration prior to the addition of H_2O_2 and t is the reaction time. A plot of $\ln(C_0/C)$ vs t gives a straight line with the slope of k_{app} the pseudo first order rate constant. The half-life ($t_{1/2}$) expresses the time (min) it takes for half of the initial concentration of the API to degrade.

$$t_{1/2} = \ln(2)/k_{app}$$

k_{app} and $t_{1/2}$ are used to compare the efficiency of different reactions and concentrations of reactants for the optimisation of removal techniques (Keane et al., 2011).

As discussed later in this chapter, at lower iron(II) concentrations the reaction fits the first order equation well with R values >0.95. This was due to an excess of H_2O_2. The cycling of Fe^{3+} back to Fe^{2+} by the halogen lamp may have been the rate determining step making Fe^{2+} the rate limiting reactant. As the iron(II) concentration was increased the R values generally decreased indicating that the reaction was fitting the pseudo-first order equation less well and moving more towards a second order model.

4.3.3 Choosing a quenching agent

4.3.3.1 Demonstrating the requirement for a quenching agent

Fenton's reaction is self-propagating with the cycling of ferric and ferrous iron together with hydrogen peroxide creating radicals. Therefore after a sample has been taken the reaction requires quenching. Failure to do this leads to inaccurate results if the analysis is not performed immediately in a time-controlled manner (Figure 4.22). However, simply controlling the time over which the analysis is performed is not ideal. Analytical issues arise such as difficulty with the analysis of the first few minutes of an experiment, the inability to rerun samples and practicalities relating to the availability of instruments. Some commonly used agents in the quenching of Fenton's experiment include methanol, acetone, sodium sulphite and sodium thiosulphate (Chu, 2005; Karimi, 2009; Khan et al., 2009; Zimbron and Reardon, 2009). All quenching experiments were carried out at 0.1 mM famotidine, 0.012 mM Fe(II) and 20 mM H_2O_2.

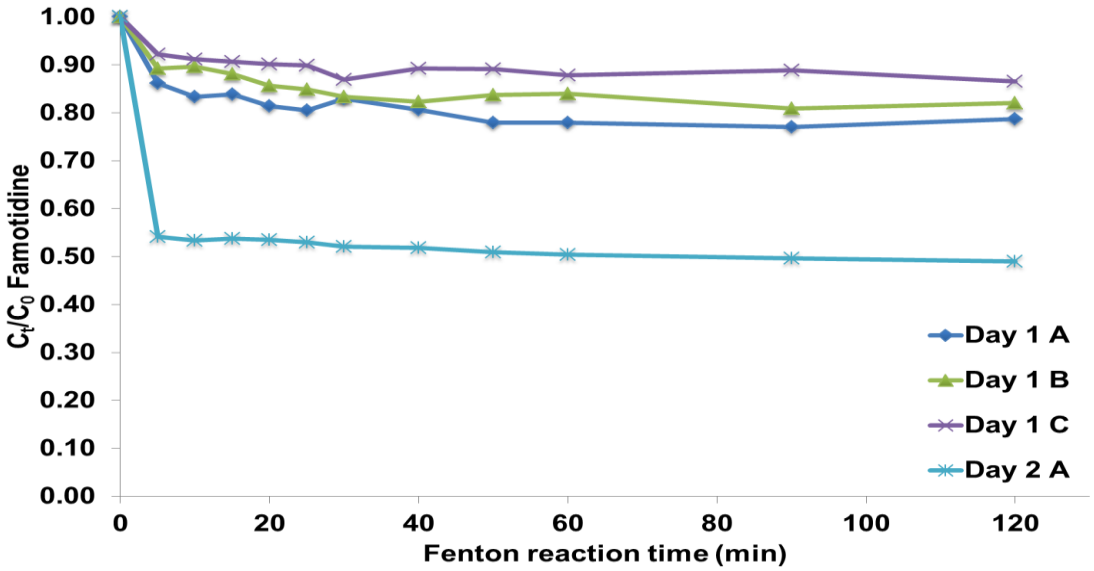


Figure 4.19: Famotidine experiment analysed on the same day vs. the following day. Three replicate experiments were carried out, all three were analysed on the same day by HPLC with about 20% removal noted. One experiment was analysed again after 18 hours. Famotidine was detected in lower concentrations (approx. 45% removal).

4.3.3.2 Sodium sulphite as a quenching agent

The oxygen scavenging agent sodium sulphite was tested as a quenching agent. One drop of 20% w/v sodium sulphite solution per 1 mL of sample was added to samples taken during photo-Fenton's oxidation. Results showed that rather than stopping the reaction the sodium sulphite enhanced degradation of famotidine as compared to unquenched samples. Samples were analysed immediately, were kept in the dark at 4°C for 18 hours and analysed again by HPLC. Samples analysed immediately had 79% of the initial concentration remaining and after 18 hours famotidine was no longer detectable in the sample.

4.3.3.3 Methanol as a quenching agent

Methanol was also investigated as a possible quenching agent. Quenching of samples using 0.1 to 1 mL of methanol per 1 mL of sample showed that concentrations of greater than 1 mL methanol per 1 mL of sample would be required (Figures 4.23 and 4.24). At 1 mL methanol peak shape was severely affected during chromatographic analysis. Therefore methanol was not considered a suitable quenching agent for use with the famotidine HPLC method used.

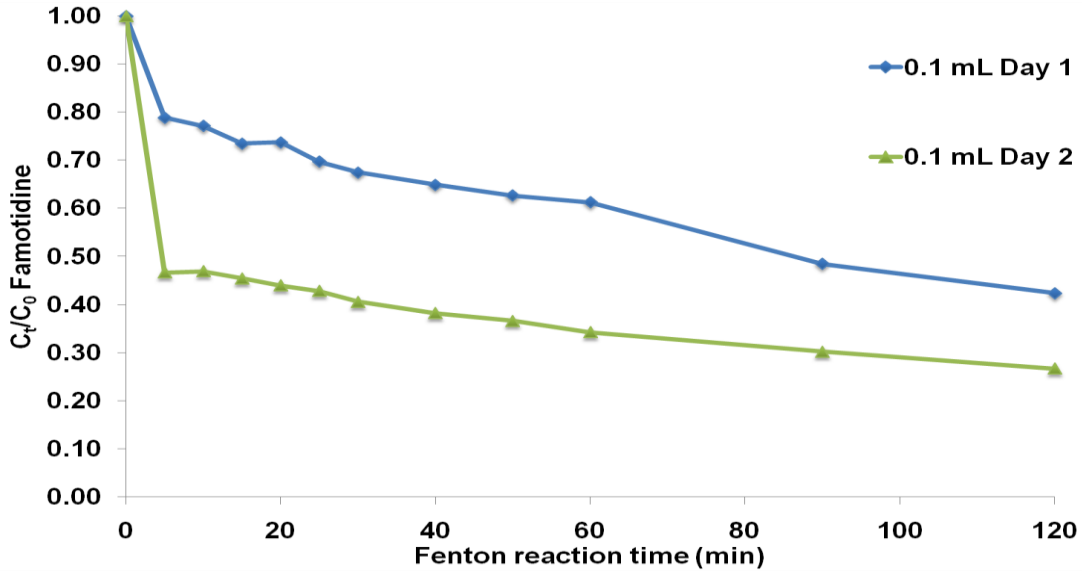


Figure 4.20: Effect of methanol on quenching. 0.1 mL methanol was unsuccessful for the quenching of 1 mL of sample.

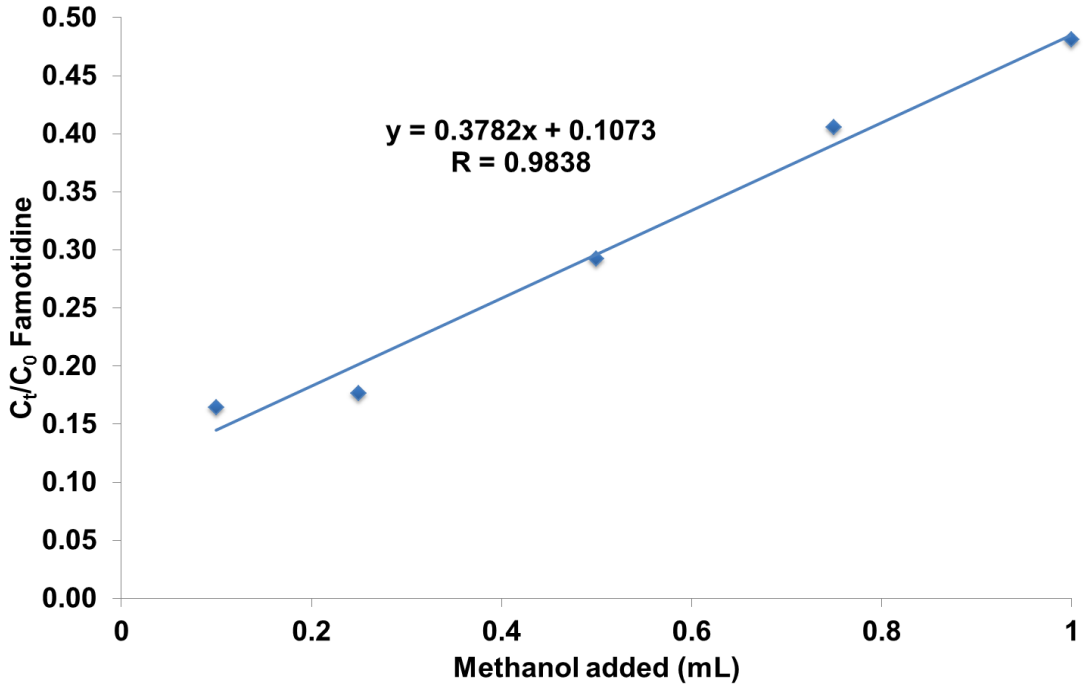


Figure 4.21: Methanol added vs C_t/C_0 for 5min sample exhibits a linear relationship. Assuming C_t/C_0 of 0.8 (from 5min sample for day 1 in Figure 4.23) is fully quenched then 1.8 mL of methanol would be required per mL of sample taken to quench the reaction.

4.3.3.4 Time-controlled experiment

Due to the difficulty finding a suitable quenching agent, no quenching agent was used and the analysis was run in a strict time-controlled manner (Figure 4.25). The chromatographic run was started at 35min into the Fenton's experiment, beginning with two blanks. Run time for each of the three analytes using individual methods was 6 minutes. While good reproducibility was achieved this method was not considered ideal. There is a number of practical considerations around the use of the HPLC, such as the inability to rerun samples or the invalidity of an experiment if the HPLC run stopped. Of particular importance is difficulty in monitoring the first few minutes of the experiment, the identification of relatively short living intermediates and kinetic analysis of results. However due to the difficulty in identifying a suitable quenching agent it was decided to proceed with the time-controlled method. A large decrease between the 0 min sample and the 5 min sample for famotidine suggested that degradation was

continued in the time between sampling and analysis on HPLC. For all further reactions for famotidine graphs take into account the time between sampling and analysis. A significant reduction was not noted between 0 and 5 minute samples for tamsulosin and solifenacin.

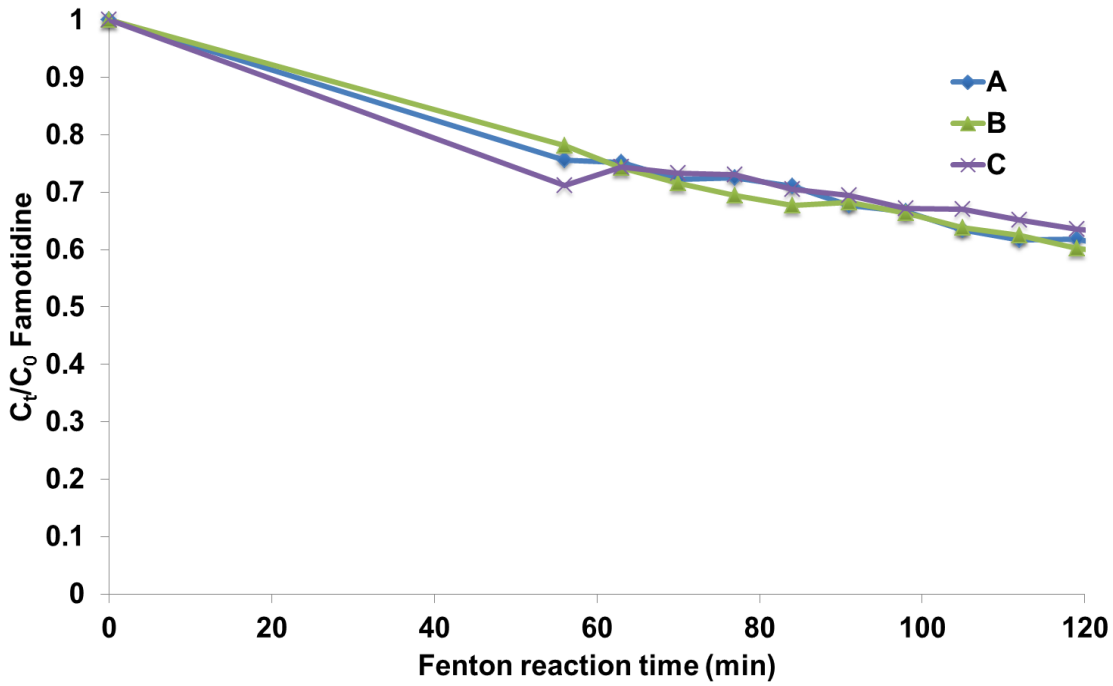


Figure 4.22: Repeat experiments conducted in time-controlled manner gave reproducible results.

4.3.4. Optimisation of Fenton's oxidation for famotidine removal

4.3.4.1 The effect of different reactants on famotidine degradation

Experiments to determine the effect of the different reactants in the photo-Fenton's process showed that photolysis, iron(II) (12µM) with and without light resulted in no degradation (Figure 4.26). The famotidine/Fe(II) solution was stable in the light or dark at pH3. Any degradation in any of the Fenton's processes was only seen on the addition of the hydrogen peroxide. At 20 mM hydrogen peroxide 11% of the famotidine was removed. A marginal improvement was observed with the use of the halogen lamp (approx. 5% after 120min). Dark Fenton's oxidation showed more than double the degradation

than hydrogen peroxide alone at 20 mM H_2O_2. Again a marginal increase in removal was noted with the use of the halogen lamp possibly due to the decomposition of hydrogen peroxide to hydroxyl radicals (approx. 5% after 120min). There is an abundance of literature which describes the use of a UV light source in the degradation of a range of micro-pollutants by photo-Fenton's oxidation due to much higher degradation rate with the use of UV lamps (Feng et al., 2005; Mèndez-Arriaga, 2010; Shemer et al., 2006; Trovo et al., 2008). The UV lamp provides greater decomposition of hydrogen peroxide to hydroxyl radicals and enhanced cycling of ferric and ferrous iron. A halogen lamp was used in this scenario to mimic sunlight for a low energy, low cost alternative. Degradation of famotidine by the processes tested were in the sequence iron(II)/H_2O_2/Light (25% removal at 60 min) > iron(II)/H_2O_2 (23% removal at 60 min) > H_2O_2/Light (12% removal at 60 min) > H_2O_2 (11% removal at 60 min) > iron(II) (0% removal at 60 min), iron(II)/light (0% removal at 60 min) and photolysis (0% removal at 60 min) (Figure 4.26).

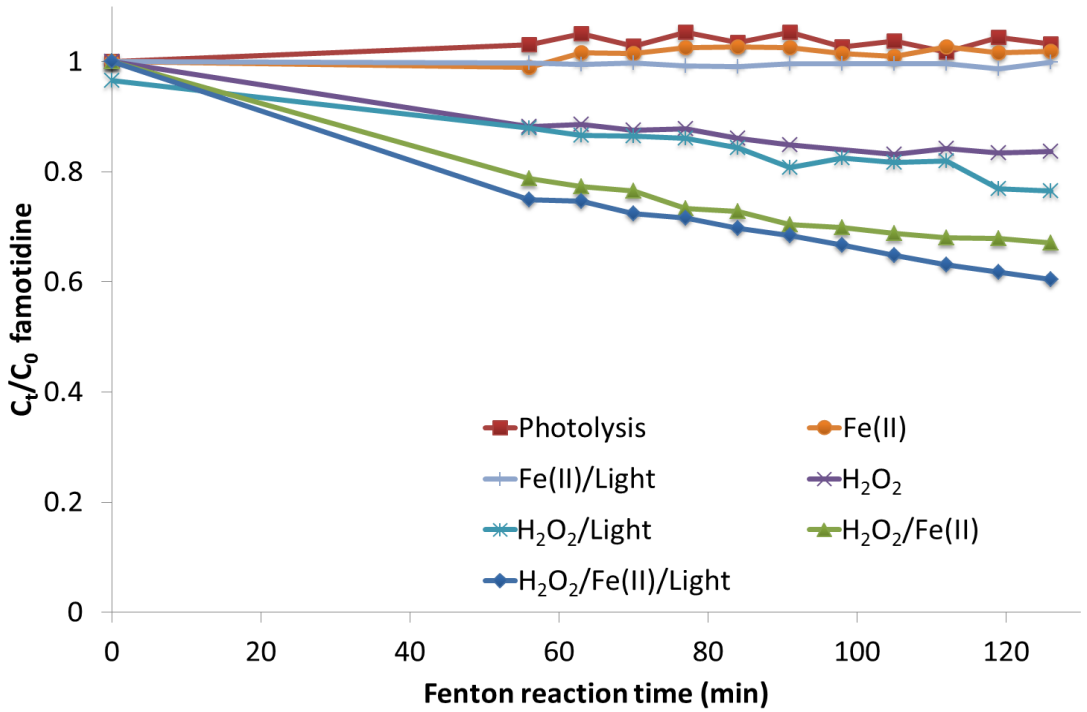


Figure 4.23: Effect of different reactants on famotidine degradation.

4.3.4.2 Famotidine light-Fenton's reactions

Photo-Fenton's optimisation began with varying Fe(II) concentrations as it is normally considered the limiting reactant (Pérez et al., 2002). Concentrations monitored were 0.012 mM, 0.03 mM, 0.06 mM, 0.09 mM and 0.12 mM Fe(II) at 20 mM hydrogen peroxide (Figure 4.27). Following initial optimisation of Fe(II) concentration, the high H_2O_2 concentration was reduced with small increases in Fe(II) concentration to ensure optimal removal was maintained e.g. 0.3 mM Fe(II) increase corresponds to a 10 mM decrease in H_2O_2. Concentrations monitored ranged between 0.12-0.18 mM Fe(II) and 5-20 mM H_2O_2 (Figure 4.26). The highest removal was seen in the sequence 0.18 mM Fe(II)/5 mM H_2O_2 (99% removal at 60 min), 0.12 mM Fe(II)/20 mM H_2O_2 (99% removal at 60 min) > 0.15 mM Fe(II)/10 mM H_2O_2 (98% removal at 60 min) > 0.15 mM Fe(II)/5 mM H_2O_2 (97% removal at 60 min) > 0.09 mM Fe(II)/20 mM H_2O_2 (90% removal at 60 min) > 0.12 mM Fe(II)/10 mM H_2O_2 (75% removal at 60 min) > 0.06 mM Fe(II)/20 mM H_2O_2 (66% removal at 60 min) > 0.03 mM Fe(II)/20 mM H_2O_2 (51% removal at 60 min) > 0.012 mM Fe(II)/20 mM H_2O_2 (25% removal at 60 min) (Figures 4.27 and 4.28).

