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PHARMACEUTICALS AND THEIR TRANSFORMATION PRODUCTS IN WATERS – ANALYTICS, HYDROLYTIC STABILITY AND THEIR ADSORPTION ONTO MULTI-WALLED CARBON NANOTUBES

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

2-OH-CBZ – 10,11-dihydro-2-hydroxycarbamazepine

2-OH-IBU – 2-hydroxyibuprofen

4-OH-DIC – 4'-hydroxydiclofenac

5-FU – 5-fluorouracil

7-OH-MTX – 7-hydroxymethotrexate

10-OH-CBZ – 10,11-dihydro-10-hydroxycarbamazepine

AC – activated carbon

ACN – acetonitrile

ac-SMX – N^4-acetylsulfamethoxazole

CBZ – carbamazepine

CBZ-Ep – carbamazepine-10,11-epoxide

CNTs – carbon nanotubes

CP – cyclophosphamide

cx-IBU – ibuprofen carboxylic acid

des-NPX – *O*-desmethylnaproxen

ESI – electrospray ionization

HPLC – high-performance liquid chromatography

IBU – ibuprofen

IDL – instrumental detection limit

IF – ifosfamide

IT – ion trap

IQL – instrumental quantification limit

LC-HRMS – high-performance liquid lhromatography coupled with high-resolution mass spectrometry

LC-MS - high-performance liquid chromatography coupled with mass spectrometry

LOD – limit of detection

LOQ – limit of quantification

MeOH – methanol

MRM – multiple ion monitoring

MS – mass spectrometry

MTX – methotrexate

MTZ-OH – hydroxymetronidazole

MWCNTs – multi-walled carbon nanotubes

NPX – naproxen

NSAIDs – nonsteroidal anti-inflammatory drugs

O-DMTRA – *O*-desmethyltramadol

PC(s) – parent compound(s)

ppm – parts per million

PTP(s) – pharmaceuticals' transformation product(s)

QqQ – triple quadrupole analyser

Q-TOF – quadrupole – time-of-flight analyser

SEM – scanning electron microscope

SIM – single ion monitoring

SPE – solid-phase extraction

TEM – transmission electron microscopy

TIC- total ion current

TRA – tramadol

UV-Vis – ultra violet–visible detector

WE – wastewater effluent

WI – wastewater influent

WWTP – wastewater treatment plant

1. INTRODUCTION

Since the early reports in the 1990s on the detection of some pharmaceuticals in sewage waters and rivers [1–4], through the rapid increase of the interest in this topic during the last two decades, nowadays, pharmaceuticals are well-known and documented pollutants of the aquatic environment. Studies revealing their presence in the environment have already made an impact on the European Union authorities, who include some of them on the official Surface Water Watch List under the Directive 2013/39/EU, to collect monitoring data on the priority substances [5]. The list was initiated in 2015 and has been updated twice since then. Initially, it included diclofenac, erythromycin, clarithromycin and azithromycin. Currently, the Watch List contains amoxicillin, ciprofloxacin, sulfamethoxazole, trimethoprim, venlaflaxine and its metabolite *O*-desmethylvenlaflaxine, clotrimazole, fluconazole and miconazole. It shows that more and more medicaments will be taken into particular consideration in terms of their presence in the surface waters.

Moreover, in recent years, more attention has been paid to other potential contaminants, namely pharmaceuticals' transformation products (PTPs). This term includes metabolites, which are being formed in the living organism after the consumption of a medicine and then excreted, as well as degradation products, which are formed through all spectrum of biotic and abiotic processes in the environment, such as hydrolysis, photolysis or biodegradation. Nevertheless, there is far less data on the presence and concentration levels of PTPs in the environment in comparison to their parent compounds (PCs). It seems that the situation is analogical to the detection of the native forms of the pharmaceuticals at the beginning of the XXI century – growing number of the experts are noticing the topic, more scientific papers with specific data are being released, but the considerable increase in interest is yet to come. Unfortunately, there are still significant limitations that hold back from wider detection of PTPs, such as multiplicity of the derivatives formed during biotic and abiotic changes (some still unknown) or the commercial availability of the chemical standards. Nonetheless, some PTPs are biologically active, like carbamazepine-10,11-epoxide [6], other can be transformed by biota back to the parent compound, like N^4-acetylsulfamethoxazole [7]. Moreover, it is important that some pharmaceuticals are metabolized to large extent, for example only 3 % of carbamazepine, 15 % of sulfamethoxazole, <1 % of propranolol or 1 % of diazepam are excreted in unchanged form [8,9]. Therefore, it is crucial to investigate the environmental fate of pharmaceuticals and their TPs to evaluate the exposure to these pollutants and the risk of their presence in the environment. The significant outcome of these efforts should also concentrate on the solutions for their effective removal.

For this reason, many different technologies for the removal of the residues of pharmaceuticals from water matrices have been developed, among which Advanced Oxidation Processes (AOPs), UV radiation or filtration processes (sand filters, micro-, ultra- or nanofiltration and reverse osmosis) have been proposed. However, these technologies pose also some disadvantages, like high operational cost [10], inefficient pharmaceuticals removal [11,12], formation of transformation products, which can be persistent and toxic [13] or require pH adjustment afterwards [14]. For these reasons, technologies based on the adsorption have been recognized as very promising alternatives, also for the removal of the pharmaceuticals and their TPs, which can be used for better water purification. The adsorption process is seen as simple, cost-effective and reliable. It does not produce side products and can be performed at various conditions [15,16]. The adsorption process based on the application of the activated carbon (AC) has already been applied for example as tertiary treatment of wastewater, but it was also found not to be sufficient for all pharmaceuticals [14]. Therefore, technologies based on the new nanomaterials as efficient adsorbents are nowadays recognized as a promising approach. In this mater, special attention has been paid to carbon nanotubes (CNTs), which are a novel material capable of removing various chemicals from water [17–19]. They can be utilised in wide pH range, are mechanically resistant, have well-defined structure with large surface area and their surface can be modified to improve their performance [15,20,21]. Moreover, large number of studies describing the adsorption of numerous pollutants indicates, that these materials are appropriate for such application. Nevertheless, there are still gaps in the knowledge in this topic, especially regarding their regeneration and multiple use.

Therefore, the main aim of this thesis was to develop novel and sensitive analytical methods for the determination of selected pharmaceuticals and their transformation products in aqueous environmental samples. The aim of this thesis was also to use developed methods in the comprehensive analysis of these compounds in different matrices, during hydrolytic stability studies and in the evaluation of their removal with multi-walled carbon nanotubes (MWCNTs).

2. THEORETICAL PART

2.1 Pharmaceuticals and their transformation products in the environment

2.1.1 Sources in the environment

In general, there are several sources of pharmaceuticals and PTPs in the environment, but the main are the inefficiently purified wastewaters [14]. Pharmaceuticals enter the municipal sewage waters due to the excretion of consumed medicaments into the sewage systems or inappropriate discharge of the unnecessary or expired products into the toilets. Naturally, it also includes significant loads of these pollutants in hospital effluents. Moreover, industrial wastewaters from the pharmaceutical manufacturing are also a source of pollution, as well as livestock breeding and even pets, where a lot of analgesics, antimicrobial agents and even hormones are applied to the animals, which they excrete. In this case, either they can get to the sewage waters or directly to the environment with manure, rain flows, through the ground water or by mixing with natural waters in the case of the aquacultures. Possible sources and routes of pharmaceuticals in the environment are presented in the **Figure 1**.

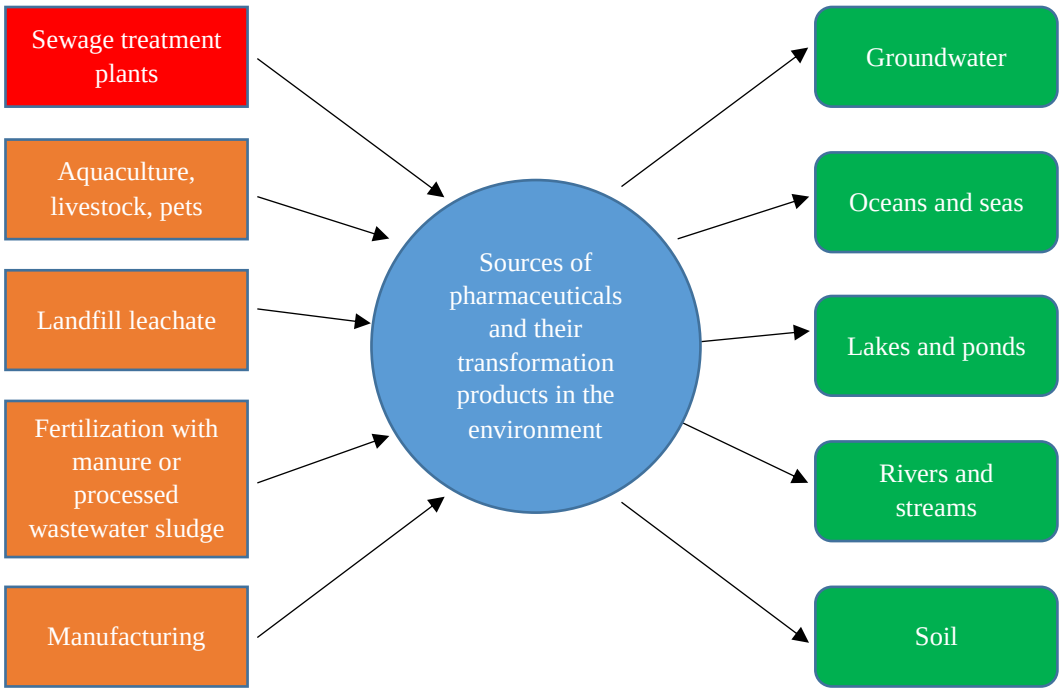


Figure 1 Sources and pathways of pharmaceuticals and their transformation products in the environment

It is worth mentioning, that consumed pharmaceuticals (both human and veterinary) undergo metabolism, which results in the production of metabolites, new compounds, in some cases only slightly different from parent compound with an additional functional group or with a part of a structure removed. In general, pharmaceuticals are mostly metabolized in the liver and excreted

via kidneys with urine [22,23]. The percentage of their metabolism vary very strongly between different pharmaceuticals. Some medicaments are excreted in unchanged forms only as several percent of initial dose. For instance, 60 % of a dose of tramadol hydrochloride is removed as metabolites [23], while less than 1 % of tacrolimus and mycophenolic acid remain intact [22], as well as 3 % of carbamazepine [24].