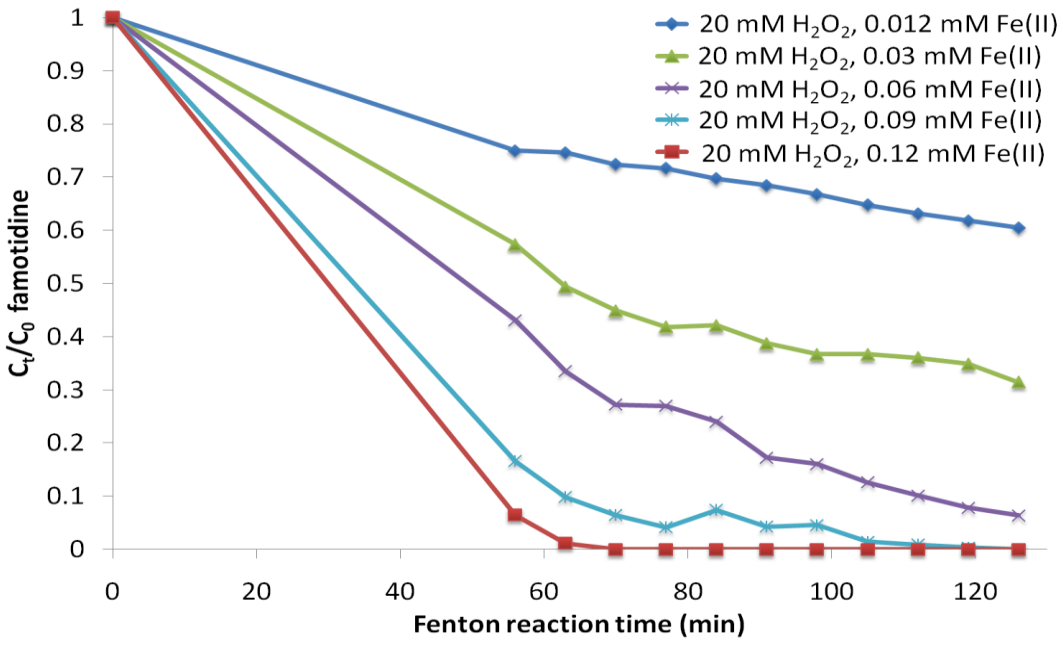


Figure 4.24: Famotidine degradation at different Fe(II) concentrations.

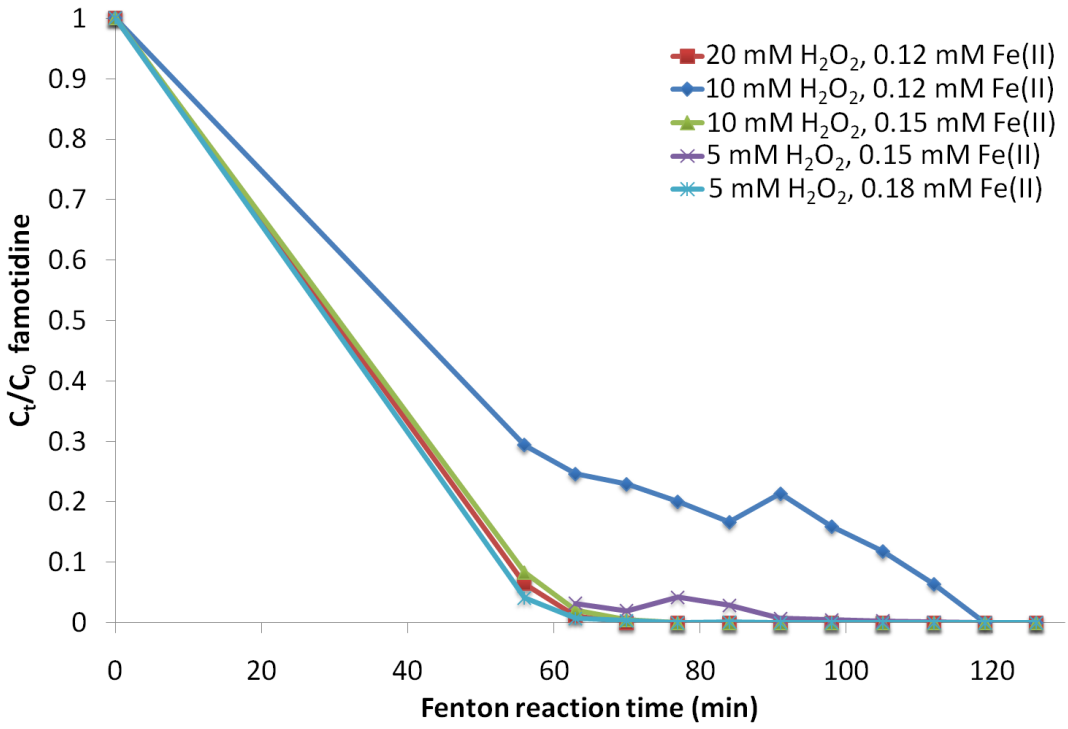


Figure 4.25: Famotidine degradation at different H_2O_2/Fe(II) concentrations

4.3.4.3 Kinetic evaluation of results

In this kinetic evaluation of results the effects of changes in initial concentrations of Fe(II) and H_2O_2 on k_{app} and $t_{1/2}$ were investigated. Degradation of famotidine using photo-Fenton's and dark-Fenton's oxidation follows pseudo-first-order degradation. The role of different Fe(II) concentrations was first investigated at 20 mM H_2O_2 and 0.1 mM famotidine. Fe(II) was varied between 0.012 mM Fe(II) and 0.120 mM Fe(II). The degradation follows the pseudo-first-order kinetic model (Figure 4.29). A graph (Figure 4.30a) of Fe(II) concentration vs. k_{app} is exponential (R=0.9774). Fe(II) is important in initiating the generation of HO· radicals. A graph of Fe(II) concentration vs $t_{1/2}$ drops exponentially with the addition of Fe(II) (R=0.9562) (Figure 4.30b). The half-life, $t_{1/2}$ is the length of time in minutes it takes half the famotidine to react.

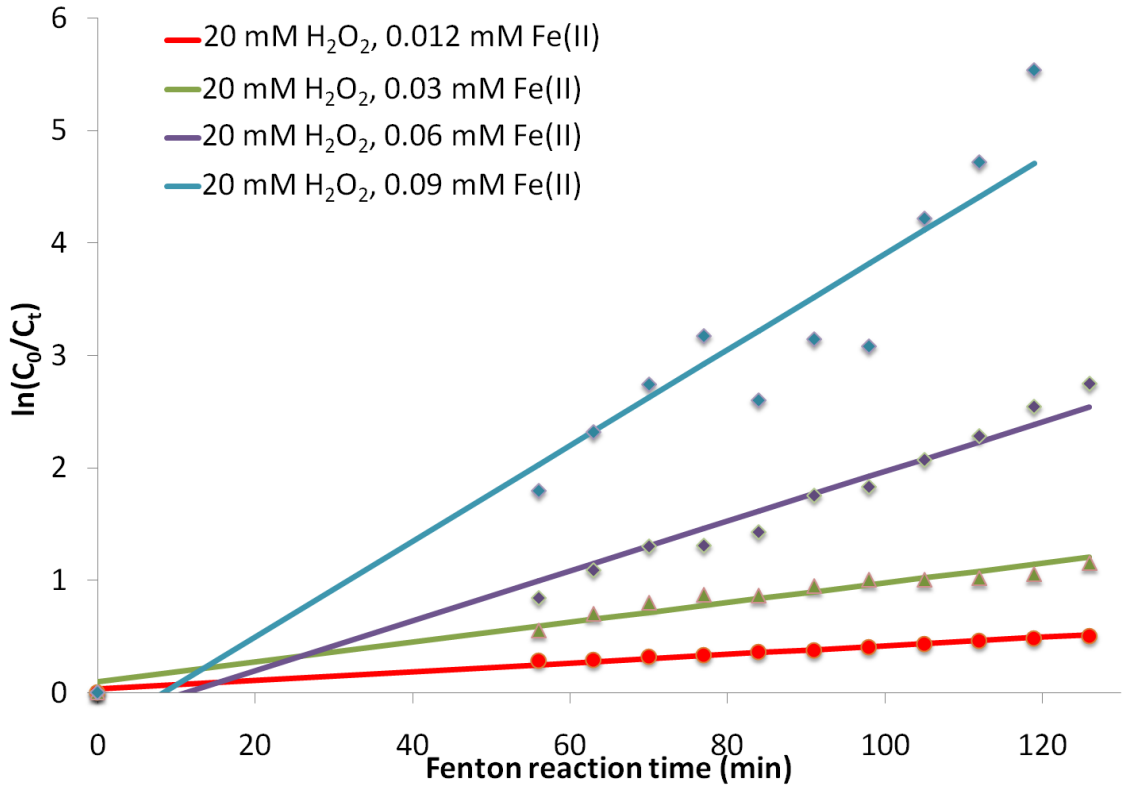
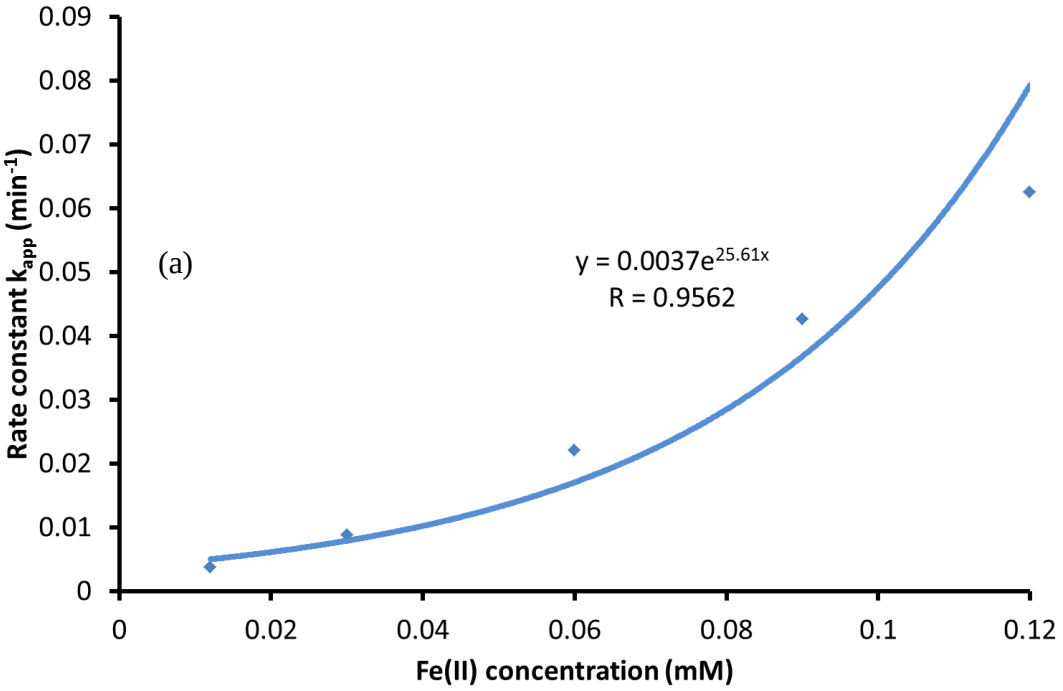


Figure 4.26: Pseudo-first-order kinetics, different Fe(II) concentrations (0.012 mM – 0.120 mM Fe(II)), 20 mM H_2O_2.



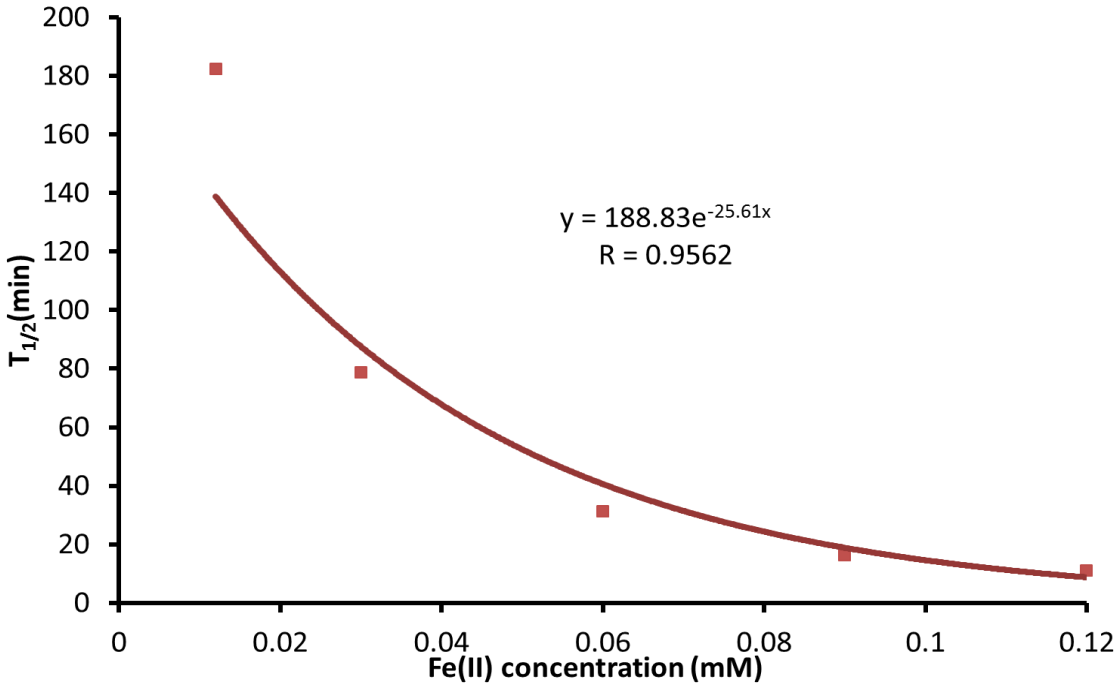


Figure 4.27: Famotidine (a) Effect of Fe(II) concentration on k_{app}, exponential relationship noted between addition of Fe(II) and k_{app}. (b) Effect of Fe(II) concentration on $t_{1/2}$. Inverse exponential trend between $t_{1/2}$ and addition of Fe(II).

Fe(II) optimisation was conducted with an excess of H_2O_2. Following initial optimisation of Fe(II), concentrations of H_2O_2 were reduced (Figure 4.31). When H_2O_2 concentrations were decreased the % removal of famotidine also decreased. In order to maintain optimum degradation, concentrations of Fe(II) were increased. Concentrations of varying Fe(II) and H_2O_2 between 0.12–0.15 mM Fe(II), 5-20 mM H_2O_2 were investigation during the optimisation process, only one concentration was changed at a time. Large decreases in H_2O_2 required only minor increases in Fe(II) to maintain optimum degradation e.g. 0.3 mM Fe(II) increase corresponds to a 10 mM decrease in H_2O_2.

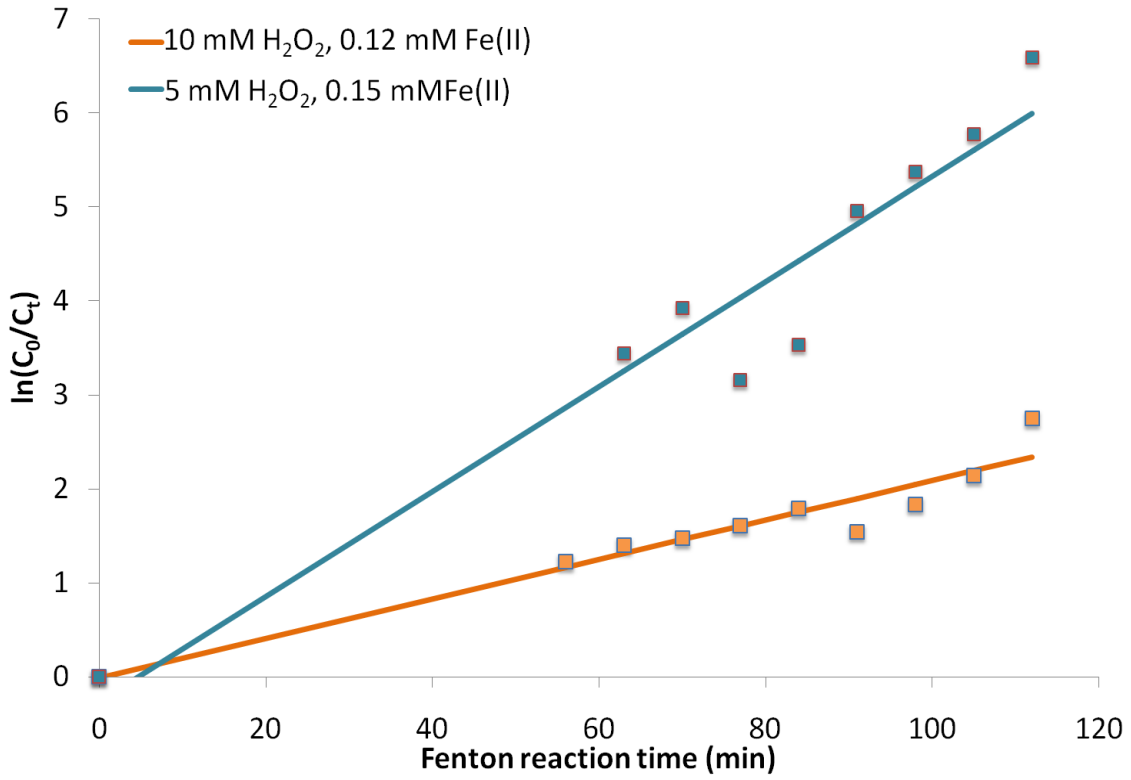


Figure 4.28: First order kinetics, different Fe(II) concentrations (0.12 mM – 0.15 mM Fe(II)), 5-10 mM H_2O_2.

4.3.5 Optimisation of Fenton's oxidation for removal tamsulosin hydrochloride

4.3.5.1 Light-Fenton's degradation of tamsulosin hydrochloride

Optimisation of tamsulosin hydrochloride degradation by photo-Fenton's oxidation began at the optimum concentration for famotidine removal at 0.12 mM Fe(II), 5 mM H_2O_2, which showed complete removal after the first sample was taken. Different concentrations of Fe(II) were investigated between 0.03 and 0.09 mM Fe(II). Optimum degradation was in the sequence 0.09 mM Fe(II) (99.7% removal at 10 min) > 0.06 mM Fe(II) (93.5% removal at 10 min) > 0.045 mM Fe(II) (49% removal at 10 min) > 0.03 mM Fe(II) (42% removal at 10 min) (Figure 4.32).

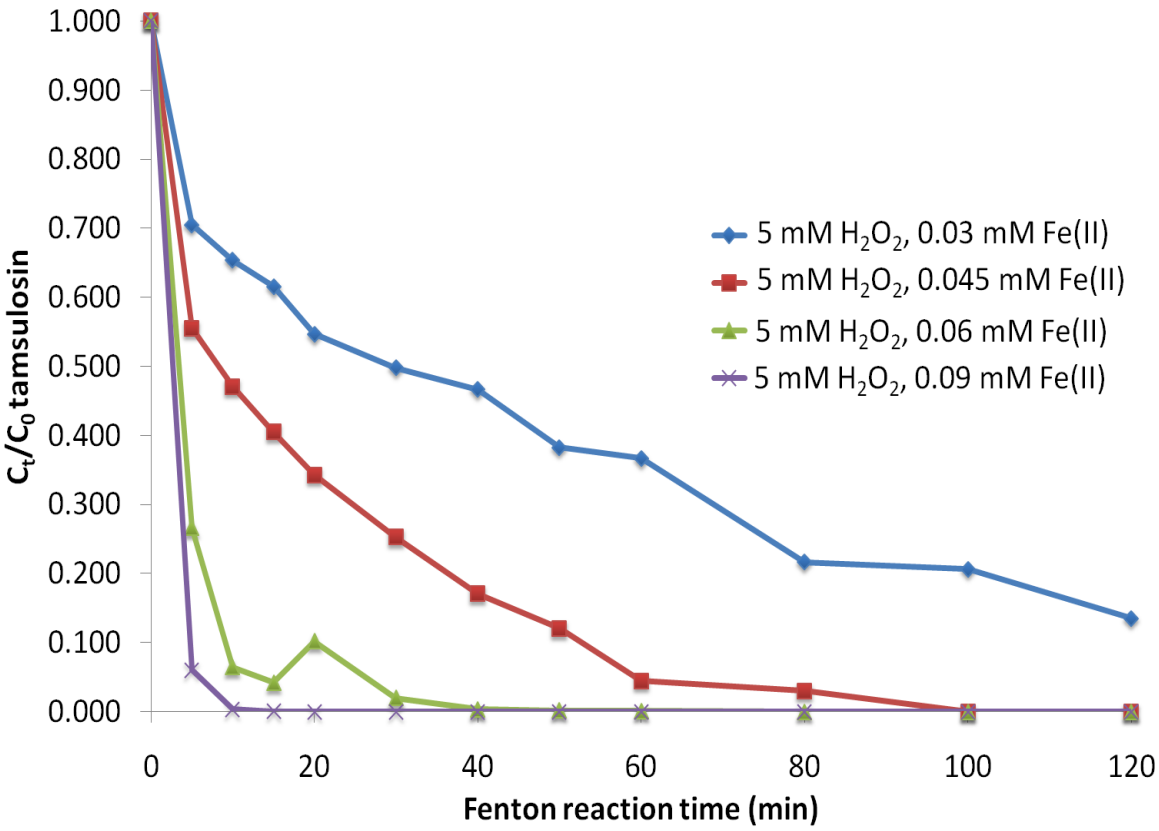


Figure 4.29: Effect of different Fe(II) concentrations on tamsulosin hydrochloride (100 µM) degradation by photo-Fenton's oxidation

4.3.5.2 The effect of different reactants on tamsulosin hydrochloride degradation

At the optimum concentration for the removal of tamsulosin hydrochloride 0.09 mM Fe(II)/5 mM H_2O_2/light the effect of the different reactants in the photo-Fenton's process were investigated. No or extremely low degradation of tamsulosin was noted at 0.09 mM Fe(II), Fe(II)/Light, 5 mM H_2O_2, H_2O_2/Light or light (Figure 4.33). At 5 mM H_2O very limited oxidation of tamsulosin hydrochloride occurred. This highlights that H_2O_2 at reasonable concentrations is not sufficient for the removal of tamsulosin hydrochloride from aqueous solutions, with or without the addition of light. Similarly to famotidine in Figure 4.24, Fe(II) and Fe(II)/Light did not degrade the pharmaceutical and Fe(II)/tamsulosin solutions were stable. When Fe(II) and H_2O_2 were combined the removal increased dramatically to 4% remaining at 10 min. Photo-Fenton's oxidation improved removal to 0.3% remaining.

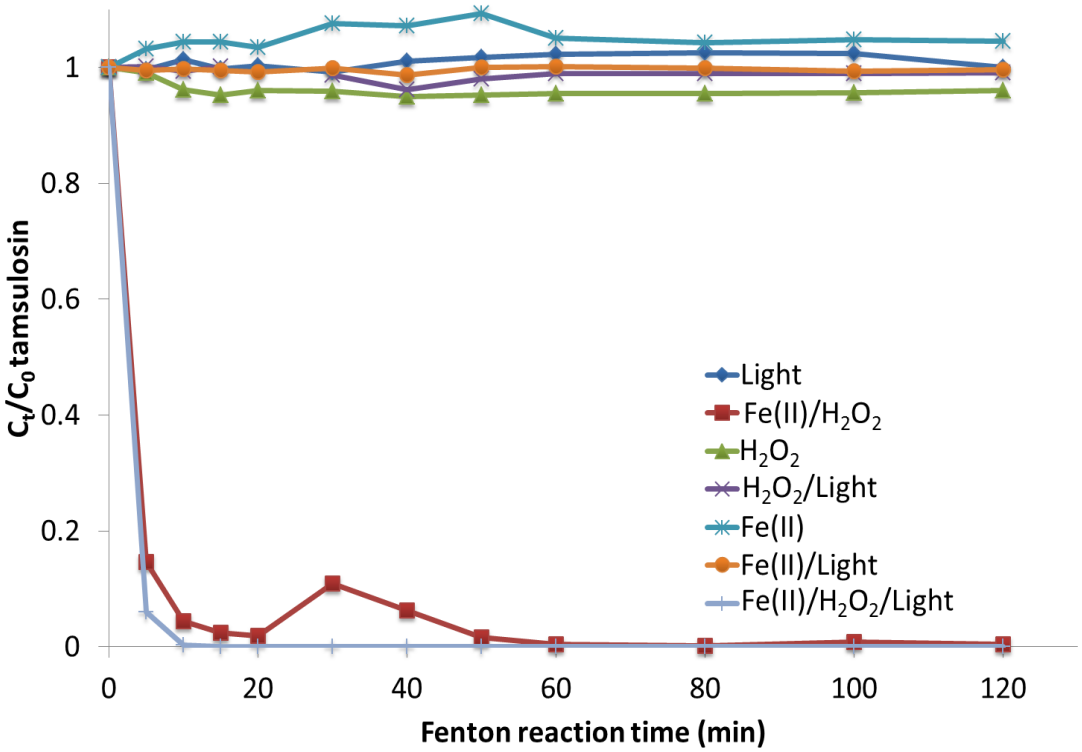


Figure 4.30: Effect of different reactants on tamsulosin hydrochloride degradation.

4.3.5.3 Kinetic evaluation of results

In this kinetic evaluation of results the effects of changes in initial concentrations of Fe(II) on k_{app} and $t_{1/2}$ were investigated. Degradation of tamsulosin hydrochloride using photo-Fenton's and dark Fenton's oxidation follows pseudo-first-order degradation. The role of different Fe(II) concentrations was first investigated at 5 mM H_2O_2 as this was the optimum determined from famotidine degradation studies and 0.1 mM tamsulosin hydrochloride. Fe(II) was varied between 0.012 mM Fe(II) and 0.09 mM Fe(II). The degradation follows the pseudo-first-order kinetic model (Figure 4.34). A graph (Figure 4.35a) of Fe(II) concentration vs. k_{app} shows exponential increase in k_{app} with Fe(II) concentration (R 0.9965). Famotidine showed a linear graph rather than the exponential seen here for tamsulosin. The conditions for famotidine used 20 mM H_2O_2 whereas 5 mM H_2O_2 was used in this set of experiments. H_2O_2 was present at much higher concentrations in the famotidine set of experiments. A graph (Figure 4.35b) of Fe(II) concentration vs. $t_{1/2}$ (min) exhibits inverse exponential degradation (R=0.9965).

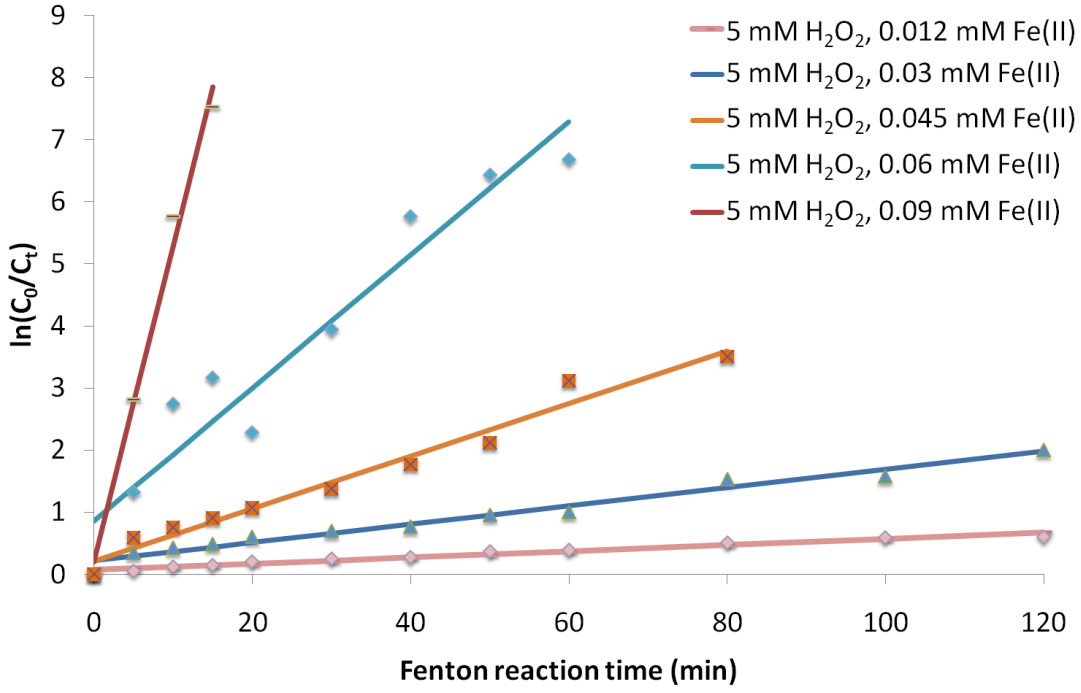


Figure 4.31: Pseudo-first-order kinetics, different Fe(II) concentrations (0.012 mM – 0.09 mM Fe(II)), 5 mM H_2O_2.

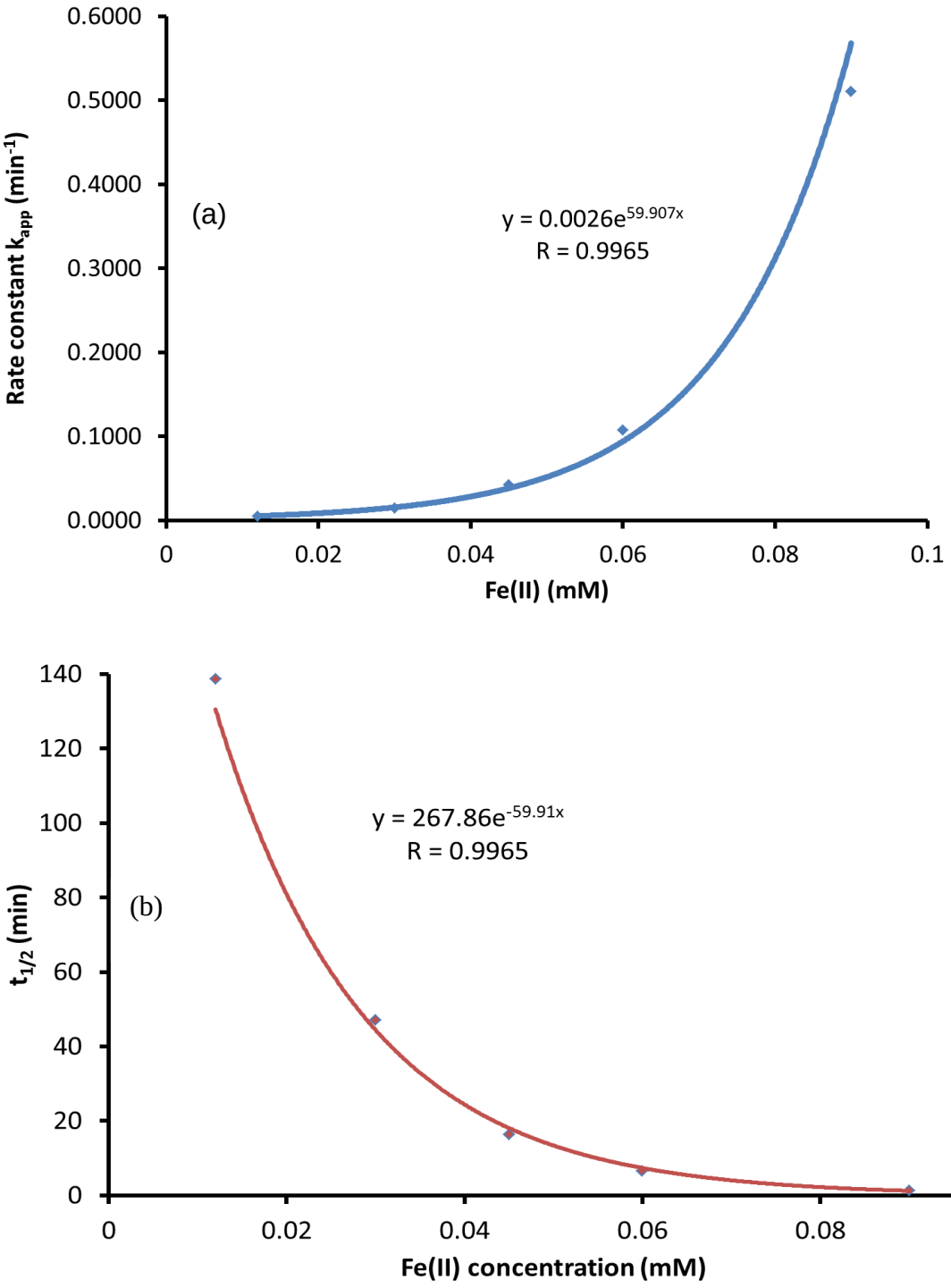


Figure 4.32: (a) Effect of Fe(II) concentration on k_{app}. An exponential relationship exists between k_{app} and the addition of Fe(II). (b) Effect of Fe(II) concentration on $t_{1/2}$. An inverse exponential plot is noted between $t_{1/2}$ and addition of Fe(II).

4.3.6 Optimisation of Fenton's oxidation for removal solifenacin succinate

4.3.6.1 Light-Fenton's degradation of solifenacin succinate

Optimisation of solifenacin succinate began at the optimum concentration for famotidine removal at 0.12 mM Fe(II), 5 mM H_2O_2, which showed complete removal after the first sample was taken. Different concentrations of Fe(II) were investigated between 0.03 and 0.09 mM Fe(II) (Figure 4.36). Optimum degradation was in the sequence 0.09 mM Fe(II), 5 mM H_2O_2 (100% removal at 10 min) > 0.06 mM Fe(II), 5 mM H_2O_2 (93% removal at 10 min)> 0.045 mM Fe(II), 5 mM H_2O_2 (86% removal at 10 min) >> 0.03 mM Fe(II), 5 mM H_2O_2 (52% removal at 10 min). Degradation at 0.03 mM Fe(II), 5 mM H_2O_2 was significantly lower than 0.045 mM Fe(II), 5 mM H_2O_2.

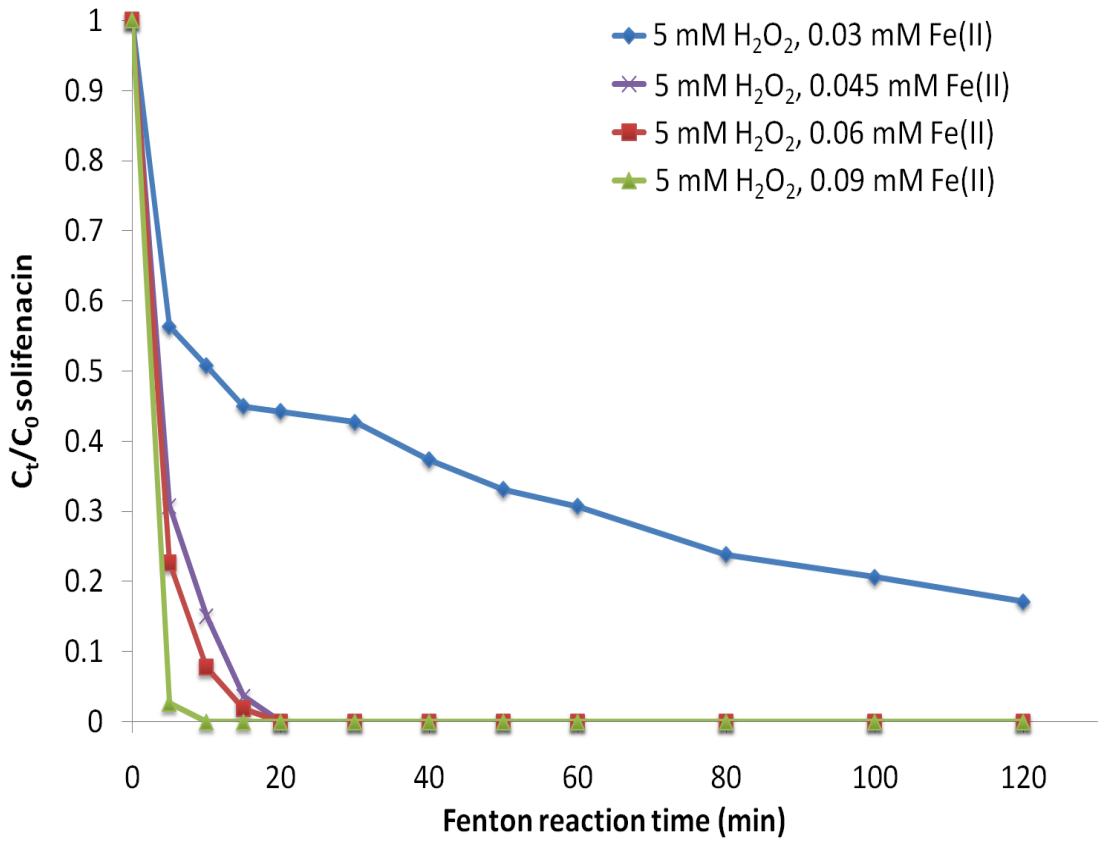


Figure 4.33: Degradation of solifenacin succinate using Fenton's oxidation.

4.3.6.2 The effect of different reactants on solifenacin succinate degradation

At the optimum concentration for the removal of solifenacin succinate 0.09 mM Fe(II)/5 mM H_2O_2/light the effect of the different reactants in the photo-Fenton's process was investigated. At 0.09 mM Fe(II), Fe(II)/light, 5 mM H_2O_2, H_2O_2/light or light no degradation of solifenacin was observed (Figure 4.37). As was the case with tamsulosin at 5 mM H_2O_2 negligable oxidation of solifenacin succinate occurred. Similarly to both famotidine and tamsulosin in Figure 4.24 and Figure 4.31, Fe(II) and Fe(II)/light did not degrade the pharmaceutical and Fe(II)/solifenacin solutions were stable. The addition of H_2O_2 and Fe(II) in combination dramatically increased to 100% with or without the addition of light.