Moreover, it must be highlighted that besides metabolic processes, transformation of parent compound may occur outside human or animal body, straightway in the environment, leading to the formation of degradation products. This may undergo through different processes, such as direct or indirect photolysis, hydrolysis or biodegradation [25]. However, the environmental changes are much less known than the metabolic ones. It is due to the complexity of processes occurring in the nature, which are very challenging to follow or to predict. Additionally, it has to be underlined, that metabolites and degradation products may undergo further transformation, which creates more transformation products and, in fact, secondary pollution. This shows how complex this process is.

2.1.2 Biotic and abiotic transformation

In general, it is impossible to fully understand the fate of a chemical in the environment *in situ*. Therefore, model laboratory experiments involving basic studies on photolysis, biodegradation or hydrolysis are necessary to evaluate these processes at least to some extent. It must be highlighted that the knowledge on the fate of parent compounds is much better than for their transformation products. Therefore, this chapter refers mainly to the available data on the stability of the PTPs. Detailed information on the susceptibility to photodegradation, biodegradation and hydrolysis of PTPs has been just recently published in the review paper, which I have co-authored [26]. It has to be mentioned, that the data is not consistent in terms of the conceptual approaches of the experiments and conditions applied during investigations. This makes it difficult to compare the data between different tests, for example, when different light intensities were used to assess the photolysis. Moreover, a majority of the available data refers mainly to the transformation of the native forms of pharmaceuticals, during which the transformation products are observed and its stability is or might be assessed. Moreover, many tests, which include some data on PTPs were prepared to enable observing a certain transformation of the native form of pharmaceuticals, like the use of high amounts of biologically active materials to observe formation of the PTPs or using high temperatures, high light intensity etc. This gives only partial or very limited information on PTPs stability. Therefore, it seems obvious that more studies should be performed aiming at assessing the stability of PTPs as target compounds.

2.1.2.1 Photolysis

This abiotic degradation process can be either direct or indirect. Direct photolysis occurs when a molecule absorbs a photon (solar energy) which leads to breaking of a chemical bond. Indirect photolysis includes other chemicals in water, like nitrates or dissolved organic matter (natural photosensitizers), which are excited by the light and form oxidant species, like singlet oxygen $^1O_2$, hydroxyl radicals $HO^\bullet$ and others. These highly reactive oxidants can then react with other organic compounds, such as pharmaceuticals or PTPs and cause their degradation [27,28].

In general, native forms of pharmaceuticals present various susceptibility to photolysis. For example, oseltamivir was found resistant to direct photolysis from natural sunlight in river water [29]. Similar conclusions were made for acetaminophen, atenolol, carbamazepine, ibuprofen and mefenamic acid, whereas propranolol, indomethacin and ifenprodil were easily degraded [30]. However, comparison of two sets of experiments performed separately in August and May (half-life of carbamazepine in first experiment was 84 h, in second 2100 h, similar dependence for ibuprofen and atenolol) using natural sun shows, that this kind of measurements cannot be accurate [30]. In general, it has to be highlighted that the experiments on photolysis are performed in various ways, differing in source and intensity of light, additives, matrix, pH, temperature and other parameters [31] (**Table 1**). The most interesting ones are those taking into account natural conditions, which prove that photolysis occurs only near the surface of the reservoir [32,33] and that laboratory data may be different from what happens in the environment [34]. Nevertheless, there is quite a lot of data on photolytic transformation of pharmaceuticals, but much less on their TPs. Therefore, the available data on that issue is presented in **Table 1**.

Table 1 Summarized collection of the data on pharmaceuticals' transformation products (TPs) and their parent compounds (PCs) regarding photolysis

Investigated pharmaceuticals and their transformation products	Identified degradation products	Crucial experimental data	Stability	Comments/conclusions	Lit.
Norfluoxetine	-	Lamp power 765 W/m^2, wavelength 300 – 800 nm.	One of the most stable in the experiment, half-life 95 h.	Faster degradation than in other study due to difference in lamp power.	[34]
Fluoxetine Norfluoxetine	Norfluoxetine	Fluorescent bulbs FL40T12-/BL; light source – sample distance 50 cm; maximum intensity at 340 nm.	Fluoxetine is quite resistant to photolysis, higher stability in acidic conditions. $t_{1/2}$ (days): pH 5 (385), pH 7 (277), pH 9 (102), synthetic humic water (21), lake waters (112, 133).	Fluoxetine seemed much more susceptible to indirect photolysis, but samples in lake water did not support that. Norfluoxetine is more susceptible than fluoxetine. Also other unknown degradation products occur.	[35]
Sulfamethoxazole N^4- acetylsulfamethoxazole 4-nitrososulfamethoxazole 4-nitrosulfamethoxazole *N*-hydroxysulfamethoxazole Sulfamethoxazole β-D-glucuronide	Sulfanilic acid (5-methylisoxazol-3-yl)sulfamate Aniline 3-amino-5-methylisoxazole 4-acetamidobenzenesulfonic acid *N*-phenylacetamide (5-methylisoxazol-3-yl)sulfamate sulfamethoxazole	Two setups: solar simulator with Xe lamp, 76 W/m^2 between 265 and 430 nm and Rayonet with lamp intensity 44.4 W/m^2 from 265 to 430 nm.	Degradation much quicker at low pH. Sulfamethoxazole β-D-glucuronide the most susceptible ($t_{1/2}$ 0.04 h), sulfamethoxazole only slightly less. Other much more stable.	Some less abundant TPs are transformed back to the PC or to main TPs, like N^4- acetylsulfamethoxazole.	[36]
N^4-acetylsulfamethazine Sulfamethazine N^4-acetylsulfapyridine Sulfapyridine	Multiple products found	HPLC grade water and WWTP effluent as media; SunTest CPS with Xe lamp, 450 W/m^2, emitted wavelengths from 200 to 800 nm.	Lowest stability observed for *N*-acetylsulfapyridine in HPLC water ($t_{1/2}$ 0.25 h) and highest for sulfapyridine in HPLC water ($t_{1/2}$ 10.93 h).	N^4-acetylsulfamethazine and *N*-acetylsulfapyridine were more susceptible to the direct photolysis than their PCs. However, they were more stable in the WWTP effluent than HPLC water, contrary to their PCs.	[37]

4-methylaominoantypirine 4-acetylaminoantipyrine 4-formylaminoantipyrine	Multiple products found	Pure water, synthetic seawater, synthetic freshwater; irradiation Suntest CPS+ 250 and 765 W/m^2, temp. 17 and 40 °C.	4-methylaominoantypirine quickly degraded ($t_{1/2}$ between 0.12 – 0.58 h) by direct photolysis in various conditions. Indirect photolysis does not occur. 4-acetylaminoantipyrine and 4-formylaminoantipyrine in pure water with half-lives of 27.9 h and 24.7 h.	-	[27]
Diclofenac, sulfamethoxazole, *N*^{4}-acetylsulfamethoxazole, acyclovir, atenolol, bezafibrate, clarithromycin, erythromycin, trimethoprim, metoprolol, tramadol, *N*-desmethyltramadol, *O*-desmethyltramadol, N,O-didesmethyltramadol, oxazepam, codeine, carbamazepine, 10,11-dihydro-10-hydroxy-carbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine, 2-hydroxycarbamazepine, 3-hydroxycarbamazepine, venlafaxine, *O*-desmethylvenlaflaxine, *N*-desmethylvenlaflaxine, *N,O*-didesmethylvenlaflaxine, fluconazole, iomeprol, iopromide, diatrizoate	-	WWTP effluents in tubes placed under the water surface on different depths.	At depth 10 cm after 6 days highest removal rates (over 90 %) were found for diclofenac, *O*-desmethyltramadol, *O*-desmethylvenlaflaxine, *N,O*-didesmethylvenlaflaxine and 3-hydroxycarbamazepine. Venlaflaxine, tramadol, *N*-desmethyltramadol, 2-hydroxycarbamazepine and *N*-desmethylvenlaflaxine were degraded in about 70 %. The rest was stable.	At the depth 40 cm no degradation was observed. Photolysis occurs only close to the surface.	[32]

Taking into account the available literature data on this topic it might be concluded that there is high variability in susceptibility to photolysis between pharmaceuticals and PTPs. For example, TPs of sulfamethoxazole were generally more stable than their PC, while norfluoxetine was less stable than fluoxetine. Moreover, matrix can have an impact on degradation rates, for example N^4-acetylsulfamethazine and N^4-acetylsulfapyridine were more stable in wastewater than in pure water [37]. It might be also suggested that further studies with more unified approach should be recommended to fully assess the susceptibility of selected transformation products of pharmaceuticals to photodegradation under environmental conditions as well as compare their fate and determine those of the highest stability and hence possibly of the highest priority in terms of their future environmental risk assessment.