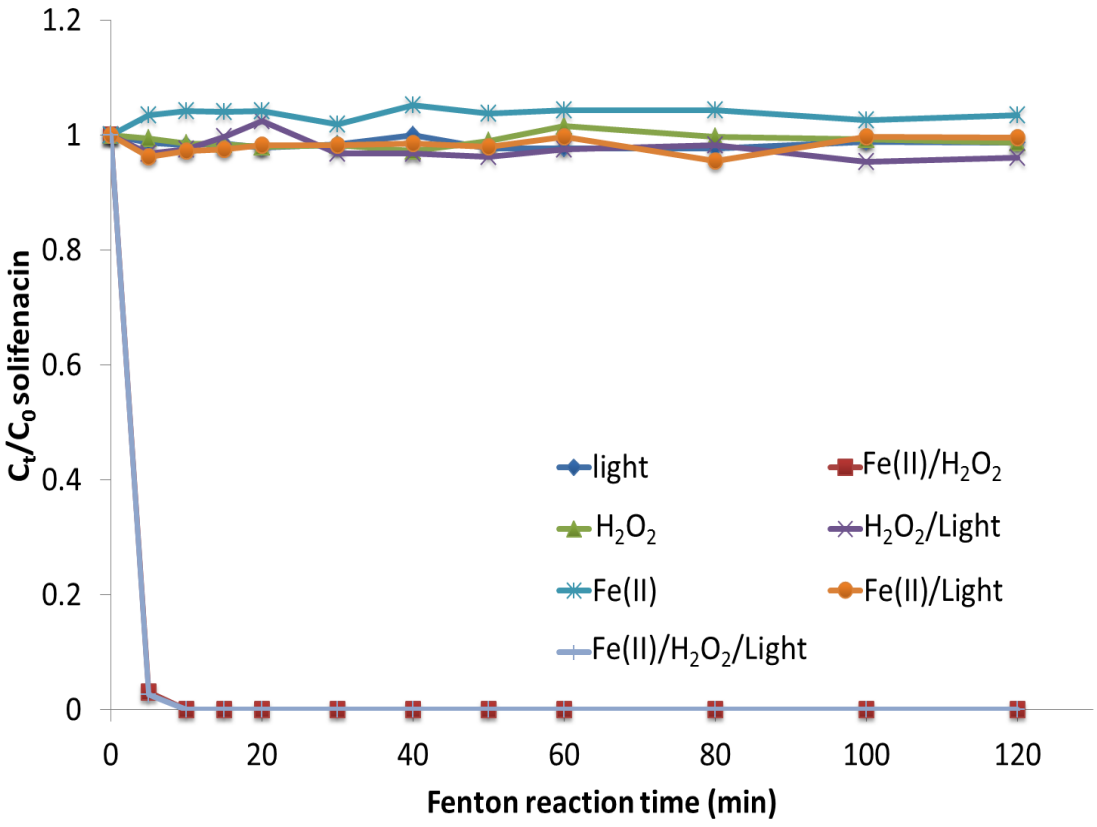


Figure 4.34: Effect of different reactants on solifenacin succinate degradation.

4.3.6.3 Kinetic evaluation of results

In this kinetic evaluation of results the effects of changes in initial concentrations of Fe(II) on k_{app} and $t_{1/2}$ were investigated. Degradation of solifenacin succinate using photo-Fenton's and Fenton's oxidation follows pseudo-first-order degradation. The role of different Fe(II) concentrations was first investigated at 5 mM H_2O_2 as this was optimum determined from famotidine and tamsulosin hydrochloride degradation studies and 0.1 mM solifenacin succinate. Fe(II) was varied between 0.03 mM Fe(II) and 0.06 mM Fe(II). The degradation follows the pseudo-first-order kinetic model (Figure 4.38). 0.03 mM Fe(II) had a much lower k_{app} than both 0.045 and 0.06 mM Fe(II). Figure 4.39 shows (a) effect of Fe(II) concentration on $k_{app.}$ and (b) effect of Fe(II) concentration on $t_{1/2}$.

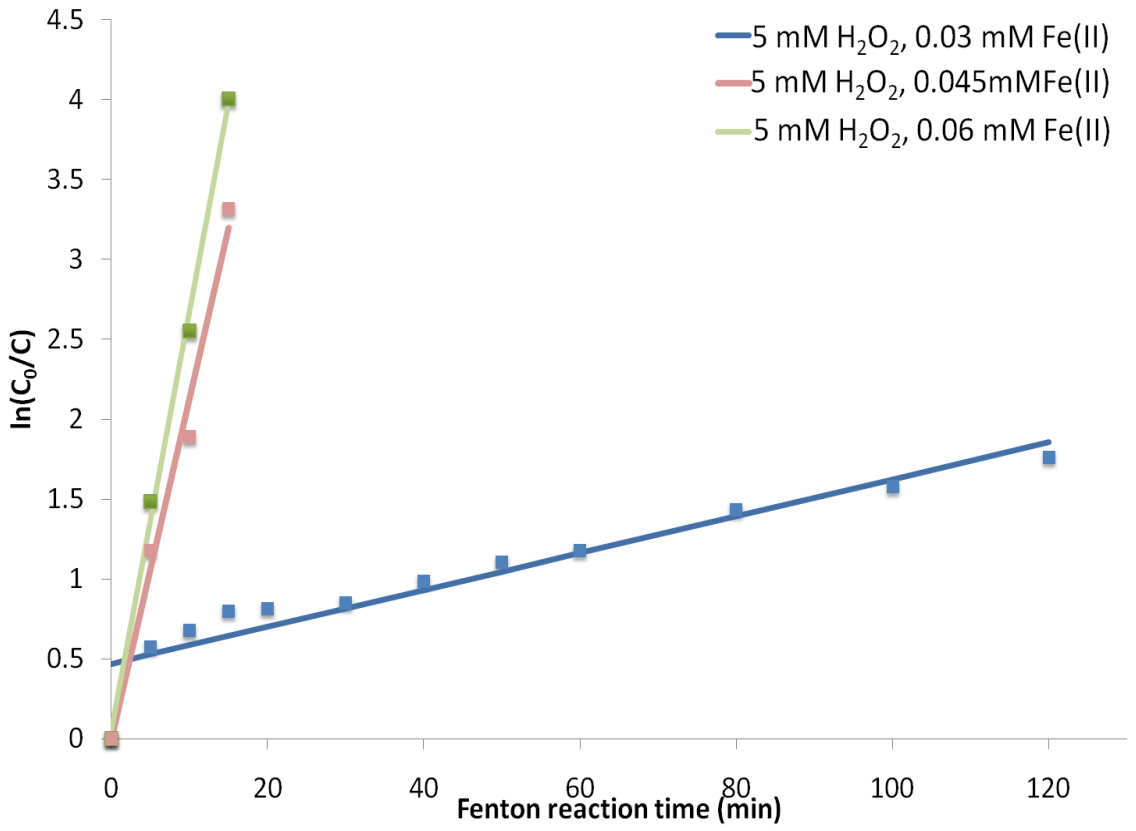
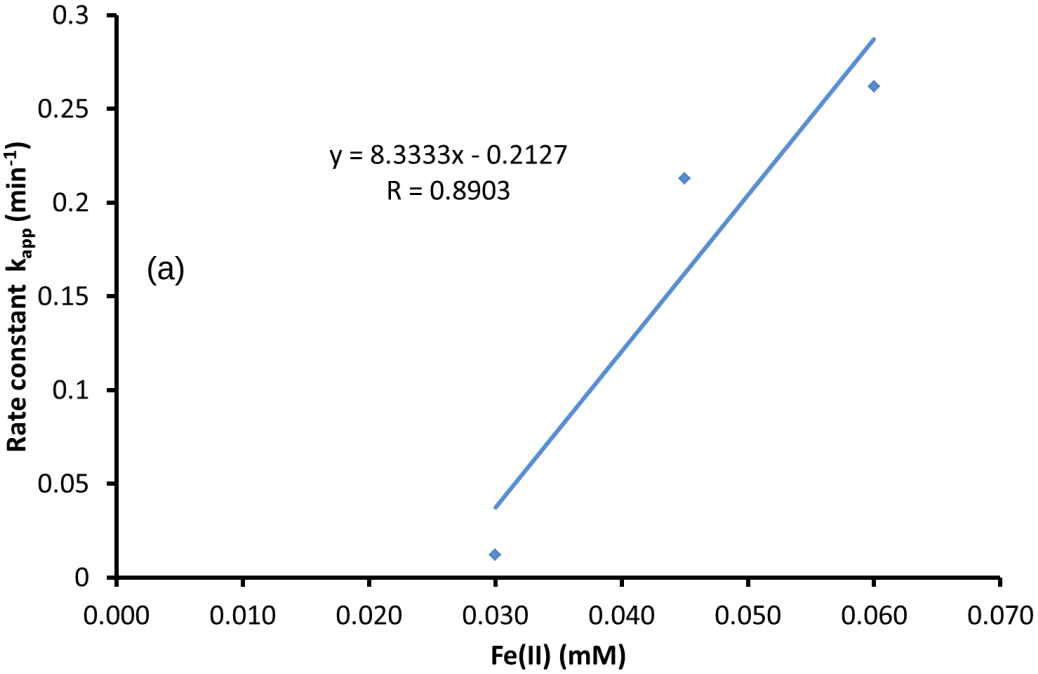


Figure 4.35: First order kinetics, different Fe(II) concentrations (0.3mM – 0.06mM Fe(II)), 5mM H_2O_2.



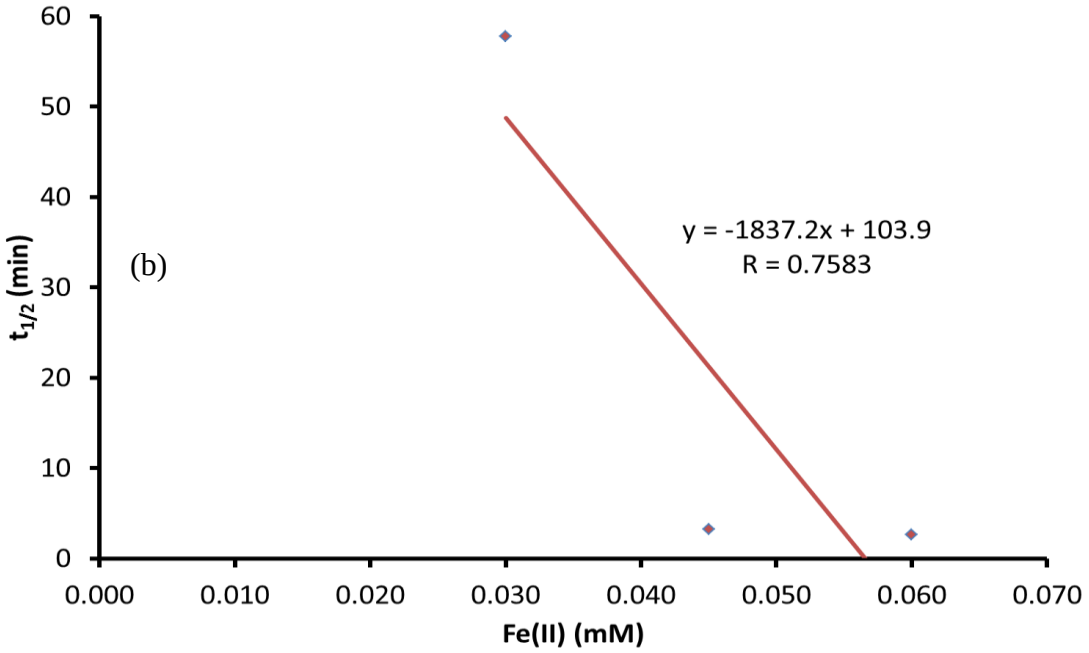


Figure 4.36: (a) Effect of Fe(II) concentration on k_{app}, fitted to linear plot (b) Effect of Fe(II) concentration on $t_{1/2}$, fitted to linear plot.

4.3.7 Summary of photo-Fenton's optimisation experiments

Photo-Fenton's oxidation effectively removed all three APIs from aqueous solutions. Tables 4.9 and 4.10 show a summary of Fenton's results including k_{app}, $t_{1/2}$, R and % removal for all Fenton's optimisation experiments. Investigation of the effect of different reactants in the Fenton's process on the removal of all three APIs showed no or very limited removal by photolysis, Fe(II) and Fe(II)/light. For famotidine at 20 mM H_2O_2, approximately 11% removal was seen with the addition of H_2O_2, which was slightly increased with the use of light. Tamsulosin and solifenacin showed no degradation at 5 mM H_2O_2 in the light or dark.

Dark Fenton's oxidation showed double the degradation of H_2O_2 alone at 0.012 mM Fe(II)/20 mM H_2O_2 for famotidine and again the use of the halogen lamp slightly improved the removal. Dark Fenton's oxidation resulted in only 4% tamsulosin remaining at 10 minutes at 0.09 mM Fe(II), 5 mM H_2O_2. The use of the halogen lamp increased removal with only 0.3% remaining. Dark Fenton's oxidation of solifenacin had 0% remaining at 10 minutes at 0.09 mM Fe(II), 5 mM H_2O_2.

In famotidine photo-Fenton's reactions between 0.012 mM Fe(II) and 0.120 mM Fe(II) at 20 mM H_2O_2 optimal removal occurred at 0.120 mM. Photo-Fenton's reactions for both tamsulosin hydrochloride and solifenacin succinate between 0.012 mM Fe(II) and 0.090 mM Fe(II) at 5 mM H_2O_2 exhibited optimal removal at 0.090 mM Fe(II).

Comparison of Fenton's processes with other photocatalytic and advanced oxidation methods in the literature and even between reports describing Fenton's process is problematic. Different conditions including different light sources, reactor types and concentrations of pharmaceuticals, iron(II) and hydrogen peroxide are common. Fenton's process such as electro-Fenton's and Fenton's-like reactions are also difficult to compare. Fenton's oxidation has also

been investigated for different wastewater types. In terms of removal of APIs and/or reduction in TOC, COD or DOC. There is no standard method for assessing efficiency of process. Time-based kinetics modelling are commonly used as the rate constant and half-life can be used to compare the efficiency of processes even between different types of process. Fenton's and photo-Fenton's degradation followed pseudo-first order degradation for all three APIs at the concentrations used.

Table 4.9: Summary of Fenton's oxidation experiments carried out showing % removal and first order kinetics of famotidine degradation at different Fe(II) and H_2O_2 concentrations.

Process	Fe/H_2O_2/Light	% remaining at 60 min	R	k_{app} (min^{-1})	$t_{1/2}$ (min)
Photo-Fenton's	0.012/20/light	75	0.9805	$3.827x10^{-3}$	182.4
	0.03/20/light	49	0.9515	$8.770x10^{-3}$	78.8
	0.06/20/light	34	0.9661	$2.213x10^{-2}$	31.4
	0.09/20/light	10	0.9046	$4.257x10^{-2}$	16.3
	0.12/20/light	1	0.9175	$6.247x10^{-2}$	11.1
	0.12/10/light	25	0.9210	$2.099x10^{-2}$	33.0
	0.15/10/light	2	0.8754	$6.656x10^{-2}$	10.4
	0.15/05/light	3	0.9266	$5.807x10^{-2}$	11.9
	0.18/05/light	1	0.9382	$6.993x10^{-2}$	9.9
Photolysis	-/-/light	100	-	-	-
Dark Fenton's	0.012/20/-	77	0.9515	$3.118x10^{-3}$	223.6
Hydrolysis	-/20/-	89	0.9137	$1.395x10^{-3}$	495.1
	-/20/light	88	0.9407	$1.570x10^{-3}$	433.2
Fe(II) processes	0.012/-/light	100	-	-	-
	0.012/-/-	100	-	-	-

Table 4.10: Summary of Fenton's oxidation experiments carried out showing % removal and first order kinetics of tamsulosin hydrochloride and solifenacin succinate degradation at different Fe(II) and H_2O_2 concentrations.

		Tamsulosin hydrochloride				Solifenacin succinate			
Process	**Fe/H_2O_2/Light**	**% remaining at 10 min**	**R**	**k_{app} (min^{-1})**	**$t_{1/2}$ (min)**	**% remaining at 10 min**	**R**	**k_{app} (min^{-1})**	**$t_{1/2}$ (min)**
Photo-Fenton's	0.012/5/light	77	0.9608	0.0050	138.6	-	-	-	-
	0.030/5/light	58	0.9707	0.0147	47.2	48.3	0.8856	0.012	57.8
	0.045/5/light	51	0.9731	0.0423	16.4	14.4	0.9852	0.213	3.3
	0.060/5/light	6.5	0.9144	0.1072	6.5	6.8	0.9962	0.262	2.6
	0.090/5/light	0.3	0.9877	0.5103	1.4	0	-	-	-
	0.120/5/light	0	-	-	-	0	-	-	-
Photolysis	-/-/light	96	-	-	-	98	-	-	-
Dark Fenton's	0.090/5/-	4	-	-	-	0	-	-	-
Hydroxylation	-/5/-	99	-	-	-	99	-	-	-
	-/5/light	96	-	-	-	97	-	-	-
Fe(II) processes	0.090/-/light	100	-	-	-	97	-	-	-
	0.090/-/-	100	-	-	-	100	-	-	-

One method for comparing optimum conditions by comparing the ratio of API: Fe(II): H_2O_2 concentration. Table 4.11 compares removal efficiency of the three APIs in this study with methods in the literature. The average API: Fe(II): H_2O_2 ratio in the methods is approximately 1:1:50. Fe(II) ratio ranged from 0.2-4.2 and H_2O_2 from 1.4-116.3. The order of magnitude between optimum API and Fe(II) ratios was typically the same. H_2O_2 was typically 1-2 orders of magnitude higher than API and Fe(II) concentration.

0.18 mM Fe(II)/5 mM H_2O_2 was required for the removal of famotidine in comparison to 0.09 mM Fe(II)/5 mM H_2O_2 for tamsulosin and solifenacin. The higher concentrations of iron required for the removal of famotidine may be due to its suspected iron chelation and antioxidant properties. Due to the presence of amine, thiozole and thioether groups on the famotidine structure it has strong complexing properties. It complexes with a number of metals including copper and iron (Muller and Burrows, 1998; Stargrove et al., 2008). This results in inhibition of HO· radicals generation in Fe^{2+}/H_2O_2 reaction mixtures by famotidine (van Zyl et al., 1993). However, from Figure 4.24 no reduction in famotidine was noted in experiments with 0.1 mM famotidine at 0.012 mM Fe(II) with and without light. However the final optimised concentration was much higher at 0.18 mM Fe(II) and perhaps at these concentrations complexing may be occurring. The antioxidant properties of famotidine have been investigated in gastric disease pathology as an inhibitor of oxidation induced damage (Ahmadi et al., 2010; Sener-Muratoglu, 2001). The exact mechanism of its action as a radical scavenger is unclear. However, it has been reported that famotidine has weak hydrogen peroxide scavenging activity (Ahmadi et al., 2010) but it acts as a HO· radical scavenger (Sener-Muratoglu, 2001).

Table 4.11: Comparison of different methods for the treatment of APIs using photo-Fenton's oxidation. Appendix D contains an extensive list of rate equations and conditions for literature examples of pharmaceuticals treated by Fenton's processes.

Pharmaceutical	pH	APIs (mM)	Fe(II) (mM)	H_2O_2 (mM)	k	k_{app} (min^{-1})	%Removal	Ratio API; Fe(II); H_2O_2	Ref.
Famotidine	3	0.1	0.15	5	1	0.05807	97	1; 1.5; 50	This study
Famotidine	3	0.1	0.18	5	1	0.06993	99	1; 1.8; 50	This study
Tamsulosin	3	0.1	0.09	5	1	0.5103	99	1; 0.9; 50	This study
Tamsulosin	3	0.1	0.06	5	1	0.1072	93.5	1; 0.6; 50	This study
Solifenacin	3	0.1	0.06	5	1	0.262	96.7	1; 0.6; 50	This study
Solifenacin	3	0.1	0.045	5	1	0.213	97.4	1; 0.45; 50	This study
Melatonin	3.0	0.086	0.1	10	1	0.25	100	1; 1.16; 116.3	Xu et al., 2009
Melatonin	3.0	0.086	0.05	10	1	0.18	100	1; 0.58; 116.3	Xu et al., 2009
Metronidazole	3.5	0.006	0.00588	0.0294	2	0.00248	73	1; 0.98; 4.9	Shemer et al., 2006
Metronidazole	3.5	0.006	0.01176	0.0294	2	0.00383	73	1; 0.2; 4.9	Shemer et al., 2006
Amoxicillin	3.5	10 mg/L	25 mg/L	500 mg/L	-	-	100	1; 2.5; 50	Ay and Kargi, 2010
Amoxicillin	3.5	105 mg/L	50 mg/L	500 mg/L	-	-	100	1; 0.48; 4.8	Ay and Kargi, 2010
Amoxicillin	3.5	105 mg/L	25 mg/L	255 mg/L	-	-	100	1; 0.24; 2.4	Ay and Kargi, 2010
Ibuprofen	3	0.87	0.32	1.2	-	-	100	1; 0.4; 1.4	Mèndez-Arriaga, 2010
							Average	1; 1.2; 42.9	

4.3.8 Intermediate study

The identification of intermediates is an important step in the evaluation of the photo-Fenton's process. Some intermediates can be more toxic than the parent compound or may be biologically active. A particularly recalcitrant intermediate may be formed and not degraded. Intermediates shown from other catalysis or oxidation studies may suggest different degradation pathways. It has been shown in the case of other APIs that degradation follows different pathways depending on the treatment applied (Perez-Estrada et al., 2005a). Calza et al., 2004a has shown that intermediate products in photodegradation studies reflect biotransformation in animal models. Therefore metabolites are a useful guide in identifying intermediates. For each API intermediates were identified during a seven hour photo-Fenton's experiment detected by LC-MS. After 5 hours a second addition of H_2O_2 was added.