2.1.2.2 Biodegradation

Biodegradation is a process of an elimination of a chemical compound through the biochemical mechanisms, mostly involving microorganisms and protozoa [28]. It can be done either by metabolic processes of an organisms, which use a compound as a source of the energy, carbon and nitrogen or by co-metabolism, in which transformation of a compound is a side-effect of metabolic changes of other substances, which provide the energy and essential nutrients for the organism [28,38]. Moreover, this process may occur in aerobic, anoxic or anaerobic conditions [12]. Biodegradation plays enormous part in the removal of the organic pollutants in the environment, since microorganisms are present almost everywhere in the ecosystems. Their abilities are widely used in wastewater and drinking water treatment, where active sludge, sediment or biofilm is utilized to purify water. However, according to the literature, pharmaceuticals are often not sufficiently removed from wastewater [39,40] or even drinking water, despite the fact that in some cases they are easily biodegradable [41]. It shows, that they are either poorly biodegradable or the purification system is not efficient enough to manage such big load of contaminants. Therefore, some laboratory tests have been made to evaluate susceptibility of the pharmaceuticals and their TPs to biodegradation. In general, there are medicaments, which are easily or quite easily transformed by microorganisms, such as ibuprofen [12,42], naproxen, β-lactam antibiotics [43] or ciprofloxacin [44], whilst carbamazepine, sulfamethoxazole, diclofenac or macrolide antibiotics are recognized as much more persistent [30,43,45]. Nevertheless, the susceptibility or degradation rates vary depending on many factors, such as the conditions of performed studies, like aerobic or anaerobic environment [46], with or without additional source of energy [47], time of the contact [43] etc. as well as the type of the microorganisms [48]. Moreover, studies focused on the evaluation of biodegradation of PTPs are very limited. Besides scarce data regarding specifically

PTPs, additional information can be deducted from biodegradation tests of their PCs, when they are being formed during experiment and observed. However, if the PC remains in the solution by the end of the test and concentration of TPs is constant or increases, which gives no information on the stability of TPs. Detailed information has been summarized in the **Table 2**.

Table 2 The available data on biodegradation of pharmaceuticals' transformation products (TPs) and selected examples concerning the information on biodegradation of their parent compounds (PCs) (<LOQ – below the limit of quantification)

Target analyte	Experimental setup	Stability	Observed TPs and their stability	Lit.
Ketoprofen	Batch test (28 days) with WWTP sludge (adapted to the removal of investigated compounds). Metabolic and co-metabolic (with nutrients) transformation investigated.	Removed through metabolic transformation, intact with the addition of nutrient.	3-(hydroxy-carboxy- methyl)hydratopic acid, quickly removed.	[47]
			3-(keto-carboxymethyl)- hydratopic acid, concentration increasing until the end of the test, but with ketoprofen still in the solution.	
Ibuprofen		Intact without nutrient, removed in 15 days through co-metabolism.	1- and 2-hydroxyibuprofen, found in small amounts, removed quite quickly.	
Bezafibrate		Intact without nutrient, removed through co-metabolism, degraded the quickest.	4-chlorobenzoic acid, gone in several days. Other undetected TPs were there basic on the dissolved organic carbon measurements.	
Naproxen		60 % eliminated in co-metabolism.	*O*-desmethylnaproxen, formed in small amount. In longer period eliminated.	
Diclofenac		Intact in both cases.	-	
Diclofenac	Fixed-bed column bioreactor with sediment from a creek receiving WWTP effluent. High sediment/water ratio.	Quickly degraded, even with multiple additions of diclofenac to column.	p-benzoquinone imine of 5-hydroxydiclofenac, with concentration decreasing in time.	[48]
p-benzoquinone imine of 5-hydroxydiclofenac		Decreased in time with biologically active and sterile sediment, possibly resistant to biodegradation.	-	
Phenazone	Batch tests with biologically active clay from drinking water treatment plant.	Complete transformation after 168 h.	1,5-dimethyl- 1,2-dehydro-3-pyrazolone, formed in small quantity, decreasing in time.	[41]
Propyphenazone		Complete transformation after 168 h.	4-(2-methylethyl)-1,5-dimethyl-1,2-dehydro-3-pyrazolone, formed in small quantity, decreasing in time.	
Dimethylaminophenazone		Rapid degradation.	1-acetyl-1-methyl-2-phenylhydrazide, detected at constant level through entire test.	
			1-acetyl-1-methyl-2-dimethyloxamoyl-2-phenylhydrazide, detected at constant level through entire test.	
			Acetoaminoantipyrine, small amounts, quick removal.	
			Formylaminoantipyrine, small amounts, quick removal.	
1,5-dimethyl- 1,2-dehydro-3-pyrazolone		Complete degradation after 168 h.	-	

1-acetyl-1-methyl-2-phenylhydrazide Acetoaminoantipyrine Formylaminoantipyrine 1-acetyl-1-methyl-2-dimethyloxamoyl-2-phenylhydrazide				
		Completely stable.	-	
Carbamazepine	Batch test (35 days) with river sediment and artificial river water with daily dose of glucose and aeration.	Almost completely stable.	Carbamazepine-10,11-epoxide, small amount, concentration increasing until the end, but carbamazepine was still present in solution.	[49]
Diclofenac		Almost complete removed by the end of the test.	4-hydroxydiclofenac, small amounts, when diclofenac was removed, significant disappearance, suggesting susceptibility to biodegradation.	
Ibuprofen		Completely removed.	2-hydroxyibuprofen and carboxyibuprofen, quick formation and quick disappearance – susceptible to biodegradation.	
Metoprolol		Around 60 % removal.	Metoprolol acid, the most abundant, increasing concentration by the day 20, then rapid degradation.	
			α-hydroxymetoprolol, increasing concentration for 10 days, then disappearance.	
			α-ketometoprolol reached maximum concentration in 10 days and remained on this level until the end.	
			Unknown TP, quite persistent.	
Acetaminophen, bezafibrate, bicalutamide, carbamazepine, chlorthalidone, clofibric acid, fluconazole, furosemide, hydrochlorothiazide, ibuprofen, ketoprofen, metoprolol, naproxen, propranolol, sotalol, sulfamethoxazole, tramadol	Laboratory-scale artificial rivers (flumes) with different sediments and water.	The lowest dissipation half-lives: acetaminophen, bezafibrate, ibuprofen, ketoprofen (1.8 – 2.2 days), the highest: bicalutamide, hydrochlorothiazide, tramadol, carbamazepine, chlorhalidone, fluconazole (49 days to complete stability). PCs migrate to the pore water, where biotransformation occurs. TPs were found in surface and pore water.	4-chlorobenzoic acid (quick degradation); carbamazepine 10,11-epoxide (stable increase); saluamine (stable increase); 4-amino-6-chloro-1,3-benzenedisulfonamide, chlorothiazide (stable increase); 2-hydroxyibuprofen carboxyibuprofen (<LOQ); metoprolol acid (increase, then decrease by the end); α-hydroxymetoprolol (<LOQ); 1-naphthol (quick increase and disappearance).	[50]

2.1.2.3 Hydrolysis

Hydrolysis is a common process of a chemical degradation due to the cleavage of chemical bonds by the reaction with water. In general, it is a reaction resulting from nucleophile attack on an electron-depleted part of a chemical structure, which in consequence leads to the production of two different products [51]. Hydrolysis may be strongly dependent on the pH of water [52,53]. As the pharmaceuticals and their TPs are mainly dissolved in water in the environment, it makes it one of the basic processes of their potential elimination. However, it must be highlighted that this process was mainly investigated in the terms of their stability as a medicament before administration of the drug, whereas their stability in water in the environmental context was recognised much later. It is worth mentioning, that pharmaceuticals detected in the environment present very diversified persistency in water. For example, sulphonamides are quite stable in water solution, with higher stability at alkaline than acidic conditions [54]. Nevertheless, at the environmentally relevant temperature of 20 °C, their degradation was negligible for 30 days of the test. On the other hand, quick amoxicillin hydrolysis at 30 °C occurs the fastest in alkaline conditions ($t_{1/2}$ = 7 h at pH 11), the slowest at pH 7 ($t_{1/2}$ = 144 h) and at moderate rate at pH 3 ($t_{1/2}$ = 89 h) [55]. Oxytetracycline is more stable, but the degradation is also pH-dependent [56]. The quickest degradation occurs at neutral conditions (over 70 % degradation in 6 days at 25 °C), while it is much more stable at low pH (only 10 % degradation at pH 3). Moreover, the degradation rates are at the same level independently from the concentration of the analyte. Furthermore, antibiotics chloramphenicol, florfenicol, spiramycin and tylosin are hydrolytically stable at environmentally relevant temperature and pH [57]. In the experiments with batch tests including river sediments, out of 16 PCs there were only two TPs of hydrochlorothiazide found in sterile and water-only controls, namely chlorothiazide and 4-amino-6-chloro-1,3-benzenedisulfonamide [49]. Dark controls of sulfapyridine, sulfamethazine, acetylsulfapyridine and acetylsulfamethazine showed that there was no hydrolytic degradation during photolysis tests [37]. Control experiments with water-only setup for an environmental fate of pharmaceuticals in artificial river showed, that during 29 days that only 5 out of 17 compounds were not fully stable [50]. Ibuprofen, bezafibrate, acetaminophen, ketoprofen and hydrochlorothiazide were removed from 7 % to 28 % of the initial concentration. In general, it was not a rapid degradation, so it could be stated that investigated pharmaceuticals are quite resistant to the hydrolysis.