4.3.8.1 Intermediates produced by photo-Fenton's oxidation of famotidine

Famotidine results are for 100 µM famotidine degraded using 0.09 mM Fe(II), 20 mM H_2O_2 and light. Figure 4.40 shows degradation with time of the ions present at 0 min. There is a number of ions present at 0 min other than the famotidine ion at m/z 338. The famotidine ion (338 m/z) is approximately 8 times more intense than the next highest present ion at 0 min. By approximately 150 min the famotidine ion is no longer detected. The other ions present are m/z 259, 340, 360, and 359 (Figure 4.41). However from Figure 4.42 it can be seen that the intermediates linger until approximately 360 min. The presence of the main famotidine impurity (A7) is noted. It has a molecular weight of 258 $gmol^{-1}$ and so is likely to be the ion 259m/z. A list of famotidine impurities is shown in Appendix E. Famotidine with the sodium adduct is also seen at 359. At 340m/z there is also a peak noted, which may be as a result of degradation of the parent compound.

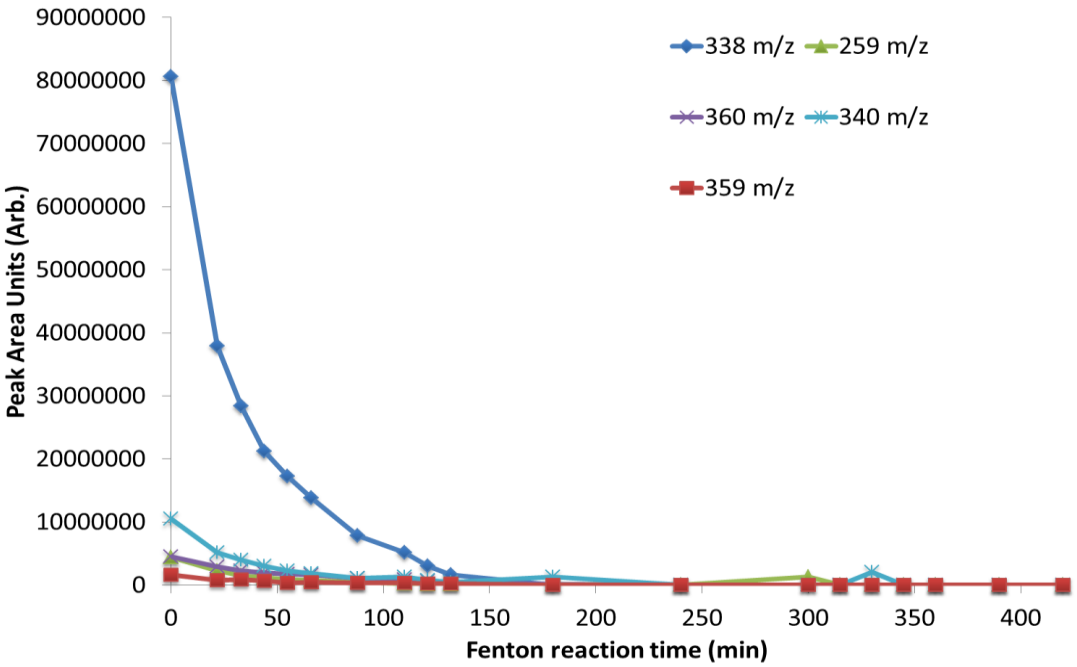


Figure 4.37: Reduction of famotidine ions present at 0min with time.

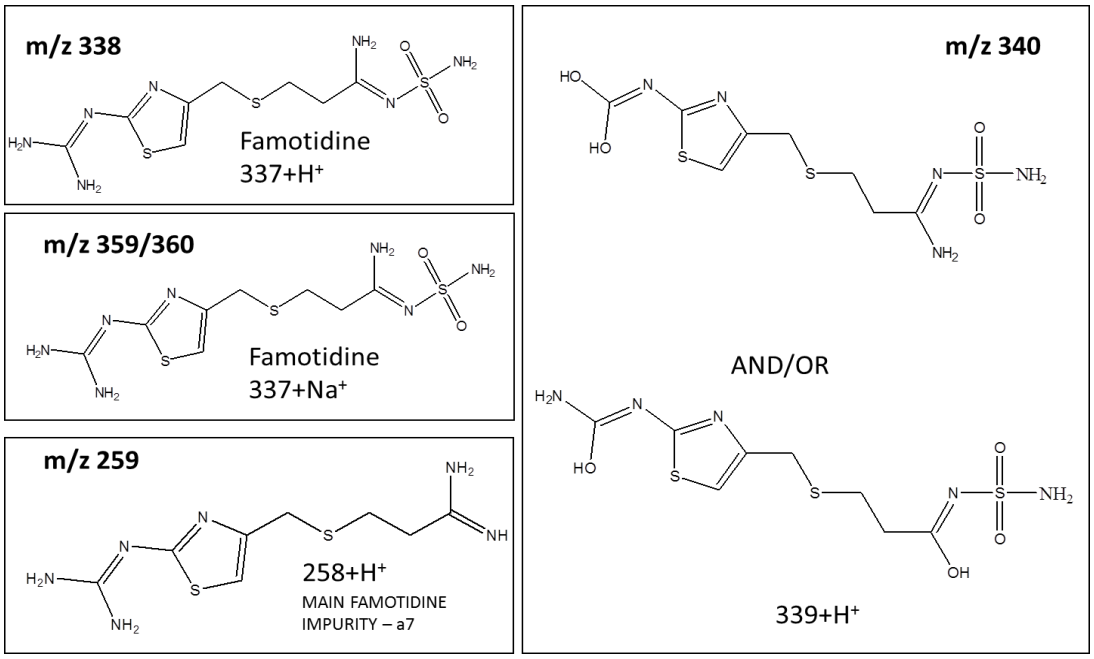


Figure 4.38: Structure of ions present at 0min for famotidine experiment.

There is formation and degradation of intermediates predominantly over the first 180 min. After 180 min more recalcitrant ions m/z 354, 370, and 376 lingered until about 360 min after which no further intermediates were detected *via* LC-MS m/z 354, 372 and 406.9 were present at the highest intensity (Figure 4.42).

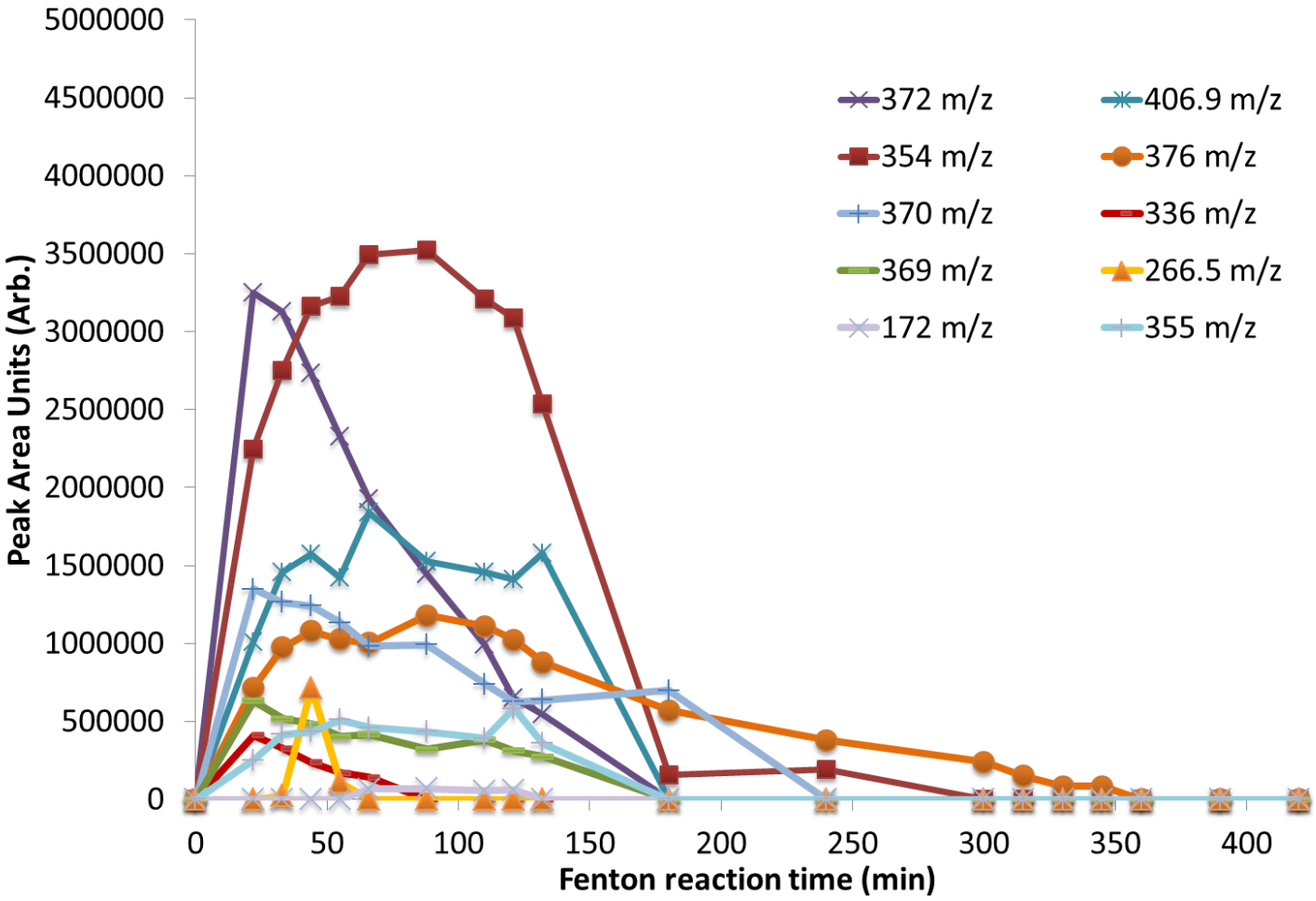


Figure 4.39: Change in peak area units of intermediates present with time.

Figure 4.43 shows the structures and proposed degradation pathways for the lower molecular weight famotidine intermediates. The possible degradation pathway is 264 → 185 → 171 $gmol^{-1}$. The higher famotidine molecular weight structures are seen in Figure 4.44. Structures are proposed for ions with molecular weight 353, 369, 370, 371, and 375 $gmol^{-1}$. One of the main intermediates 354 m/z is likely to have the same structure as the impurity A6 (Appendix E). Transformation of the guanidine is as proposed by Calza et al., 2004b. No major famotidine metabolites are known to use as a comparison.

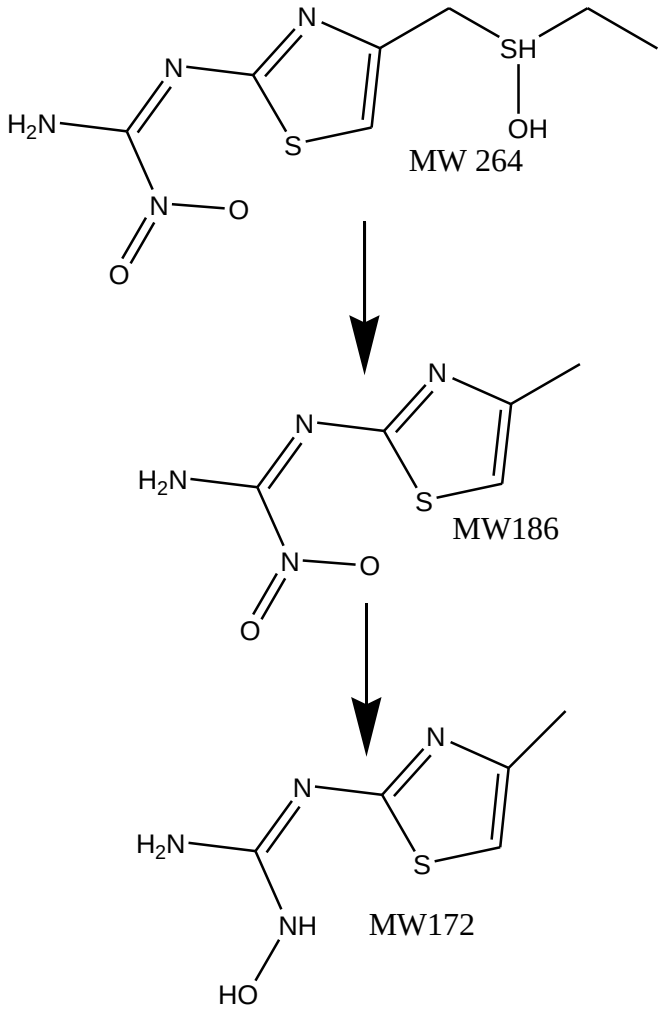


Figure 4.40: Low molecular weight intermediates of famotidine as identified by LC-MS.

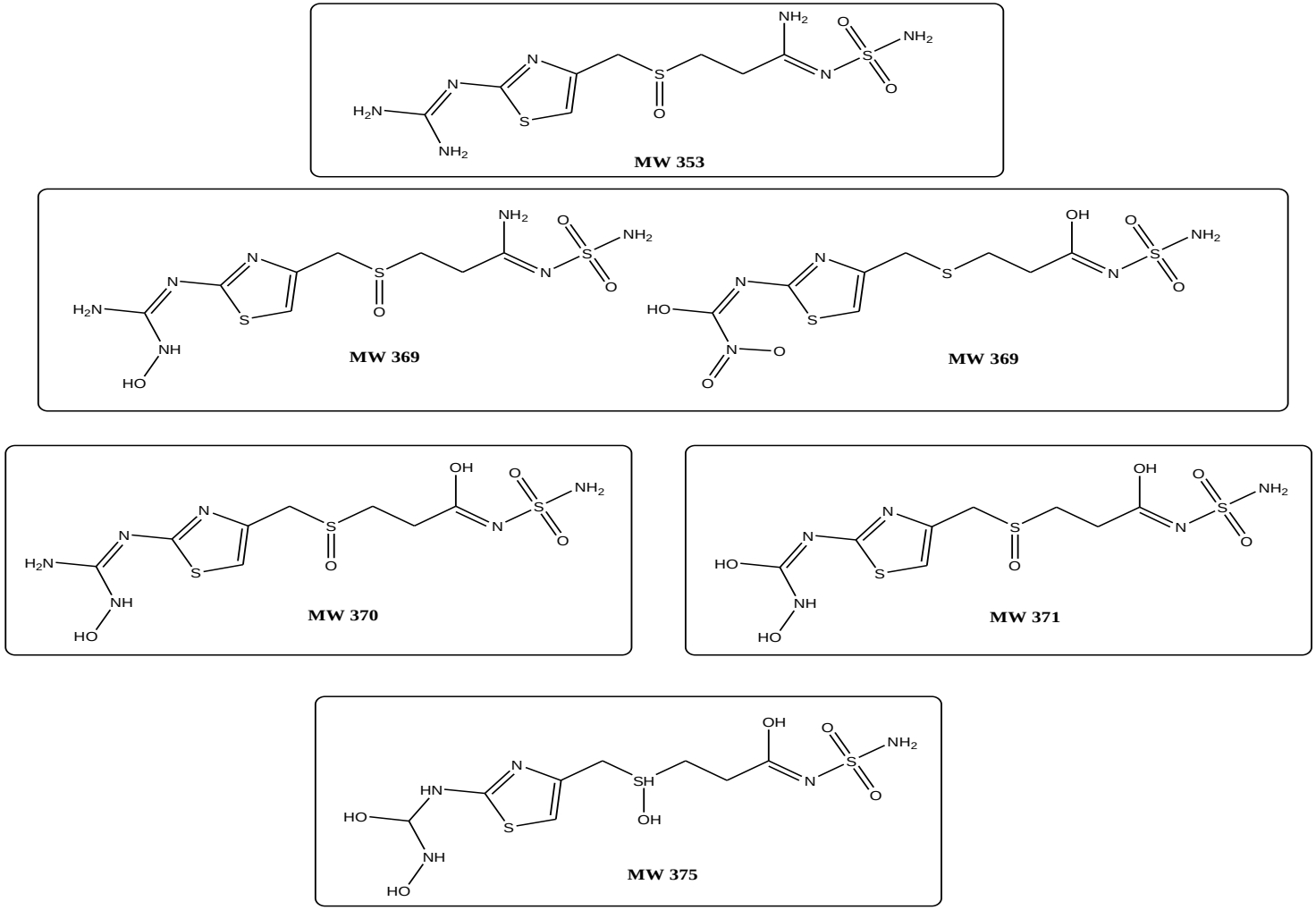


Figure 4.41: High molecular weight intermediates of famotidine as identified by LC-MS.

4.3.8.2 Intermediates produced by photo-Fenton's oxidation of tamsulosin hydrochloride

Conditions used for the tamsulosin experiment are 100 µM tamsulosin hydrochloride, 0.045 mM Fe(II) and 5 mM H_2O_2. The experiment showed formation and degradation of intermediates in first 180 min. A large number of intermediates were formed. This is due to the large number of hydroxylation sites. 13 different ions were noted. No intermediates were noted after 180 min. Ions present at 0 min are shown in Figure 4.45.

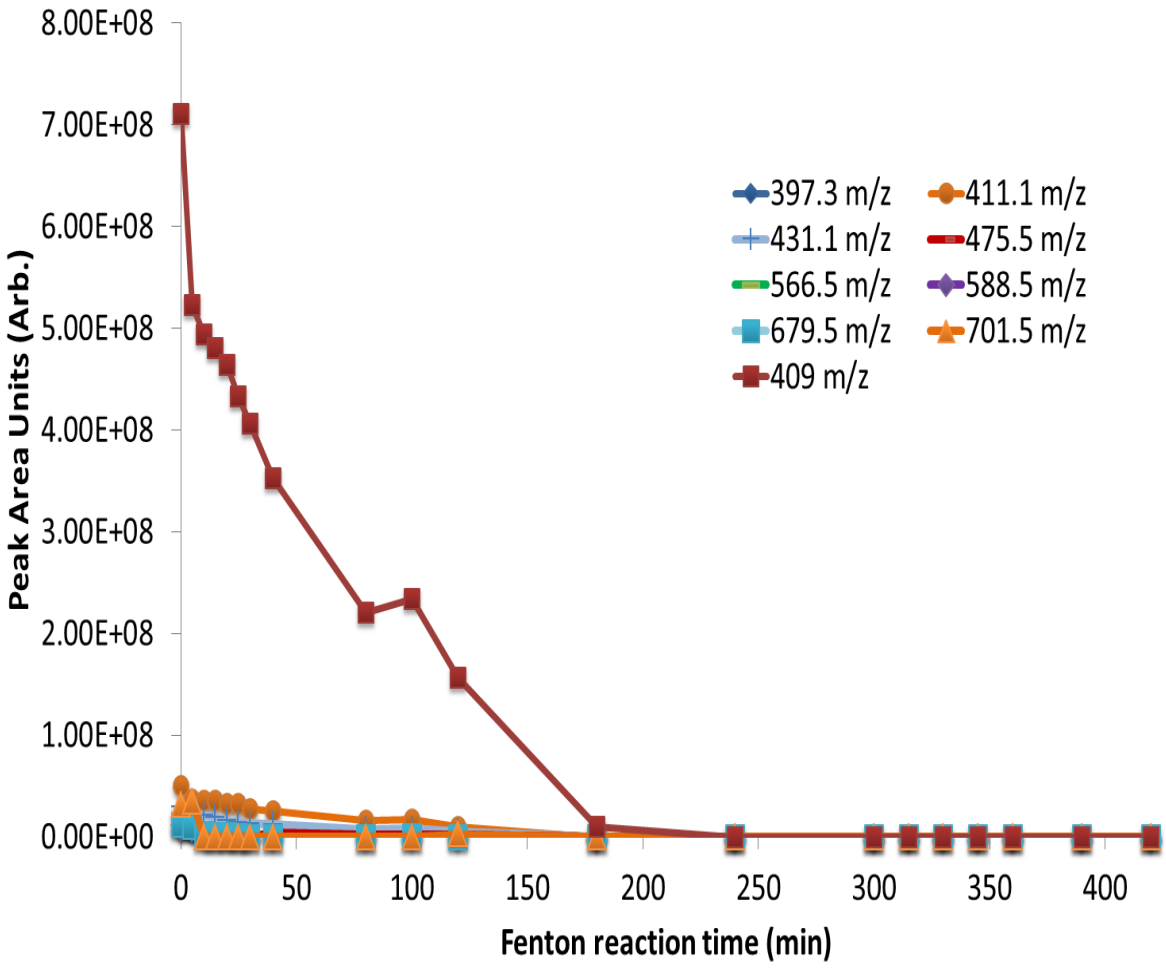


Figure 4.42: Degradation of tamsulosin ions present at 0 min.

Tamsulosin (m/z 409), is approximately 14 times more intense than the next highest ion at 0 min. By approximately 180 min the tamsulosin ion is no longer detected. Intermediates are also no longer detected at 180 min. Nine other ions are present at 0 min. The other ions present are m/z 397, 411, 431, 566.5, 588.5, 679.5 and 701.5. Tamsulosin hydrochloride has a large number of impurities. The impurities may be reflected in the large number of ions noted at 0 min. LC-MS data showing change in intermediates with time during the photo-Fenton's oxidation experiment are shown in Figure 4.46.

Intermediates and degradation pathways for tamsulosin are presented in Figure 4.47. Proposed structures are mainly as a result of HO· radical attack. Tamsulosin is readily metabolised in the body. Some of the proposed structures are the same as the human metabolites M1 and M4. Main human metabolites of tamsulosin are shown in Appendix F. These metabolites are pharmokinetically active with a potency in terms of activity in the body of tamsulosin ≈ M4 > M1 > M2 ≈ M3 >>AM1 (Taguchi et al., 1997).

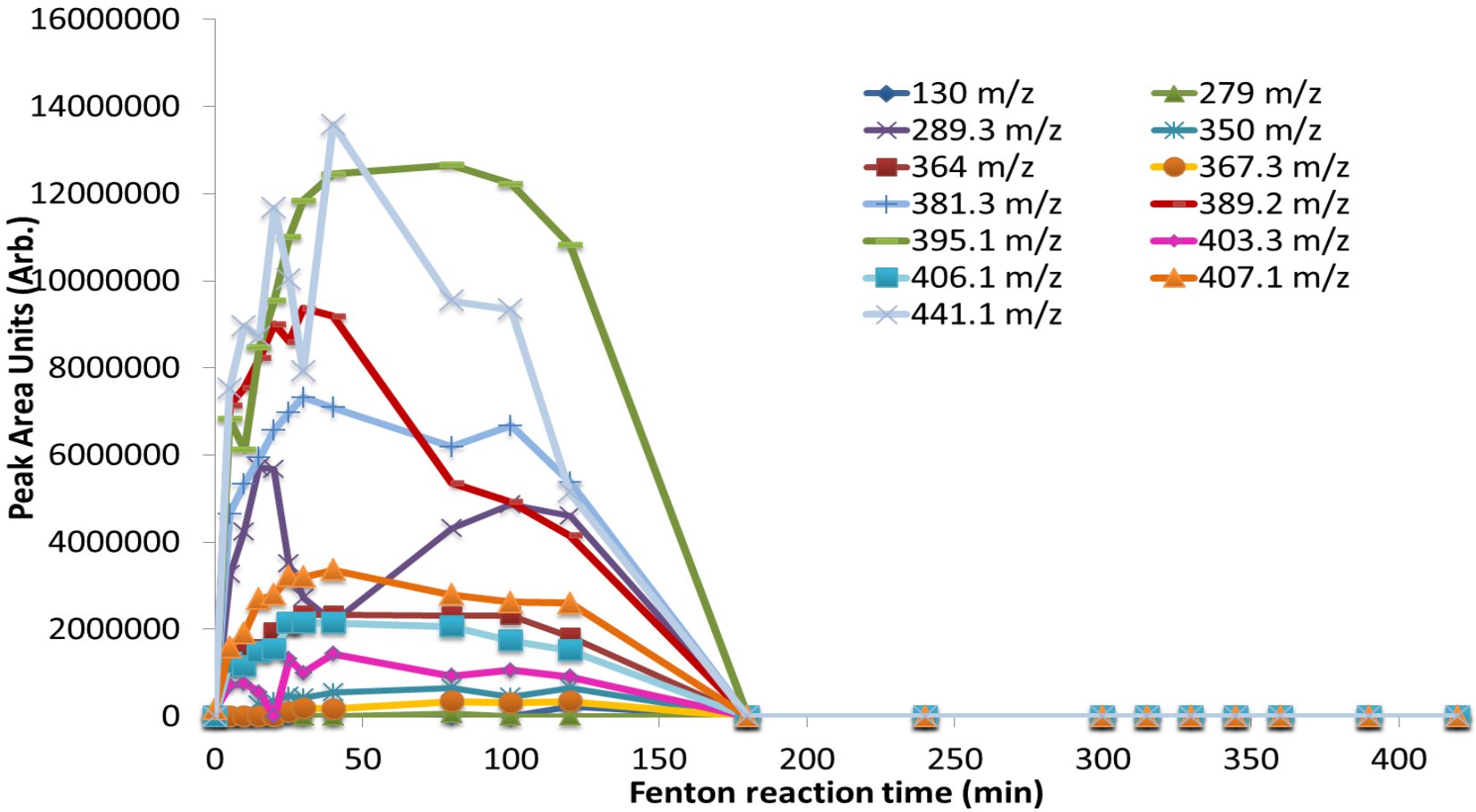


Figure 4.43: Change in peak area units of intermediates present with time.

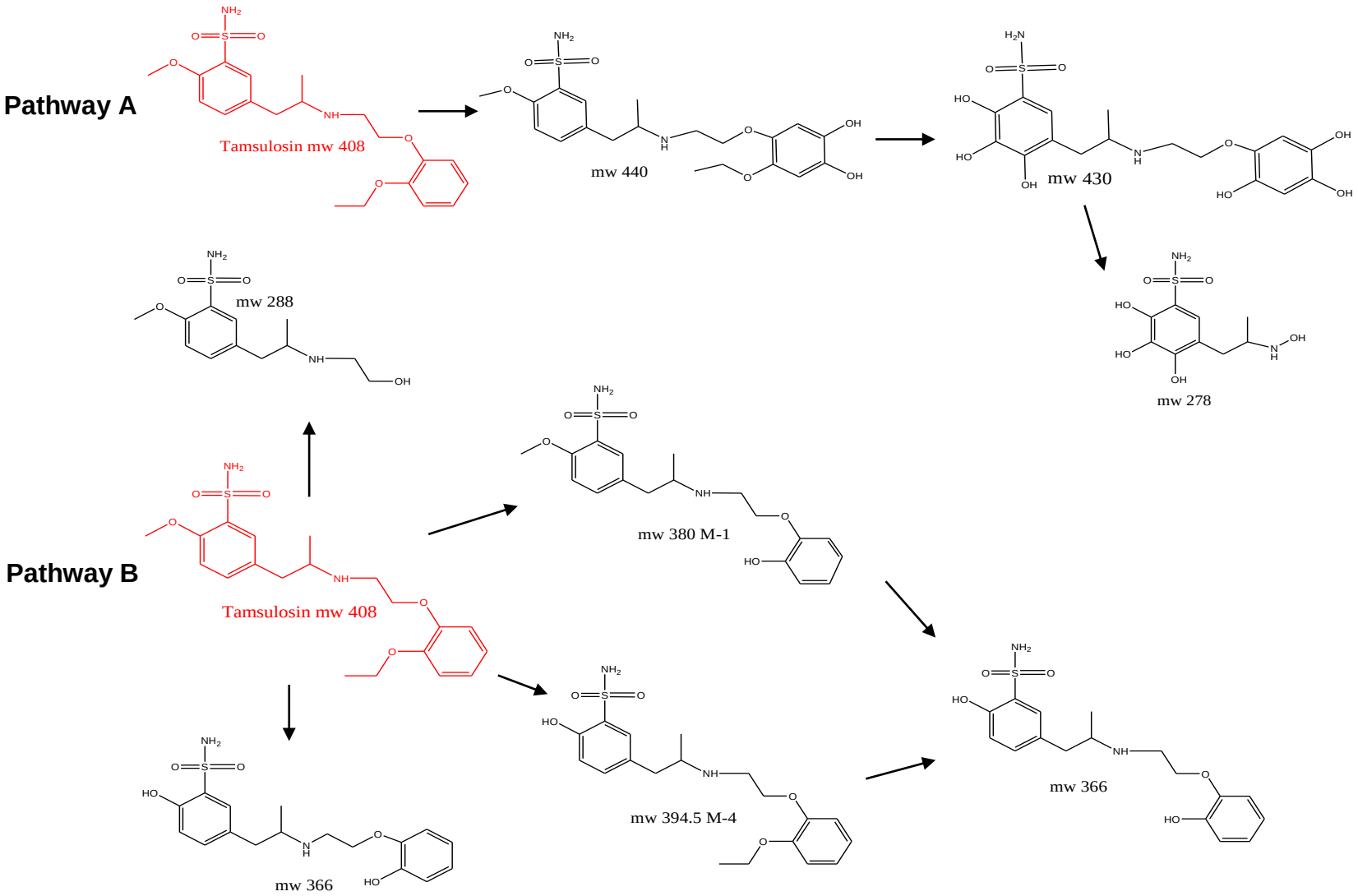


Figure 4.44: Proposed degradation pathways for tamsulosin hydrochloride.

4.3.8.3 Intermediates produced by photo-Fenton's oxidation of solifenacin succinate

The conditions for the identification of solifenacin succinate intermediates were 100 µM solifenacin, 0.045 mM Fe(II) and 5 mM H_2O_2. Ions present at 0 min are shown in Figure 4.48. The ion with the highest intensity is m/z 363 (solifenacin succinate). By approximately 30 min the solifenacin ion is no longer detected. There are ten other ions present at 0 min. The other ions present are m/z 701, 566, 476, 445, 395, 379, 377, 363, 347 and 301. LC-MS data showing change in intermediates with time are shown in Figure 4.49. Intermediates were also no longer detected at 180 min. Formation and degradation of intermediates occurred in first 180 min. 17 different ions were noted. Many of them have an m/z that reflects the molecular weight of metabolites. Intermediates and degradation pathways for solifenacin are presented in Figure 4.50. Appendix G shows solifenacin metabolites. No intermediates were noted after 180 min.

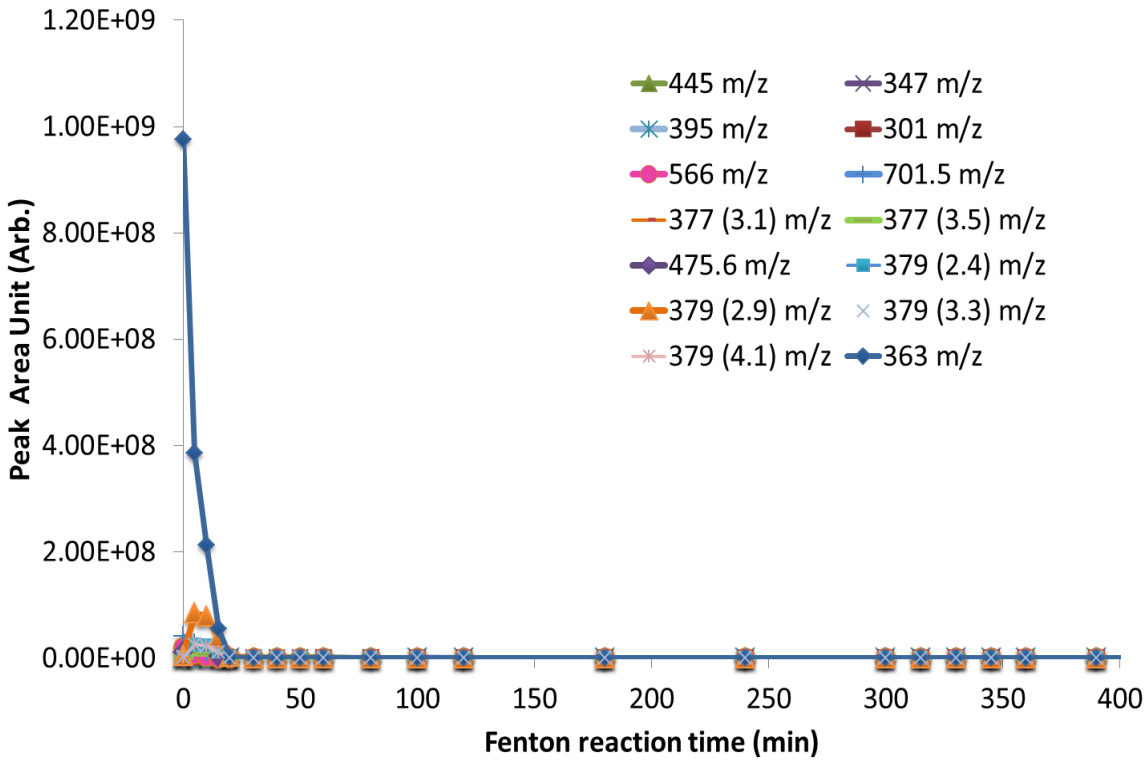


Figure 4.45: Solifenacin ions present at 0 min. Where multiple peaks for same molecular weight exist the retention time of peaks are shown in brackets.

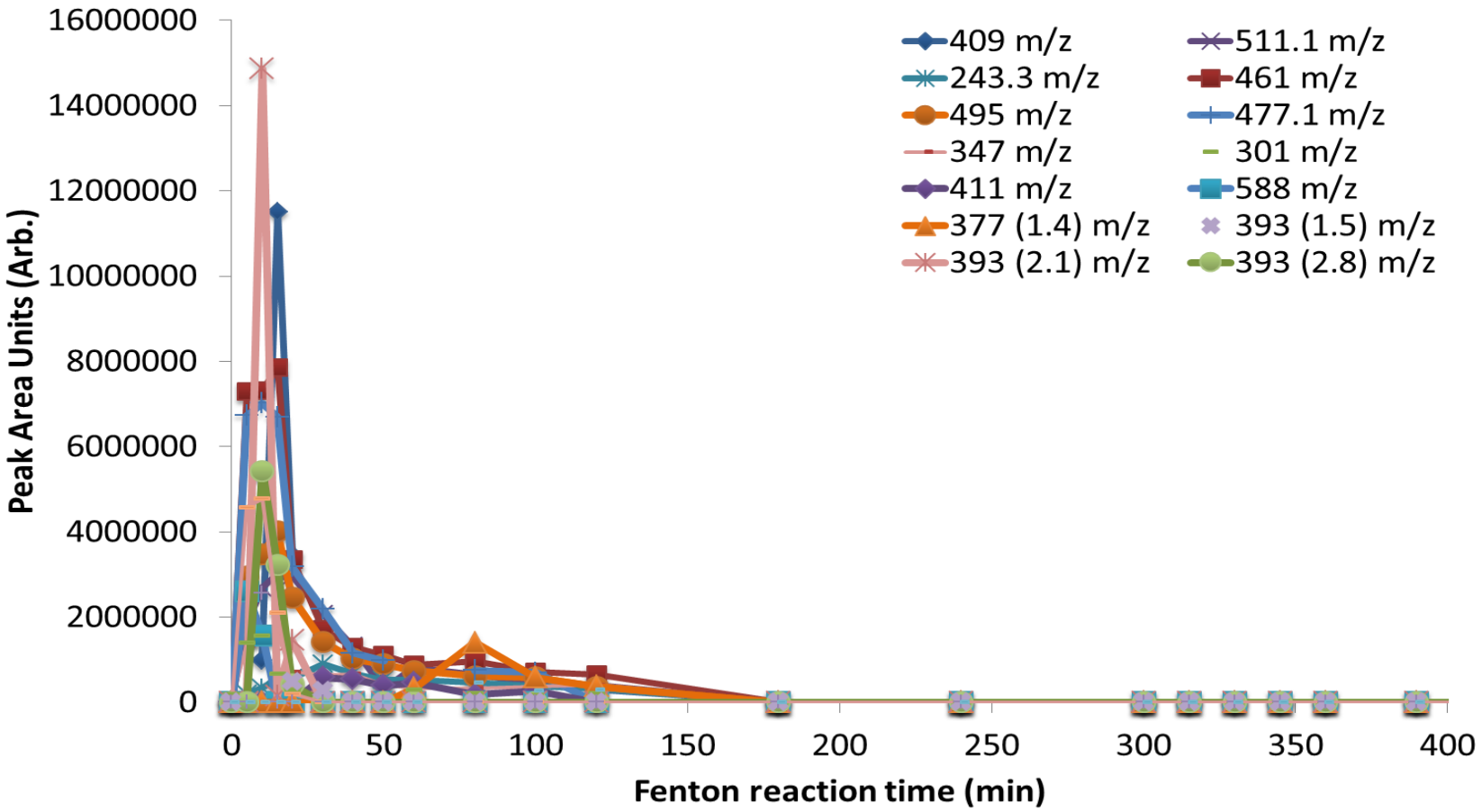


Figure 4.46: Change in peak area units of solifenacin intermediates present with time. Where multiple peaks for same molecular weight exist the retention time of peaks are shown in brackets.