Similarly to the state of knowledge on the photodegradation or biodegradation, also in this case data on PTPs susceptibility to hydrolysis is much more limited. Often, some conclusion can be drawn from other tests (like biodegradation or photolysis experiments), where control solutions

are prepared and verified to monitor the stability of the analytes. For example, incubation of the control sample of p-benzoquinone imine of 5-hydroxydiclofenac without sediment showed almost no decrease of concentration, which excludes hydrolysis [48]. 4-methylaominoantypirine was also stable as a dark control sample to the photolysis tests [27]. In the case of data targeted at hydrolysis of PTPs, it was proved that ricobendazole degradation rates are pH-dependent with a V-shape rate profile (pH 2 – 12), with a lowest degradation rate at pH 4.8 ($t_{1/2}$ = 96 months at 55 °C), which indicates high stability under these conditions. More rapid degradation was observed at alkaline conditions. Additionally, concentration of phosphate buffer had no influence on the degradation rates [52]. On the other hand, phosphoramide mustard degradation at 25 °C was fast [58]. In the pH range 5 – 12, the hydrolysis rates were constant, but with the acidification the rates decreased significantly ($t_{1/2}$ were 5.5 h at pH 1.7, 1.6 h at pH 7.4 and 1.7 h at pH 12). There has also been a chloride stabilization effect observed; degradation decreased with the increase of the chloride ions concentration. Other observations indicate that hydrolysis of 2-dechloroethylifosfamide and 3-dechloroethylifosfamide is also pH-dependent [59,60]. They both degrade quickly in strongly acidic conditions, but the process slows down with the increasing pH up to 6, in which the degradation rates are the lowest. It is worth mentioning, that various anticancer drugs and some of their metabolites were investigated in terms of their stability [61]. In general, after 24 h of incubation at 4, 15 and 25 °C, the least stable was 5-(3-*N*-methyltriazen-1-yl)-imidazole-4-carboxamide, which degraded almost completely even at 4 °C. Other PTPs were stable at 4 °C. However, at 25 °C hydroxymethotrexate was not detected in the solution, while 35 % of its PC methotrexate was still observed, which means that TP is less stable than PC. On the other hand, tamoxifen was completely removed, while (Z)-4-hydroxytamoxifen and endoxifen remained intact in 70 and 75 %, respectively. Moreover, 87 % of hydroxypaclitaxel, while only 78 % of paclitaxel were still observed after 24 h. Nevertheless, as all investigated compounds were in the same solution, it cannot be excluded that PCs were transformed into TPs, which makes it harder to evaluate the stability of TPs. Taking into consideration all of the data available, there is still necessity to evaluate hydrolytic stability of pharmaceuticals and especially their TPs in unified conditions that could relate to the environmental conditions, which could allow the comparison of the obtained/presented data.

2.1.3 Biological activity and toxicity of transformation products of pharmaceuticals

There are some premises that PTPs should be taken into account alike native forms of pharmaceuticals in terms of their presence in the environment and potential harmful effects of that. One of the factors indicating that PTPs may be pollutants of high concern is the activity preserved from their native forms; hence, they can still act as medicaments, which might have negative influence on biota. There are various PTPs which pharmacological activity have been already proved, for example clofibric acid (TP of clofibrate), 2-hydroxyatorvastatin (TP of atorvastatin), carbamazepine-10,11-epoxide (TP of carbamazepine), 10,11-dihydro-10-hydroxycarbamazepine (TP of oxcarbazepine), desmethyldiazepam and oxazepam (TPs of diazepam), enalaprilat (TP of enalapril), trans-4-hydroxyglyburide (TP of glyburide), 7-hydroxymethotrexate (TP of methotrexate), hydroxyl-desmethyltamoxifen and 4-hydroxytamoxifen (TPs of tamoxifen) and many more. These last two metabolites of tamoxifen are actually 100 times more active than its PC [62–64].

Besides pharmacological activity, Maculewicz et al. made a thorough review of the current state of the knowledge on ecotoxicological studies regarding PTPs [26]. In general, for some of the studied PTPs, like acridine, *N*-desmethylclarithromycin, norfluoxetine, norsertraline or 4-hydroxytamoxifen, the EC_{50} was below 1 mg/L, which according to the European Directive EC 93/67/EEC is the value which labels the compounds as very toxic to the aquatic organisms [65]. Moreover, it was found that PTPs such as carboxycyclophosphamide, *N*-acetylsulfapyridine or mixture of photodegradation products of naproxen, carbamazepine, tetracycline and others were more toxic than their PCs [26]. In other cases EC_{50} was on the different levels of mg/L, like 5 mg/L determined for sulphanilamide towards *L. minor*, which classifies it as toxic to aquatic organisms or carboxycyclophopshamide and *S. leopolinensis* at a level of 17 mg/L, which classifies it as harmful to aquatic organisms, according to the European Directive EC 93/67/EEC [65]. Some compounds, like carboxycyclophopshamide or 4-hydroxytamoxifen were also found either genotoxic or had an influence on size reduction or morphological abnormalities of the test organisms. It indicates that PTPs may be directly toxic to biota, however, it must be highlighted that many of them have an influence on test organisms at much higher concentration than usually found in the environment. Nevertheless, there is still a lot to investigate in terms of mixture and chronic effects of prolonged exposure to low doses of these compounds.

2.1.4 Data on the presence of selected pharmaceuticals and their transformation products in the environment

There has been a significant number of data published on the presence of pharmaceuticals in the environment worldwide. The review papers have been regularly published for many years to summarize the state of the art up to date [2,9,66,67]. As the number of studies increases over the years, the reviews became often focused on certain group of pharmaceuticals [68,69], environment compartment [70] or on combining the data on presence with their toxicity, removal, fate or limiting to the data from a certain geographic region [40,71,72]. Some studies have also included data on PTPs [28,73,74] and their number is growing. However, the data on the PTPs is far less available than for their PCs. Therefore, available information on PTPs found in sewage and natural waters have been summarized in **Table 3** and **Table 4**, respectively. Due to the fact, that analytical papers and reviews often include quite significant load of data, like number of compounds, matrixes, and additional information, the data in the **Table 3** and **Table 4** is restricted to the PTPs that were included in the research within this doctoral thesis.

Table 3 Summarized selected data on the presence of pharmaceuticals; transformation products and their parent compounds in wastewaters worldwide (<LOQ – below the limit of quantification; <LOD – below the limit of detection; WI – wastewater influent; WE – wastewater effluent)

TPs	Country	Sample	Conc. range [ng/L]	Mean (median) ng/L	Lit.
Sulfamethoxazole	Various	WI	<LOD – 2 260	-	[9]
		WE	<LOD – 37 700	-	[9]
		WI	100 – 100 000	-	[71]
		WE	90 – 10 000	-	[71]
N^4- acetylsulfamethoxazole	Spain	WE (24 h composite)	<LOQ – 324	105	[75]
		WI (24 h composite)	95 – 1 558	-	[76]
		WE (24 h composite)	30 – 960	(90)	[77]
	Switzerland	WI (24 h composite)	850 – 1 600	(1 400)	[78]
		primary WE (24 h composite)	570 – 1 200	(890)	[78]
		secondary WE (24 h composite)	<LOQ – 150	(40)	[78]
		tertiary WE (24 h composite)	<LOQ – 180	(10)	[78]
	Australia	WI (grab samples)	390 – 445	-	[79]
		MBR electro-chlorination effluent (grab samples)	27 – 35	-	[79]
		UV-disinfected effluent (grab samples)	8 – 47	-	[79]
		primary WE (72 h composite)	518 – 943	-	[80]

		secondary WE (72 h composite)	<LOD – 86	-	[80]
		tertiary WE (72 h composite)	71 – 82	-	[80]
	UK	WE (grab samples)	690 – 2 200	-	[81]
Ibuprofen	Various	WI	<LOD – 2 109 880	-	[46]
		WE	<LOD – 28 000	-	[46]
		WI	1 100 – 200 000	-	[71]
		WE	500 – 90 000	-	[71]
1-hydroxy ibuprofen / 2-hydroxy ibuprofen	Spain	WE (24 h composite)	141 – 1 200	618	[75]
1-hydroxyibuprofen		WI (grab sample)	2 550 – 5 780	-	[82]
		WE (grab sample)	110 – 1 410	-	[82]
2-hydroxyibuprofen		WI (grab sample)	1 210 – 9 398	-	[82]
		WE (grab sample)	390 – 5 870	-	[82]
	USA	WE (grab sample)	<LOD – 200	-	[83]
Hydroxyibuprofen	Portugal	WI (grab sample)	<LOD – 334	115 – 179	[84]
		WE (grab sample)	<LOD – 780	322 – 780	[84]
	Norway	WI (grab sample)	1 320	-	[85]
		WE (grab sample)	210 – 1 130	-	[85]
		Hospital effluent	1 590 – 5 010	-	[85]
	Germany	WI (grab sample)	6 840	-	[85]
		WE (grab sample)	90	-	[85]
	Sweden	WI (grab sample)	990	-	[86]
		WE (grab sample)	50	-	[86]
Carboxyibuprofen	Norway	WI (grab sample)	1 630	-	[85]
		WE (grab sample)	70 – 1 270	-	[85]
		Hospital effluent	10 600 – 18 400	-	[85]
	Germany	WI (grab sample)	23 000	-	[85]
	Portugal	WI (grab sample)	7 215 – 120 365	56 165	[84]
	Spain	WI (grab sample)	<LOQ – 38 400	-	[82]
		WE (grab sample)	<LOQ – 10 650	-	[82]
	Sweden	WI (grab sample)	10 750	-	[86]
		WE (grab sample)	430	-	[86]
Carbamazepine	Various	WI	150 – 8 500	-	[71]
		WE	90 – 1 100	-	[71]
		WE		-	[71]
Carbamazepine-10,11-epoxide	Spain	WE (24 h composite)	506 – 810	632	[75]
		WI (24 h composite)	30 – 93	-	[76]
		WE (24 h composite)	0 – 87	-	[76]
		WE (24 h composite)	-	10 – 12	[87]
		WE (24 h composite)	18 – 939	-	[88]
	Portugal	WI (grab sample)	<LOD – 45	(44)	[84]
		WE (grab sample)	<LOD – 88	(63 – 88)	[84]
		WI (24 h composite and grab samples)	-	(48)	[89]
		WI (24 h composite and grab samples)	-	(50)	[89]
	Canada	WI (24 h composite)	-	47	[90]
		WE (24 h composite)	-	52	[90]

		WI (24 h composite)	-	39	[91]
		WE (24 h composite)	-	19	[91]
	Norway	WE (24 h composite)	83 – 110	-	[92]
	France	WI (24 h composite and grab samples)	<LOD – 27	-	[93]
		WE (24 h composite and grab samples)	<LOQ – 29	-	[93]
	Germany	WI (24 h composite and grab samples)	-	(59)	[89]
		WI (24 h composite and grab samples)	-	(087)	[89]
2-hydroxycarbamazepine	Canada	WI (24 h composite)	-	59	[91]
		WE (24 h composite)	-	70	[91]
		WI (24 h composite)	-	121	[90]
		WE (24 h composite)	-	132	[90]
	Spain	WI (24 h composite)	24 – 2 261	-	[76]
		WE (24 h composite)	<LOQ – 64	-	[76]
	Serbia	WE (composite)	15 939	-	[94]
	France	WI (24 h composite and grab samples)	<LOD – 39	-	[93]
		WE (24 h composite and grab samples)	<LOQ – 48	-	[93]
	Germany	WI (24 h composite and grab samples)	-	(170)	[89]
		WI (24 h composite and grab samples)	-	(140)	[89]
	Portugal	WI (24 h composite and grab samples)	-	(90)	[89]
		WI (24 h composite and grab samples)	-	(97)	[89]
10-hydroxycarbamazepine	Canada	WI (24 h composite)	-	9	[90]
		WE (24 h composite)	-	9	[90]
		WI (24 h composite)	-	22	[91]
		WE (24 h composite)	-	33	[91]
	France	WI (24 h composite and grab samples)	<LOD – 1 115	-	[93]
		WE (24 h composite and grab samples)	<LOQ – 1 170	-	[93]
	Germany	WI (24 h composite and grab samples)	-	(490)	[89]
		WI (24 h composite and grab samples)	-	(500)	[89]
	Portugal	WI (24 h composite and grab samples)	-	(270)	[89]

		WI (24 h composite and grab samples)	-	(270)	[89]
Diclofenac	Various	WI	<LOD – 44 230	-	[46]
		WE	<LOD – 131 150	-	[46]
		WI	500 – 100 200	-	[71]
		WE	50 – 1 250	-	[71]
4-hydroxydiclofenac	Spain	WI (24 h composite)	396 – 12 398	-	[76]
		WE (24 h composite)	4 – 7 016	-	[76]
	Norway	WE (24 h composite)	1 844 – 3 032	-	[92]
Metoprolol	Various	WI (24 h composite)	160	-	[86]
		WE (24 h composite)	190	-	[86]
		WI	0 – 4 723	-	[9]
		WE	6 – 4 470	-	[9]
Metoprolol acid	Spain	WI (24 h composite)	725 – 1 094	-	[76]
		WE (24 h composite)	1 078 – 2 008	-	[76]
		WI (24 h composite)	119 – 298	-	[95]
		WE (24 h composite)	1 107 – 2 506	-	[95]
Metronidazole	Various	WI	5 – 1 500	-	[71]
		WE	1 – 50	-	[71]
Hydroxymetronidazole	Portugal	Hospital effluents	<LOD – 11 344	121 – 1 604	[96]
		WI (24 h composite)	<LOD – 145	63	[96]
		WE (24 h composite)	65 – 158	102	[96]
Tramadol	Various	WI	~ 600	-	[71]
		WE	~ 650	-	[71]
O-desmethyltramadol	Croatia	WI (24 h composite)	298 – 671	-	[97]
		WE (24 h composite)	859 – 890	-	[97]
Methotrexate	various	WE (24 h composite)	<LOD – 13	-	[98]
		Hospital effluent (24 h composite)	<LOD – 4 689	-	[64]
7-hydroxymethotrexate	Spain	Hospital effluent (composite)	79 – 846	326	[99]
Naproxen	Various	WI	<LOD – 611 000	-	[46]
		WE	<LOD – 33 900	-	[46]
O-desmethylnaproxen	Germany	WE (24 h composite)	230	-	[100]

Taking into account the main routes of pharmaceuticals and their TPs release to the environment, it is completely justified why wastewaters are one of the most commonly investigated samples for their presence. Among many different medicaments and their transformation products it seems that the most frequently investigated analytes are TPs of the anticonvulsant carbamazepine:

2-hydroxycarbamazepine, 10-hydroxycarbamazepine and carbamazepine-10,11-epoxide; the TPs of non-steroidal anti-inflammatory drug ibuprofen:1/2-hydroxyibuprofen and carboxyibuprofen; as well as the TPs of sulfonamide antibiotic sulfamethoxazole: N^4-acetylsulfamethoxazole. In the case of carbamazepine, it is frequently detected in the wastewater and environmental samples, probably due to high persistency and consumption. Additionally, its TPs are well-known human metabolites and microbial transformation products. Moreover, only around 3 % of carbamazepine is excreted in unchanged form, which suggests that TPs should not be found in high amounts, at least in WWTP influent. In fact, based on the data presented in the **Table 3**, it might be observed that its metabolites have been detected in the concentration even up to several μg/L [76,94]. Also in the case of ibuprofen, one of the most popular medicaments (sold over the counter) applied for pain, inflammation or fever, commonly detected in many different environmental samples, its TPs (1-hydroxy-, 2-hydroxy-, 1/2-hydroxy- or just hydroxyibuprofen) were found in both influents and effluents, often on the levels of μg/L. Similar concentrations were also reported for the N^4-acetylsulfamethoxazole. Concentrations of other PTPs, less commonly analysed, show that they can be at very high levels, reaching μg/L even in the effluents.

It must be also highlighted that during the WWTPs processes measured concentrations are a resultant of various processes, like abiotic transformation, biodegradation and adsorption to the sludge, but also transformation of some PTPs back to their PCs. Presented results show, that monitoring of PTPs in the wastewaters should be performed in combination with their PCs in order to further assess the possible risk posed by these chemicals released into the environment.

Table 4 Summarized selected data on the presence of the pharmaceuticals' transformation products and their parent compounds in natural waters worldwide (<LOQ – below the limit of quantification; <LOD – below limit of detection)

TPs	Country	Sample	Conc. range [ng/L]	Mean (median) ng/L	Lit.
Sulfamethoxazole	Various	Various	0 – 38 850	-	[101]
			0 – 5 320	-	[102]
			<LOD – 1 820	-	[9]
N^4- acetylsulfamethoxazole	Spain	River or tributary	<LOQ – 74	5	[62]
	UK	Upstream before WWTP discharge	<50	-	[81]
		Downstream after WWTP discharge	240	-	[81]
Ibuprofen	Various	Various	1 – 84 600	-	[101]
			150 – 10 000	-	[103]
			<LOD – 3 110	-	[46]
1-hydroxyibuprofen	Spain	River	<LOD – 560	-	[82]
2-hydroxyibuprofen	France	River	<LOQ – 19	10.2	[104]
	Spain	River	<LOD – 1 460	-	[82]
Hydroxyibuprofen	Portugal	River	<LOD – 317	(17)	[84]
		Ocean	22 – 287	39 (35)	[105]
	Norway	Sea	<LOD – 2	-	[85]
	Germany	River	18 – 101	-	[106]

	Sweden	Dams after sewage discharge	280 – 380	-	[86]
		River	20 – 60	-	[86]
Carboxyibuprofen	Germany	River	<LOQ – 32	-	[106]
	Norway	Sea	<LOD – 7	-	[85]
	Portugal	Ocean	<LOD – 1 227	400 (270)	[105]
	Spain	River	<LOD – 3 950	-	[82]
	Sweden	Dams after sewage discharge	2 390 – 4 090	-	[86]
		River	230 – 680	-	[86]
Carbamazepine	Various	Various	1 – 11 581	-	[101]
			0 – 11 600	-	[103]
			0 – 3 240	-	[102]
Carbamazepine-10,11-epoxide	Spain	River or tributary	<LOQ – 1 667	1 and 7	[62]
	Serbia	Rivers, canals, lake	<LOQ – 932	-	[94]
		Untreated drinking water	128	-	[94]
	Portugal	River	<LOD – 44	(34)	[84]
	Denmark	River	17	-	[107]
		Pre-treated drinking water	6	-	[107]
	Norway	Sea (Fjord)	17 – 77	-	[92]
	Spain	Groundwater	-	7 – 34	[87]
		River	<LOD - 71	54	[108]
		Drinking water	<LOD - 2	1	[108]
		Groundwater	<LOD – 8	-	[109]
	Germany	River	60 – 80	-	[110]
	Sweden	Rivers	1 – 22	-	[110]
2-hydroxycarbamazepine	Spain	River or tributary	<LOQ – 62	77 and 212	[62]
		Groundwater	<LOD – 48	-	[109]
	Serbia	Rivers, canals, lake	160	-	[94]
10-hydroxycarbamazepine	China	Rivers	<LOD – 2	-	[111]
		Lakes	1 – 46	-	[111]
Diclofenac	Various	Various	0.0 – 57 160	-	[101]
			2 – 31 300	-	[103]
			<LOD – 380	-	[46]
4-hydroxydiclofenac	Spain	River or tributary	<LOQ – 48	1 and 6	[62]
		Groundwater	<LOD – 147	-	[109]
Metoprolol	Various	Various	1 – 3 960	-	[101]
			30 – 240	-	[86]
Metoprolol acid	China	Rivers	<LOD – 12	-	[111]
		Lakes	<LOD – 203	-	[111]
	Germany	River	400 – 890	-	[110]
	Sweden	Rivers	52 – 550	-	[110]
Tramadol	Various	Various	<30 – 5 970	-	[101]
O-desmethyltramadol	Denmark	River	17	-	[107]
	Croatia	River	8 – 10	-	[97]
Naproxen	Various	Various	0 – 12 300	-	[101]
			<LOD – 128	-	[46]
			<LOD – 7 189	-	[102]
O-desmethylnaproxen	Pakistan	Rivers, drainage, harbour	40 – 1 360	-	[100]