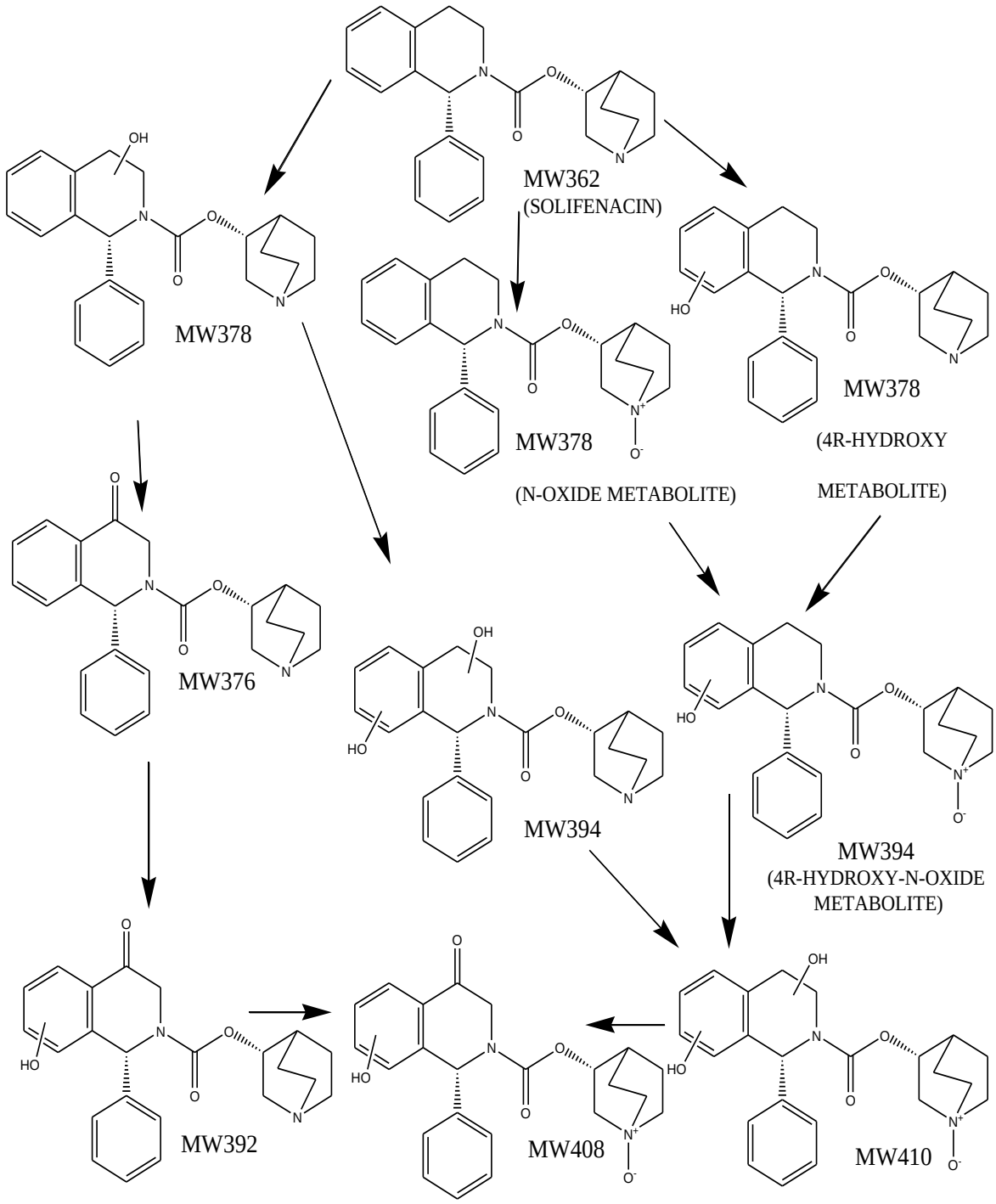


Figure 4.47: Solifenacin degradation pathways of ions seen by LC-MS analysis. The 4-R-hydroxy metabolite is pharmokinetically active.

Solifenacin has four major metabolites, one metabolite that is pharmokinetically active (4R-hydroxy solifenacin) and three inactive metabolites (N-glucuronide, N-oxide, and 4R-hydroxy-N-oxide) (Yanagihara et al., 2007). The N-glucuronide metabolite is not relevant to this study. The N-oxidation of the quinuclidin ring and the 4R-hydroxylation of the tetrahydroisoquinoline ring are likely intermediates to occur as a result of OH· radical attack. Both the 4-hydroxy solifenacin and N-oxide solifenacin have the same molecular weight at 378 $gmol^{-1}$) and likely the same m/z (379 m/z). Figure 4.51 shows the chromatogram of the 379 m/z ion with multiple peaks. The multiple peaks are likely to represent different structures with slightly different affinities to the PFP stationary phase. It is likely that these two metabolites are among the structures of the ions seen. The extra peaks may be caused by the hydroxyl group being attached to different locations on the structure. The three larger peaks possibly represent the structures shown in the figure however this would be difficult to show definatively without further investigation. The n-oxide metabolite also has the same molecular weight this may also be present here. The different structures may represent hydroxyl groups at different locations resulting in slightly different polarities.

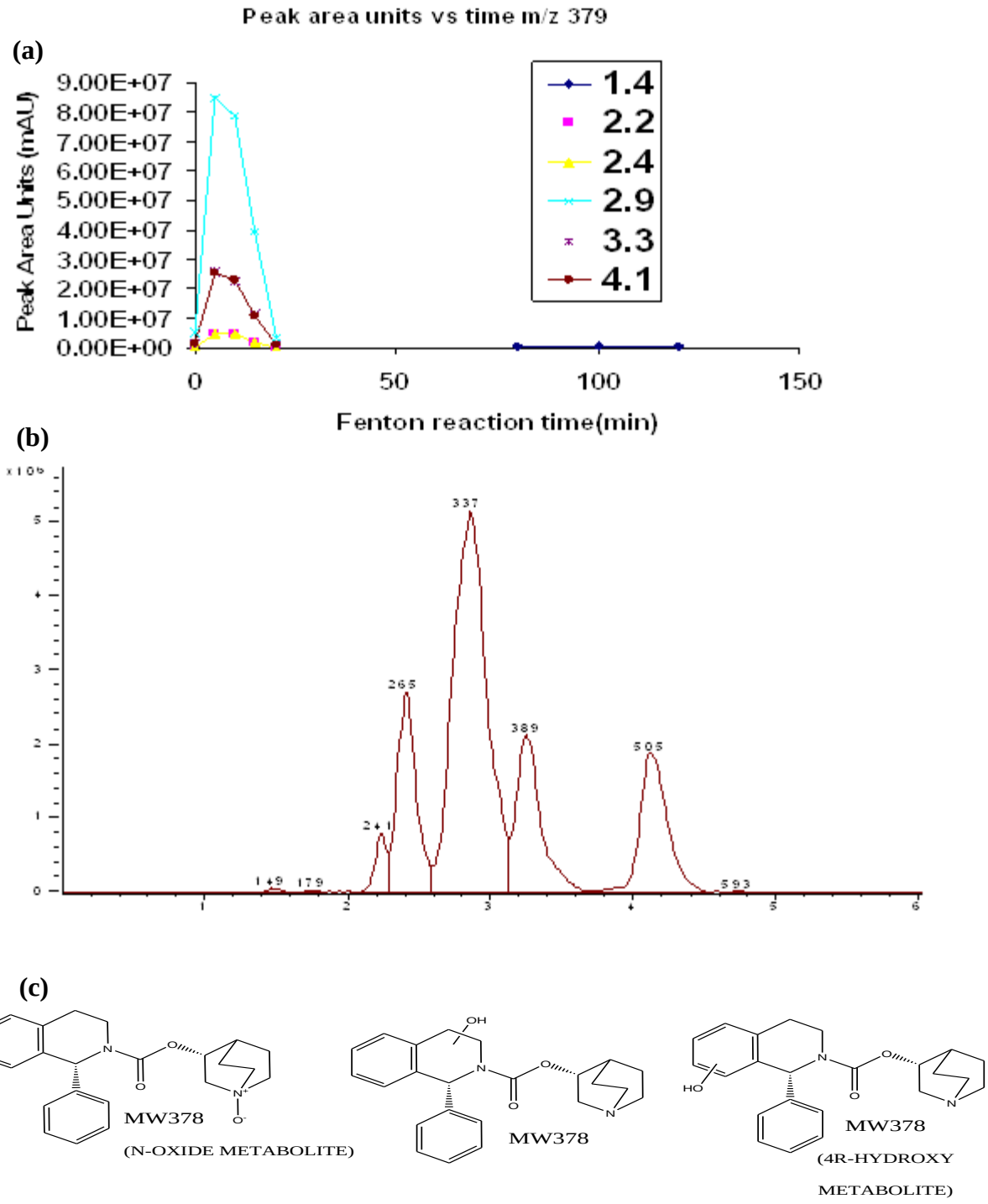


Figure 4.48: Six different peaks for ion at m/z 379 suggests different structures at same molecular weight. (a) shows formation and reduction of peaks with fenton reaction time (b) EIC for m/z 379 showing multiple peaks (c) possible structures including metabolite

4.3.8.4 Summary of the intermediate study

For each of the three APIs formation and subsequent degradation of intermediates in a classic bell shaped curve occurred. Proposed intermediates for famotidine showed cleavage of the C-C bonds, HO· radical substitution for amine groups and guanidine transformation. A large number of intermediates was observed for tamsulosin due to the large number of hydroxylation sites as well as cleavage of the C-C bonds. Low molecular weight intermediates were not seen for solifenacin. HO· radical attack on the ringed structures was a prominent characteristic of the solifenacin degradation including N-oxidation of the quinuclidin ring and the 4R-hydroxylation of the tetrahydroisoquinoline ring.

There were no intermediates identified that formed and did not degrade within the seven hour experiment. Earlier reported results indicated that the parent compounds were removed successfully by photo-Fenton's oxidation. These results indicate that a more complete degradation of all three APIs is occurring. No particularly recalcitrant intermediates are formed that are not removed by photo-Fenton's oxidation. While toxicity studies were outside the scope of this particular study, some intermediates that are pharmokinetically active for both tamsulosin and solifenacin were proposed.

5. Conclusions and further research

5.1 Main findings and conclusions

As discussed in Chapter 1 the project had three primary aims:

1. to develop a single method for the monitoring of all three APIs
2. to monitor the concentrations in wastewater of the APIs
3. to investigate a method for the removal of the APIs from the wastewater.

The extent to which these aims were met and the main findings are outlined below:

1. **A new SPE-LC-MS/MS method was successfully developed and validated in influent and effluent samples.** Coefficients of correlation for each analyte were >0.9301 confirming the linearity of the method. The method had a LOQ of 400 µg/L, 1.0 µg/L, 6 µg/L, repeatability of 2.5% CV, 14.8% CV and 11.9% CV was determined for famotidine, tamsulosin and solifenacin respectively in influent. The method had a LOQ of 400 µg/L, 0.8 µg/L, 6 µg/L repeatability of 2.0% CV, 11.0% CV and 10.9% CV was established for famotidine, tamsulosin and solifenacin respectively in effluent. SPE recovery was >90% for the three APIs in both matrices. The developed method was applied to the six month monitoring programme.

2. **All three APIs were detected frequently in both influent and effluent from the onsite wastewater treatment plant at Astellas Ireland Pharmaceutical Limited** in Mullhudart Co. Dublin. Famotidine (average 2.6 mg/L in effluent) and solifenacin (average 0.079 mg/L in effluent) were detected at higher concentrations than is typically found for other pharmaceuticals in municipal and hospital wastewater ((Lacey et al., 2008; Suarez et al., 2009). Tamsulosin was typically detected at only trace concentrations in the low µg/L range. The concentrations of the three APIs fluctuated considerably depending on production regimes, changes in production methods and the production processes. Samples were collected in plastic bottles and were transferred to glass bottles immediately on return to the laboratory. This may have resulted in a slightly lower concentration recorded for the more hydrophobic APIs. All three

APIs were detected in both influent and effluent samples at different times during the study. Famotidine and tamsulosin were present in almost every sample taken (85-100%). Solifenacin was out of production during the first two months of the six month sampling programme and was detected in 65% of both influent and effluent samples.

Famotidine was detected at higher concentrations than expected at an average concentration of 1.6 mg/L in influent and 2.6 mg/L in effluent. Solifenacin was also detected at concentrations much higher than those typically detected in surface and municipal wastewaters at a max effluent concentration of 79 µg/L. Tamsulosin was typically detected at only trace concentrations in the low µg/L range. Tamsulosin is produced in a small multipurpose facility for the manufacturing of small amounts of valuable pharmaceuticals. The relatively large loss of famotidine and solifenacin represents a monetary loss for the pharmaceutical company. As stated previously the maximum famotidine influent concentration of 5.8 mg/L corresponds to 1.65 kg of lost product per batch. The concentrations of the three APIs fluctuated considerably depending on production regimes, changes in production methods and the production processes. This highlights the requirement for the six-month monitoring programme. Other monitoring programme lengths were considered including monitoring daily over a production cycle and monitoring over a longer period up to a year. 26 weekly samples were considered sufficient to see trends over the approximately two week production cycles. It also covered a shut-down period to show minimum concentrations of the APIs released to the environment.

The on-site wastewater treatment facility was broadly ineffective for the removal of the three APIs. Effluent concentrations of famotidine exceeded influent 77% of the time because wash water from the famotidine production process was used to make a feed for the activated sludge lagoons and that contained high concentrations of dissolved famotidine (20 kg in 1700 L). Famotidine remained in the effluent at an average of 2.6 mg/L. Tamsulosin remained at trace

concentrations throughout the monitoring period. The average concentration of tamsulosin in the influent was only marginally higher than the effluent at 0.005 mg/L in comparison to 0.004 mg/L. There is evidence of up to 40% removal for solifenacin, which is more non-polar and is less metabolically resistance.

Literature documenting releases of pharmaceuticals from industrial sources are rare. Available reports are generally from emerging economies with less stringent environmental laws such as Korea or India. Such reports may be looking at massive production facilities producing hundreds of tonnes of pharmaceuticals for sale worldwide. It could therefore be argued that they may be looking at more extreme cases of pollution and are not representative of the industry as a whole. More studies are needed focusing on varying type and size of production facilities in different geographical locations. As discussed in Chapter 4 concentrations of commonly monitored APIs are generally 1-2 but can be as much as 4 orders of magnitude higher than hospital or municipal sources, depending on the size of the production facility. This study focused on a medium sized production facility in a developed country. The concentrations of famotidine detected were at least three orders of magnitude higher than typically detected in municipal effluent. Concentrations of APIs in the wastewater were reflective of the amount of API being produced as shown in Table 5.1.

Table 5.1: Approximate production and influent concentrations during monitoring period from November 2009 to December 2010.

API	Production (tonnes)	Influent concentration (mg/L)
Famotidine	9-10	1.6
Tamsulosin	0.18	0.005
Solifenacin	2	0.04

Not only are the concentrations released by a source of API contamination of the environment important in relation to environmental fate but also the source type. The pharmaceuticals released from municipal plants may have undergone

metabolism in the body before being excreted, but pharmaceuticals released from industrial sources are in the original, unmetabolised form. This is important in understanding the environmental form to be evaluated, designing fate and effects testing strategies, interpreting results, predicting environmental fate and carrying out environmental risk assessments (Kümmerer, 2004).

3. **Photo-Fenton's oxidation successfully removed all three APIs from water.** Higher Fe(II) concentrations were required for the removal of famotidine (0.18 mM Fe(II)/5 mM H_2O_2) than tamsulosin and solifenacin (0.09 mM Fe(II)/5 mM H_2O_2). This may be due to iron chelating or antioxidant properties of famotidine. The intermediate study showed complete removal of all intermediates generated by photo-Fenton's oxidation. Pharmokinetically active intermediates were identified. To compare the efficiency of the Fenton's oxidation of the three APIs, the ratios of the API concentration to the concentration of the reactants were compared with different reported examples (Ay and Kargi, 2010; Mèndez-Arriaga, 2010; Xu et. al., 2009; Shemer et al., 2006). The order of magnitude between optimum API and Fe(II) ratios was typically the same. H_2O_2 had an average of 1.2 orders of magnitude higher than the API and Fe(II) concentrations. Time-based kinetic modelling are commonly used as the rate constant and half-life can be used to compare the efficiency of processes (even between different types of process such as TiO_2 photocatalysis and ozonation). Fenton's and photo-Fenton's degradation followed pseudo-first order degradation for all three APIs at the concentrations used. Rate constants and half-lives for the optimised photo-Fenton's conditions are shown in Table 5.3.

Table 5.2:Summary of rate constant k_{app} and half-life $t_{1/2}$ for photo-Fenton's oxidation.

API	k_{app} (min^{-1})	$t_{1/2}$ (min)
Famotidine (0.18 mM Fe(II)/5 mM H_2O_2)	$6.993x10^{-2}$	9.9
Tamsulosin (0.09 mM Fe(II)/5 mM H_2O_2)	0.5103	1.4
Solifenacin (0.06 mM Fe(II)/5 mM H_2O_2)	0.262	2.6

5.2 Main implications of study

There are 2 main implications of this project:

1. **The effect of the concentrations detected in the pharmaceutical wastewater on current understanding of the sources of pharmaceuticals in the environment** The concentrations of pharmaceuticals released from the Astellas facility are broadly similar to concentrations detected at industrial facilities in Korea and India (SIm et al., 2011; Larsson et al., 2007). The levels detected are higher than typical surface waters and municipal waste waters. This may indicate that pharmaceutical releases represent higher concentrations than generally acknowledged. Studies of concentrations of pharmaceuticals released from industrial facilities in Europe and America are few or not publically available. This project may prompt further study to assess the impact of releases from pharmaceutical facilities and the role overall loading of pharmaceuticals released from industrial facilities play on the environment.

2. **The application of photo-Fenton's oxidation to the treatment of pharmaceutical wastewater** This project showed the effective removal of all three APIs produced at one industrial facility using photo-Fenton's oxidation. While numerous studies are available monitoring the breakdown of pharmaceuticals as a result of photo-Fenton's oxidation using UV lamps there is less work done on the breakdown of pharmaceuticals using halogen lamps to mimic solar light. The use of the halogen lamp showed little improvement over dark Fenton. Fenton's reagent is an effective method for the removal of micro-pollutants from aqueous solutions.

5.3 Further research

Further investigation of actual releases of pharmaceuticals by industrial facilities is required. It is generally accepted that the most significant entry route of pharmaceuticals into the environment is through municipal wastewater treatment

effluents. However the results of this study and others (Larsson et al., 2007; Sim et. al., 2011) indicate that larger concentrations than released by municipal facilities may be released locally by production facilities. Monitoring of production facilities in particular those producing pharmaceuticals that are thought to be of greater environmental concern such as EDCs and antibiotics as well as fermentation, R&D facilities and facilities that recover APIs from natural resources is required. The large concentrations of APIs released cause not only potential environmental problems but also represent a loss of valuable product to the pharmaceutical company. This suggests scope for further research in the area of efficient and cost effective product recovery from waste streams.

The wastewater treatment facility at the manufacturing plant was unsuccessful for the removal of the three APIs from the wastewater. As indicated in the literature review, operating activated sludge plants at higher HRT may provide more success in the removal of APIs from wastewater. Methods for the adaption of existing treatment facilities for the enhanced removal of APIs from wastewaters such as pre-treating of wastes with AOPs is an active area of research.

The three APIs investigated in this work are not known to have high environmental toxicity. Synergistic effects of cocktails of low concentrations of pharmaceuticals in the environment are largely unknown and represent a significant knowledge gap. In this particular scenario it is important to note that the wastewater is being released to the municipal sewer and so will be diluted and treated again before being released to the environment. It may be useful to examine the concentrations of specific APIs detected in municipal facilities if there is a pharmaceutical facility in its catchment area. It has been suggested by Enick and Moore (2007) that up to 50% of pharmaceutical wastewater is released to the environment totally untreated. The release of such wastewaters worldwide is largely unregulated. Further research is required on strategies for the reduction in releases of untreated wastewater and wastewater high in API

concentration. On-going research to characterise pharmaceuticals according to risk based on toxic effects (Cooper et al., 2008) may result in future restrictions on pharmaceuticals releases directly into the environment (Larsson and Fick, 2009).