Based on the data presented in the **Table 4** it might be concluded that the number of reports on the presence of PTPs in the natural waters in comparison with the wastewaters is more limited. In general, the same compounds are most frequently investigated and detected (although some of the

literature sources are the same for wastewaters and natural waters, so it is obvious that it will be similar distribution of data; however, not all of them overlap). Among the investigated different samples/sources, waters collected from rivers definitely dominate. It is mostly due to the fact, that these samples are frequently collected downstream of a wastewaters discharge points. Therefore, in many cases these natural waters are constantly provided with a load of treated wastewater. In general, concentrations of PTPs in natural waters are lower than in wastewater, but there are cases when they have been detected at the levels of μg/L.

The data presented above suggests that worldwide analytical trends should be followed on a local level. Research carried out as such can feed into evaluation at a global level and enable interpretation of results from both a local and a global perspective. In Poland, the knowledge about concentration levels of pharmaceuticals, and especially their TPs, in the environment is even more limited. Just recently comprehensive studies have been published on this topic [112,113]. Previously, the studies were focused only on few analytes or one group, like nonsteroidal anti-inflammatory drugs (NSAIDs) or antibiotics or were focused mostly on developing the analytical method. Nevertheless, the summary of this knowledge is presented in **Table 5**. The compounds included in this research are also limited to the chosen ones, similarly to the previous tables.

Table 5 Summarized selected data on the presence of the pharmaceuticals' transformation products and their parent compounds in different waters in Poland (<LOQ – below the limit of quantification; <LOD – below limit of detection)

Compound	Sample	Concentration range [ng/L] or only identification	Mean (median) ng/L	Lit.
Sulfamethoxazole	Digested activated sludge	2 910	-	[114]
	Digested manure	240	-	[114]
	Lake and stream	<LOQ – 76	-	[115]
	WI (24 h composite)	1 225 – 1 778	-	[116]
	WE (24 h composite)	508 – 1 226	-	[116]
N^4- acetyl-sulfamethoxazole	WI (24 h composite)	1 358 – 3 349	-	[116]
	WE (24 h composite)	<LOQ – 196	-	[116]
Ibuprofen	WI (24 h composite)	7 479 – 11 463	-	[112]
	WE (24 h composite)	16 – 77	-	[112]
	Drwina River upstream WWTP discharge	180 – 3 730	2 909	[112]
	Drwina River downstream WWTP discharge	76 – 131	106	[112]
	Wisla River upstream WWTP discharge	60 – 85	67	[112]
	Wisla River downstream WWTP discharge	56 – 62	59	[112]
	WI (24 h composite)	3 838	-	[117]
	WE (24 h composite)	228	-	[117]
Diclofenac	WI (24 h composite)	3 416 – 40 570	-	[112]
	WE (24 h composite)	1 499 – 2 906	-	[112]
	Drwina River upstream WWTP discharge	666 – 894	794	[112]
	Drwina River downstream WWTP discharge	1 225 – 1 353	1 304	[112]
	Wisla River upstream WWTP discharge	123 – 161	136	[112]
	Wisla River downstream WWTP discharge	165 – 184	175	[112]
	WI (24 h composite)	3 268	-	[117]
	WE (24 h composite)	4 773	-	[117]
	Soil	<LOD – <LOQ	-	[118]
Metoprolol	WI (24 h composite)	<LOD	-	[117]
	WE (24 h composite)	518		[117]
Tramadol	Water phase of WWTP activated sludge	-	362 – 435	[112]
	Solid phase of WWTP activated sludge	-	202 – 401	[112]
	Drwina River upstream WWTP discharge	167 – 205	181	[112]
	Drwina River downstream WWTP discharge	274 – 469	328	[112]
	Wisla River upstream WWTP discharge	50 – 65	55	[112]
	Wisla River downstream WWTP discharge	53 – 71	61	[112]
O-desmethyl-tramadol	WI (24 h composite)	201 – 453	-	[112]
	WE (24 h composite)	567 – 1 167	-	[112]
	Water phase of WWTP activated sludge	-	571 – 1 030	[112]
	Solid phase of WWTP activated sludge	-	<LOQ	[112]
	Drwina River upstream WWTP discharge	258 – 346	287	[112]
	Drwina River downstream WWTP discharge	534 – 636	599	[112]
	Wisla River upstream WWTP discharge	92 – 120	105	[112]
	Wisla River downstream WWTP discharge	102 – 121	111	[112]
Methotrexate	Digested activated sludge	262	-	[114]
	Digested manure	18	-	[114]
Naproxen	WI (24 h composite)	19 593 – 177 704	-	[112]
	WE (24 h composite)	55 – 302	-	[112]
	Drwina River upstream WWTP discharge	436 – 1 092	874	[112]
	Drwina River downstream WWTP discharge	233 – 259	235	[112]
	Wisla River upstream WWTP discharge	51 – 65	57	[112]
	Wisla River downstream WWTP discharge	55 – 61	57	[112]

2.2 Analytical methods for the determination of pharmaceuticals and their transformation products in water samples

Analytical methods for the determination of pharmaceuticals in water samples have been intensively developed for over last 20 years. In such trace analysis of environmental samples liquid chromatography coupled with mass spectrometry (LC-MS) is by far the dominant technique. In the model, laboratory scale experiments also high-performance liquid chromatography (HPLC) coupled with different detectors is usually used. In this case, UV-Vis or photodiode-array detectors are commonly utilized [119–121]. Other detectors, like refractive index, fluorescence, chemiluminescence are far less used; usually when there is a lack of chromophore groups in the analytes, like in antibiotic tobramycin [122] or the analytes have the structural properties that allow effective application of such detectors, like macrolide antibiotics and coulimetric array detectors [123]. Nevertheless, in the case of environmental studies on the determination of pharmaceuticals, LC-MS technique has become standard instrumentality worldwide, necessary to keep up with the analytical trends. It can be seen when phrase "LC-MS + pharmaceuticals" typed at popular database Science Direct (https://www.sciencedirect.com/search?qs=LC-MS%20pharmaceuticals) shows growing number of papers published in that area (**Figure 2**). This is mainly due to the fact that mass spectrometry ensures high credibility of obtaining results.

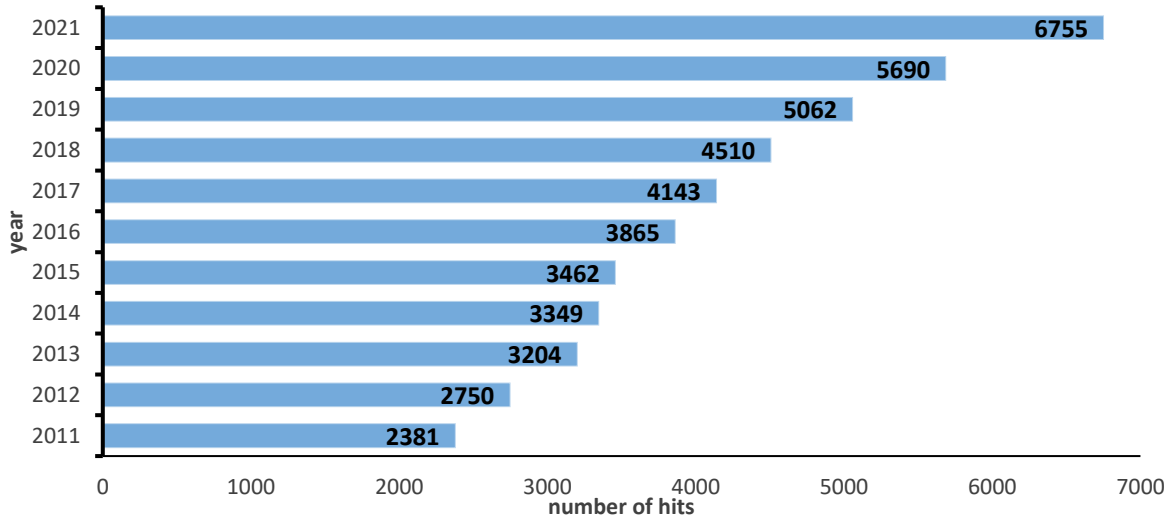


Figure 2 Number of "LC-MS + pharmaceuticals" hits in the sciencedirect.com database from 2011 to 2021

In general, this technique is based on converting sample components into ions in the ion source, passing them through ion analyser and detecting their monoisotopic masses (more specifically mass to charge ratio, *m/z*). In the vast majority of cases *m/z* refers to pseudomolecular ions $[M+H]^+$ or $[M-H]^-$ depending on the applied polarisation. Nevertheless, there can be also other ions produced, mostly through various adducts, such as $[M+Na]^+$, $[M+K]^+$, $[M+NH_4]^+$, $[M+3H]^{3+}$,

$[M+2H+Na]^{3+}$, $[M-H_2O-H]^-$, $[M+Na-2H]^-$, $[M+Formic\ Acid-H]^-$, $[M+Cl]^-$ and others [124]. It has to be highlighted, that there are different modes of data collection, which have different applications and influence on sensitivity and level of certainty on the detection of the analytes:

- SCAN, Full scan, Total Ion Current (TIC), scanning – single MS mode (LC-MS); entire mass spectra are collected and saved. It is not selective mode. Recording big number of signals causes high background noise and relatively low sensitivity;
- Selected Ion Monitoring (SIM) – single MS mode (LC-MS); only ions of interest are detected and recorded. This method is selective and much more sensitive than full scan.
- Multiple Reaction Monitoring (MRM), Selected Reaction Monitoring (SRM) – tandem mass spectrometric technique (LC-MS/MS); requires suitable mass analyser (like ion trap) or a combination of analysers, such as triple quadrupole (QqQ), in which multiple transitions between pseudo-molecular ions (called precursor ions) and fragment ions are observed. In general, precursor ions of interest are subjected to collision induced dissociation (CID), which causes their degradation and formation of fragmentation ions. Then, fragmentation ions of interest are collected and their mass spectra obtained. This technique gives high confidence of positive detection of the analytes.

Regarding current trends in pharmaceuticals determination in environmental samples, there are basically two main approaches involving different modes of data collection and qualitative or quantitative analysis, namely target analysis based strictly on MRM mode in LC-MS/MS technique, which has been developed for many years to mainly quantify concentrations of target compounds in the investigated samples, as well as suspect non-target and non-target, which are based on full scan mode performed using liquid chromatography – high resolution mass spectrometry (LC-HRMS) technique, which provides very accurate mass (*m/z*) measurement and fast detection of thousands of signals. It has to be highlighted, that the majority of the analytical methods available for pharmaceuticals and PTPs are dominated by target analysis with SIM and MRM mode (95 % of the studies present target methods [101]). Only quite recently suspect/non-target analysis have been developed and used.

Furthermore, analytical methods for the determination of pharmaceuticals and their TPs include also the step of sample preparation, which might also be different depending on the applied approach (target or suspect/non-target analysis). However, even though various techniques are available to isolate and concentrate target analytes from water samples, as well as purify them and remove part of the interferents that can disturb during the analytical process, solid phase extraction (SPE) technique dominates in this area. In general, samples are put through the columns or discs

with stationary phase, which isolates analytes from matrix, or, in some cases, retain interferents and only target compounds pass through the solid phase. If the target compounds are retained in the solid phase, they are washed out in the further step, often with organic solvent with eluting strength high enough to flush out desirable analytes. There are different types of sorbents used in SPE, such as reversed or normal phases as well as ion exchangers and other, more specific for unusual analysis. Nevertheless, Oasis® HLB (Hydrophilic – Lipophilic Balanced) (Waters, USA), is the most widely used cartridge for pharmaceuticals analysing in both target and suspect/non-target approaches. This adsorbent is a copolymer made of *N*-vinylpyrrolidone (which is hydrophilic) and divinylbenzene (which is lipophilic) [9]. This packing is a reversed-phase sorbent, but water-wettable with increased retention of polar compounds, such as pharmaceuticals and their metabolites [125]. Mostly high recoveries between 70 % to 100 % have been reported when using this columns for pharmaceuticals extraction [79,126], but it has to be mentioned that there are cases where other SPE cartridges exhibited better recovery, like for metronidazole [127]. Therefore, other SPE columns are also used in all determination approaches. Moreover, the extraction and purification method can be prepared in many ways by choosing for example sample volume, pH of the sample, clean up steps, elution solvent etc. Therefore, the approaches of target and suspect/non-target analysis and their implementations are described in the following chapters.

2.2.1 Target analysis

This approach means that only known, chosen analytes are determined in the investigated samples. In other words, the analysis is "targeted" at specific compounds. It can be performed in SIM and MRM mode. SIM is used when detection only of pseudo-molecular ion is required. It is mostly utilized in qualitative analysis. In rare cases, it is used in quantitative analysis if it is not possible to fragment the precursor ion. However, in the environmental analysis of pharmaceuticals and PTPs in water samples, the most utilized mode in target analysis is MRM (LC-MS/MS), which provides high sensitivity and selectivity. Such method is developed by analysing chemical standards of target analytes and obtaining transition ions from precursor (pseudo-molecular ion) to product (fragment) ions, which are specific for each compound. Usually, two transitions (mostly the most abundant) per compound are used, one for quantification and one for confirmation [108,116].

However, before final analysis samples need to be properly prepared in order to effectively isolate target analytes, concentrate them and remove the interferents that could disrupt analytical measurements. In the case of quantitative methods for pharmaceuticals and their transformation products determination in water samples, various sample treatments are used. First of all, the

volumes of the analysed water samples (wastewaters or natural waters) are usually between 50 and 500 mL [75,128–131]. Water samples are filtered before extraction with glass fibre filters [132], cellulose filters [131] or nylon filters [133]. Water samples have often to be adjusted pH in the range of 1.5 – 7.5 [110,126]. Then the SPE is performed. Usually, various sizes of Oasis® HLB cartridges are used, such as 500 mg/6 mL [110], 200 mg/6 mL [108] or 500 mg and 1000 mg [134]. However, other types of solid phases are also used or at least tested, such as Oasis® MAX (Mixed-mode Anion Exchange), Oasis® MCX (Mixed-mode Cation Exchange), Oasis® WCX (Weak Ion Exchange), Strata-X (Polymeric Reversed-Phase), Strata-XL-AW (Weak Anion), Isolute ENV+ (hydroxylated polystyrene-divinyl benzene copolymer), Bond Elut C18, Bond Elut Plexa, Bond Elut PPL and other [83,134–136]. In most cases the sorbent is pre-cleaned and conditioned with some organic solvents and water, then sample is put through the cartridge under the vacuum. After that, cartridge is washed by various solutions with low elution strength to remove some interferents; afterwards, column bed is dried and finally the elution of target compounds is performed [75,112]. Various solvents are used for the elution of pharmaceuticals, for example methanol (MeOH) [112,128], 2 % ammonium hydroxide with acetonitrile (ACN) [129], ethyl acetate [93], MeOH with 2 % NH_3, MeOH with 0.5 % NaOH, MeOH/methyl tert-butyl ether [126], MeOH/ MeOH with 5 % ammonium hydroxide [136], ACN with 5 % ammonium hydroxide [83] and so on. Additionally, some other steps can be mentioned, like quantitative transfer of the eluate to concentrate samples to higher degree, evaporation of volatile solvents and dissolving in different solvent (often in the initial LC mobile phase of the analytical method) or final filtration of the sample. Such prepared samples are ready to be injected into LC-MS system.

Furthermore, various LC features are used for the determination of pharmaceuticals and their TPs. Gradient elution with mobile phases like H_2O/MeOH with 0.01 % formic acid, H_2O with 0.1 % formic acid/ACN, H_2O with 10 mM acetic acid/ACN with 10 mM acetic acid, H_2O with 1mM ammonium acetate/ACN, H_2O with 0.1 % formic acid/MeOH with 0.1 % formic acid, buffer acetate 20 mM, pH 5/MeOH – ACN (50/50, v/v) with 0.05 % acetic acid, formate buffer 2mM, pH 3/ACN etc. are utilised [61,77,79,93,110,111,127,133]. Moreover, various chromatographic columns are used which differ in length, diameter, particle size and stationary phase, like XBridge Amide column (5 μm, 4.6 mm × 150 mm) (Waters), ACQUITY UPLC BEH C18 column (1.7 μm, 1 mm × 100 mm) (Waters), ACE C18 PFP (3 μm, 150 mm × 3 mm) (Avantor), UltraSep ES Phen1 column C18 (5 μm, 250 mm × 2.1 mm) (SepServ), Ultra Biphenyl column (5 μm, 100 mm × 2.1 mm) (Restek® Corporation), Phenomenex GeminiC18 (3 μm, 150 mm × 2 mm), Synergi MAX-RP (2.5 μm, 100 mm × 2 mm) (Phenomenex) [24,89,135,137–139]. Therefore, it is mostly

reversed phase chromatography, often with C18 stationary phase (sometimes with additional groups and modifications), differing in dimensions and particle sizes.

Moreover, the mass spectrometer is equipped in most cases with the electrospray ionization (ESI), working in positive or negative mode, however, positive mode is dominant [13,140]. Ionized compounds are transferred to the analyser, where filtration for ions of interest takes place. For qualitative analysis, various equipment can be used. If the obtained mass can be given as full units, low resolution MS like single quadrupole (Q) or ion trap (IT) (resolving power up to 1000) can be used. If the mass needs to be more accurate and mass spectra more detailed, high resolution MS (HRMS) should be used, like time of flight (TOF), Fourier transform ion cyclotron resonance (FTICR) or Orbitrap (resolving power over 10,000) [141]. Nevertheless, even tandem mass spectrometry (MS/MS) can be used for this purpose, like triple quadrupole (QqQ), quadrupole-time of flight (Q-TOF), ion trap-TOF (IT-TOF), IT, FTICR, Orbitrap and their hybrids [141]. In the case of quantitative methods for pharmaceuticals determination, tandem mass spectrometry is usually applied and indisputably, QqQ-MS is the most widely used equipment. Tandem MS as a whole is set to MRM mode, with three stages of operation. On the example of QqQ, first quadrupole works in SIM mode, searching for parent ions, second quadrupole works as a collision cell, where fragment ions are formed, and third quadrupole works again in SIM mode, filtering only ions of interest. Such setup allows monitoring multiple transitions within very short time, without necessity of full chromatographic separation of the analytes. Multiple parameters can be adjusted in MS, such as collision energy for fragment ion obtaining, speed of scanning, capillary voltage in ion source, voltage of filtering quadrupoles and many more.