The photo-Fenton's experiments conducted represent proof of concept type experiments showing photo-Fenton's is effective for the treatment of the three APIs. Kinetic evaluations allow the treatment efficiency to be compared with different treatment methods. There is a large scope for further investigation in this area. Further opportunities directly related to this project include monitoring the degradation of the three APIs in one mixture and with real wastewater. Other options include monitoring mineralisation and toxicity over the degradation process and the possibility of up-scaling. Different reactors types using dark-Fenton's, UV or solar light may be investigated at small scale, up scaling to pilot and full scale. Feasibility studies would be necessary to determine the economic viability of up-scaling.

Investigation of the effects of different reactants showed that very high concentrations of hydrogen peroxide are required for removal of the APIs. Use of other reactants (Fe(II) and H_2O_2) alone showed no removal. The use of the halogen light used as a substitute for sunlight made little difference to the removal efficiency; UV light would be expected to be more successful but is more expensive than sunlight. However it may reduce the concentrations of reactants required and thus the overall cost of removal after treatment. Experiments directly related to this project could be carried out to determine the percentage greater removal UV lamps would have over halogen lamps. Cost benefit analysis would be required to determine the cost efficiency of using higher concentrations of reactants in comparison to higher energy costs of UV lamps.

To allow for a more comprehensive comparison between Fenton's and other treatment methods, other methods for the removal of these particular APIs

should be investigated. The main disadvantages to the use of the Fenton's reaction are the requirement of a method for the removal of iron following treatment and the sensitivity of the Fenton's process to pH. For Fenton's oxidation pH must be kept low because when the pH is increased chemical coagulation occurs. This can be used to aid removal of the iron following treatment. However, there is scope for further investigations into the immobilisation of Fe(II) onto beads or other surfaces. This may improve the pH range over which Fenton's reaction could be performed and eliminate the disadvantage of removing the iron following treatment.

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Appendices

Appendix A: Monthly summaries of results

Period	API	WW	Hits	% Freq	Min (mg/L)	Max (mg/L)	Median (mg/L)	Average (mg/L)
November (4 weekly samples)	Famotidine	Influent	4	100	1.500	3.100	2.575	2.413
		Effluent	4	100	1.600	2.800	2.400	2.288
	Tamsulosin	Influent	4	100	0.002	0.004	0.003	0.003
		Effluent	4	100	0.003	0.007	0.004	0.004
	Solifenacin	Influent	2	50	<LOQ	0.013	0.007	0.007
		Effluent	4	100	0.009	0.017	0.013	0.013
December (5 weekly samples)	Famotidine	Influent	5	100	0.500	5.800	1.600	2.556
		Effluent	5	100	1.500	7.300	3.350	4.304
	Tamsulosin	Influent	5	100	<LOQ	0.005	0.003	0.002
		Effluent	5	100	0.002	0.004	0.003	0.003
	Solifenacin	Influent	1	20	<LOD	0.007	0.000	0.002
		Effluent	1	20	<LOD	0.007	0.000	0.002
January (4 weekly samples)	Famotidine	Influent	3	75	<LOD	2.400	0.418	0.809
		Effluent	4	100	1.200	5.000	3.390	3.245
	Tamsulosin	Influent	3	75	<LOD	0.019	0.002	0.006
		Effluent	4	100	0.003	0.004	0.003	0.003
	Solifenacin	Influent	2	50	<LOD	0.027	0.001	0.007
		Effluent	0	0	<LOD	<LOD	0.001	0.001

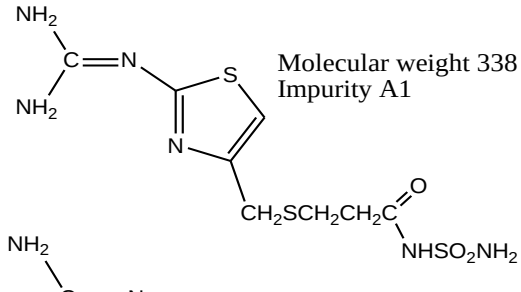
February (4 weekly samples)	Famotidine	Influent	3	75	<LOD	1.700	1.253	1.048
		Effluent	4	100	1.150	1.650	1.370	1.385
	Tamsulosin	Influent	4	100	0.003	0.019	0.007	0.009
		Effluent	4	100	0.004	0.005	0.004	0.004
	Solifenacin	Influent	4	100	0.032	0.106	0.056	0.062
		Effluent	4	100	0.007	0.019	0.011	0.012
March (5 weekly samples)	Famotidine	Influent	3	60	<LOD	0.700	0.485	0.387
		Effluent	5	100	0.700	3.900	2.155	2.213
	Tamsulosin	Influent	5	100	0.002	0.018	0.006	0.007
		Effluent	5	100	0.002	0.032	0.005	0.009
	Solifenacin	Influent	5	100	0.009	0.056	0.046	0.038
		Effluent	4	80	<LOD	0.079	0.039	0.047
April (4 weekly samples)	Famotidine	Influent	3	75	<LOD	3.100	0.430	0.363
		Effluent	4	100	1.350	2.800	1.598	1.599
	Tamsulosin	Influent	2	50	<LOD	0.004	0.001	0.000
		Effluent	4	100	0.0004	0.007	0.002	0.002
	Solifenacin	Influent	3	75	<LOD	0.174	0.032	0.059
		Effluent	4	100	0.033	0.075	0.036	0.041

Appendix B: Fenton oxidation literature examples

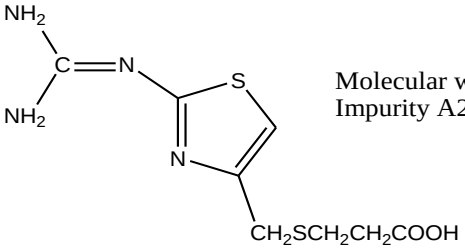
Pharmaceutical	Process	pH	APIs	Fe(II) mM	H_2O_2 mM	k	k_{app}	%Removal	Ref.
Melatonin	UV	7.0	20 mg/L	-	-	1	0.0030	28	Xu et al., 2009
Melatonin	UV	10.0	20 mg/L	-	-	1	0.0048	32	Xu et al., 2009
Metronidazole	UV	3.5	6 mg/L	-	-	1	$3.84x10^{-4}$	12	Shemer et al., 2006
Amoxicillin	UV	5	104 mg/L	-	-	-	-	2.9	Elmolla and Chaudhuri, 2009
Ampicillin	UV	5	105 mg/L	-	-	-	-	3.8	Elmolla and Chaudhuri, 2009
Cloxacillin	UV	5	103 mg/L	-	-	-	-	4.9	Elmolla and Chaudhuri, 2009
Melatonin	UV/H_2O_2	4.0	20 mg/L	-	10	1	0.018	70	Xu et al., 2009
Melatonin	UV/H_2O_2	3.0	20 mg/L	-	10	1	0.017	69	Xu et al., 2009
Metronidazole	UV/H_2O_2	3.5	6 mg/L	-	25 mg/L	1	0.00618	58	Shemer et al., 2006
Metronidazole	UV/H_2O_2	3.5	6 mg/L	-	50 mg/L	1	0.00777	64	Shemer et al., 2006
Amoxicillin	UV/H_2O_2	3.5	105 mg/L	-	500 mg/L	-	-	100	Ay and Kargi, 2010
Ibuprofen	UV/H_2O_2	6.25	0.87 mM	-	0.32	-	-	40	Mèndez-Arriaga, 2010
Melatonin	Fenton	3.0	20 mg/L	0.1	10	1	0.082	100	Xu et al., 2009
Melatonin	Fenton	3.0	20 mg/L	0.1	5	1	0.028	83	Xu et al., 2009
Melatonin	Fenton	3.0	20 mg/L	0.1	15	1	0.070	99	Xu et al., 2009
Melatonin	Fenton	3.0	20 mg/L	0.05	10	1	0.055	98	Xu et al., 2009
Melatonin	Fenton	3.0	20 mg/L	0.2	10	1	0.062	98	Xu et al., 2009
Metronidazole	Fenton	3.5	6 mg/L	0.00588	0.0294	2	$9.48x10^{-4}$	73	Shemer et al., 2006

Metronidazole	Fenton	3.5	6 mg/L	0.01176	0.0294	2	0.0011	73	Shemer et al., 2006
Ibuprofen	Fenton	3	0.87 mM	1.2	0.32	-	-	80	Mèndez-Arriaga, 2010
Melatonin	Photo- Fenton	3.0	20 mg/L	0.1	10	1	0.25	100	Xu et al., 2009
Melatonin	Photo- Fenton	3.0	20 mg/L	0.05	10	1	0.18	100	Xu et al., 2009
Metronidazole	Photo- Fenton	3.5	6 mg/L	0.00294	0.0294	2	$6.71x10^{-4}$	53	Shemer et al., 2006
Metronidazole	Photo- Fenton	3.5	6 mg/L	0.00588	0.0294	2	0.00248	73	Shemer et al., 2006
Metronidazole	Photo- Fenton	3.5	6 mg/L	0.01176	0.0294	2	0.00383	73	Shemer et al., 2006
Amoxicillin	Photo- Fenton	3.5	10 mg/L	25 mg/L	500 mg/L	-	-	100	Ay and Kargi, 2010
Amoxicillin	Photo- Fenton	3.5	200 mg/L	25 mg/L	500 mg/L	-	-	90	Ay and Kargi, 2010
Amoxicillin	Photo- Fenton	3.5	10 mg/L	0 mg/L	255 mg/L	-	-	90	Ay and Kargi, 2010
Amoxicillin	Photo- Fenton	3.5	10 mg/L	50 mg/L	255 mg/L	-	-	85	Ay and Kargi, 2010
Amoxicillin	Photo- Fenton	3.5	105 mg/L	50 mg/L	500 mg/L	-	-	100	Ay and Kargi, 2010
Amoxicillin	Photo- Fenton	3.5	105 mg/L	25 mg/L	255 mg/L	-	-	100	Ay and Kargi, 2010
Ibuprofen	Photo- Fenton	3	0.87 mM	1.2 mg/L	0.32 mg/L	-	-	100	Mèndez-Arriaga, 2010

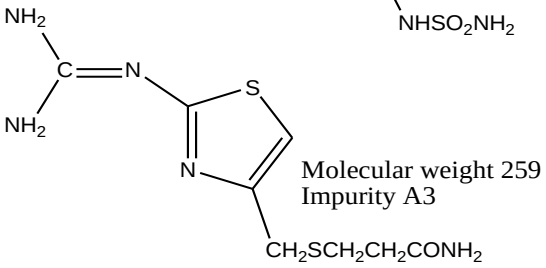
Appendix C: Famotidine impurities



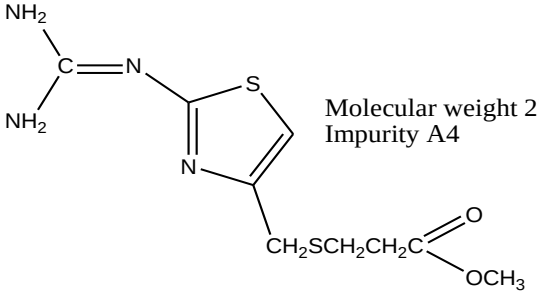
Molecular weight 338
Impurity A1



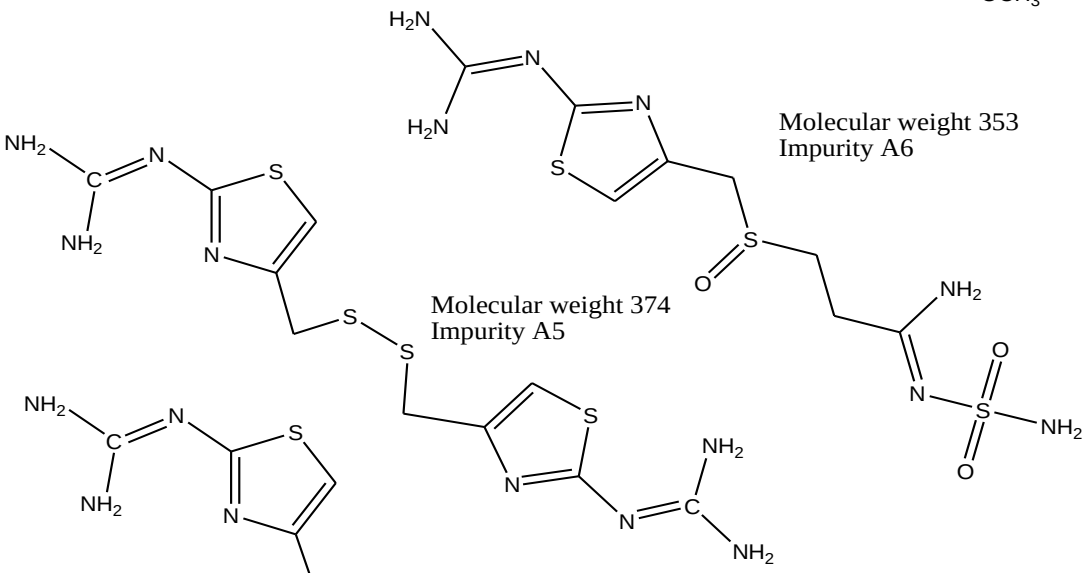
Molecular weight 260
Impurity A2



Molecular weight 259
Impurity A3



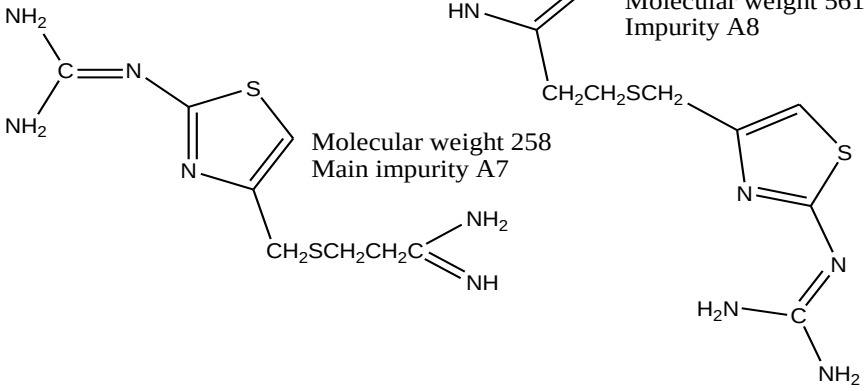
Molecular weight 274
Impurity A4



Molecular weight 353
Impurity A6

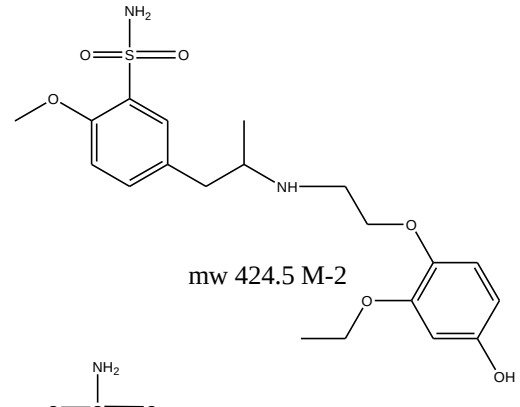
Molecular weight 374
Impurity A5

Molecular weight 561
Impurity A8

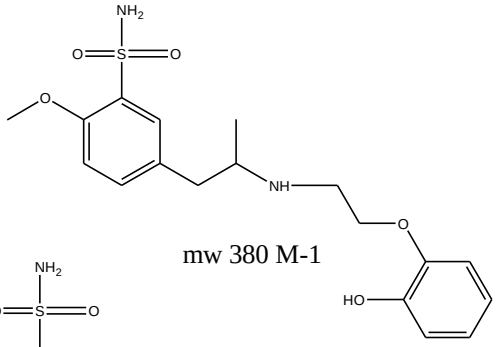


Molecular weight 258
Main impurity A7

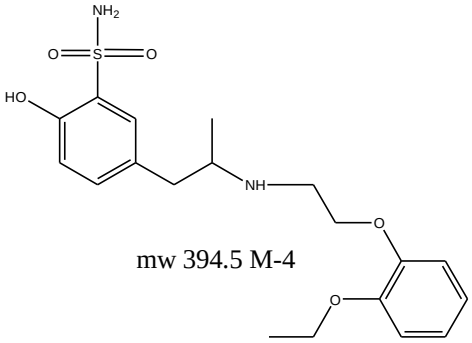
Appendix D: Main tamsulosin metabolites



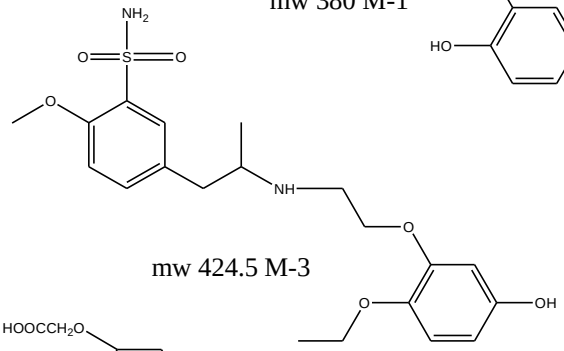
mw 424.5 M-2



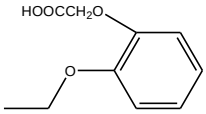
mw 380 M-1



mw 394.5 M-4

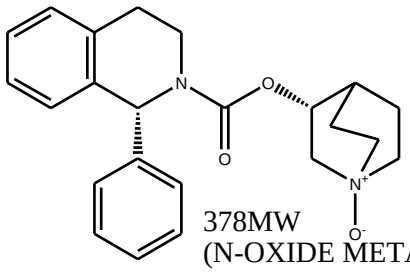


mw 424.5 M-3

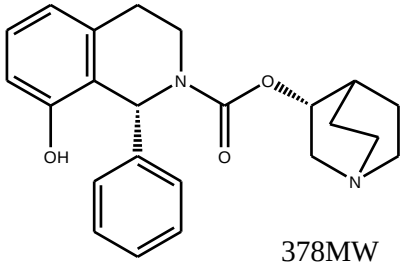


mw 196.1 AM-1

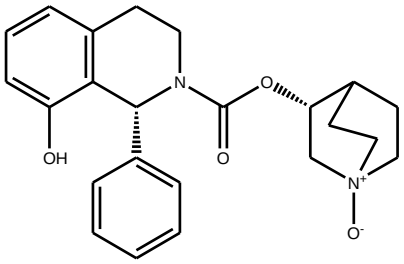
Appendix E: Main solifenacin metabolites



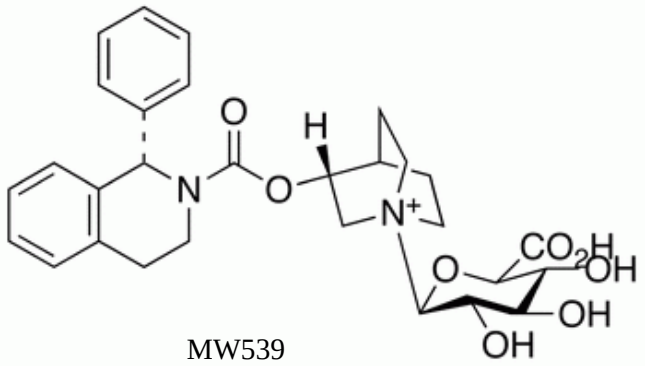
378MW
(N-OXIDE METABOLITE)



378MW
(4R-HYDROXY METABOLITE)



MW394
(4R-HYDROXY-N-OXIDE
METABOLITE)



MW539
(SOLIFENACIN N-GLUCURONIDE)