It must be also highlighted that in target analysis of pharmaceuticals and their TPs after the whole analytical procedure is selected and the best conditions for their analysis have been selected, such methods are subjected to the validation procedure which includes the determination of various parameters, like linearity, limits of quantification (LOQ), limits of detection (LOD), precision, accuracy, repeatability etc. [142,143]. Afterwards, the method can be applied to the routine analysis.

2.2.2. Suspect/non-target analysis

These are relatively new approaches to the analysis of environmental (and other) samples. The general idea is based on obtaining quality data with LC-HRMS, which provides very accurate mass measurements of the compounds (more specifically *m/z*) and can record enormous quantity of ions with high resolution [131]. For this purpose several analysers can be useful, such as TOF, Q-TOF, IT-TOF, Orbitrap or FTICR and their hybrids [141,144]. The data collection is made in full scan

mode, as it is desired to collect all the ions (signal of compounds present in the analysed sample) possible. There is lack of standardized procedures in sample preparation due to the relatively recent application of such methods. Nevertheless, the sample treatment prior analysis should be as non-selective as possible to prevent loss of the analytes [145]. On the other hand, purification is needed to remove interferents from complicated matrix, such as wastewater. Recent review on sample preparation techniques applied for suspect and non-target analysis of various chemicals, including pharmaceuticals, provides up-to-date data on this topic [145]. From 106 quality studies, it is clear that SPE is common method of samples purification and Oasis® HLB is the most widely used solid phase also for this kind of analysis. However, other sorbents were also applied; especially a combination of several different cartridges seems to be good approach to obtain wide range of chemicals. For example, Chromabond HR-X, HR-XAW and HR-XCW (neutral, anionic and cationic exchange resins) were used in the multi-laboratory experiments and over 600 compounds were identified [146]. In other study, combination of Oasis® HLB, Isolute ENV+, Strata-X-AW and Strata-X-CV resulted in detection of 2316 emerging contaminants [147]. Lately, approach without any sample preparation, besides filtering to remove solid particles, was also proposed [148,149].

After the sample preparation, the detection method is utilised. Suspect non-target is determined as the analysis of "known unknowns". It means that there is some information on the analytes suspected to be in the sample, like exact mass, isotope composition or expected adducts, but reference standards are not used. However, in the non-target screening there is no previously prepared information about composition of the sample and all data is extracted from mass spectra [145]. The suspect target screening gives a possibility to detect multi-class/multi-residue analytes much easier compared to the target analysis. However, completely non-target analysis gives possibility to detect compounds not expected in a sample, which could be omitted in the suspect or target approach. Nevertheless, final identification of the signals in non-target analysis is usually based on the library/database search, which in fact is a limiting factor. Therefore, signals that were not matched with any possible compounds after database search should be taken into consideration as potential pollutants, transformation products etc., which were not included in the database. However, investigating each signal one by one and projecting what kind of organic molecule was found is rather impossible. Therefore, the line between suspect and non-target is blurry at some points, which would probably be improved in the future. Nevertheless, the main challenge in this approach is the data processing. It is crucial to extract interesting information from such data-reach results. It is accomplished by a workflow, which restricts number of ions detected to those potentially relevant. It is done by rejecting the background noise signals, narrowing down to the

masses of interest (in the case of pharmaceuticals there is no point of searching for masses above 1000 – 1500 Da), keeping only those which are found in parallel analysis/samples, and then evaluating the quality of signals (peak shapes, presence of isotopic ions). Afterwards, remaining signals can be compared to the databases (suspect target), and when the compounds match the most accurately mass and isotopic pattern, they are recognized as tentative hits. In the case of non-target analysis, much harder work begins to identify completely unknown compounds. In fact, 5 confirmation levels have been proposed to evaluate the confidence of identifying a compound (**Figure 3**) [150]. Determining exact mass and molecular formula based on that are level 4 and 5 (the lowest). Level 3 is a basic confirmation in suspect screening, in which suspects were found by matching obtained masses and molecular formulas with compounds. Level 2 refers to probable structure, evaluated basing on the fragment ions obtained for selected compounds as an additional identifying step (so MS/MS mode is utilised). The highest confidence gives level 1, where the structure is confirmed by reference standard.

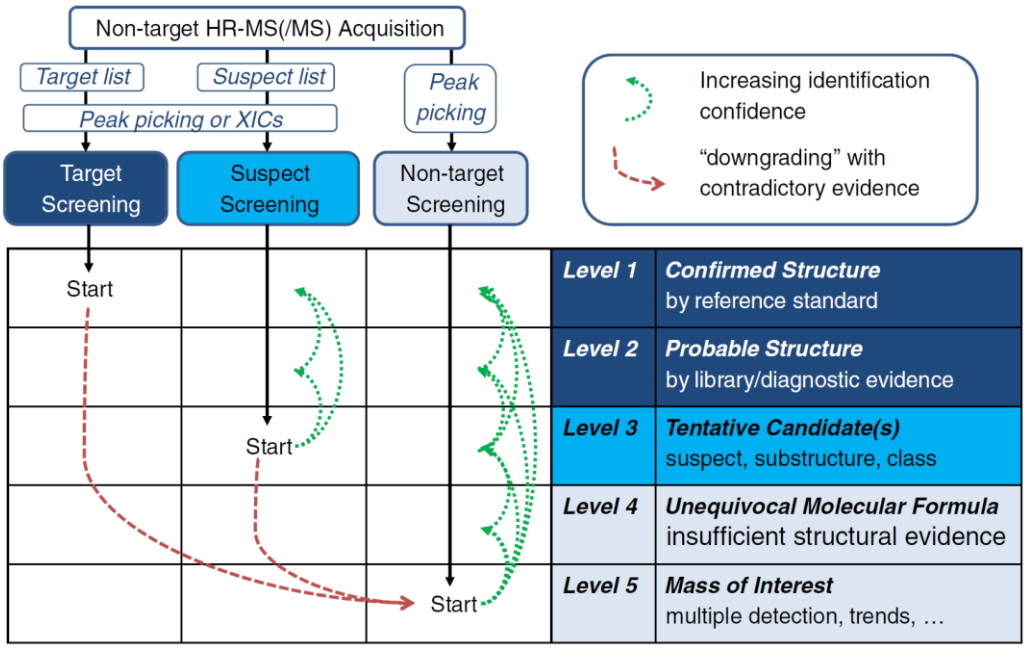


Figure 3 Differences between target, suspect, and non-target screening, with levels of confidence of detecting the analyte (XICs – extracted ion chromatograms). The Figure was published by Schymanski et al., 2015 [146]

Moreover, the data from non-target screening can be used to observe trends, differences between samples, some markers etc. This approach requires appropriate statistical models, chemometric calculations and careful data interpretation. Nevertheless, both suspect and non-target analysis require specialized software to process the data. Since it is quite new technique used in environmental sciences, parameters of data processing are not fully unified and each research presents its own set, which includes for example peak area filtering over chosen number of counts,

absolute abundance of a signal, *m/z* restrictions, mass tolerance or retention time tolerance [113,147,151].

Some studies used suspect/non-target approaches to investigate pharmaceuticals and their TPs in environmental samples. For instance, 33 degradation products of antibiotics were found in wastewaters and river water through non-target analysis [113]. These findings provided data, that many of these compounds are susceptible to wastewater treatment or are present in the river waters even before the discharge of the WWTP effluent. Other study implemented non-target analysis to find many less investigated pharmaceuticals and some transformation products in the freshwater sediments impacted by wastewaters from pharmaceutical industry [152]. Moreover, interesting example of suspect screening proved that 27 additional pharmaceuticals were found in WWTP effluent, which were omitted in the target method [153]. These examples show, that suspect/non-target approach can be successfully applied in the determination of pharmaceuticals and their TPs and it allows to detect many analytes, which would be not included in the target analysis.

2.3 Adsorption processes for the removal of pharmaceuticals and their transformation products from water samples

Adsorption is a separation process, which occurs on the surface of the adsorbent, where the two phases meet and the compounds from one phase concentrate on the surface of another. Therefore, it may be also defined as a process of purification (the solvent/diluent) or separation of dissolved compounds in the solution from the solution. Maximum adsorption performance is reached when the equilibrium between two phases is settled in a given system. It can be described by the ratio between overall amount of the substance on the adsorbent versus the amount in the solution, called distribution coefficient, K_d [154]. The adsorption efficiency depends on mass transfer of the sorbate (adsorbed substance) from water to the adsorbent surface and the rate of the process [155]. In the case of porous materials, the diffusion into the pores precedes the adsorption described by various interactions [16]. Furthermore, the interactions between adsorbent surface and the molecules are the driving forces of the adsorption, which draw molecules to the surface of the adsorbent, for example hydrophobic interactions, hydrogen bonding or electrostatic interactions [156].

The adsorption is recognized as superb technique for water purification, due to its ease of use, low cost and in many cases friendliness to the environment [157]. The adsorption in wastewater treatment is usually used at the end of the treatment process, after mechanical-biological stage (eventually after coagulation, filtration and other additional processes) [158]. This treatment stage, often called tertiary, is dedicated to remove small pollutants from wastewater, which are not

susceptible to classic treatment processes, such as pharmaceuticals. Unfortunately, most of the WWTPs operate without additional purification technology [159]. Nevertheless, the most thoroughly investigated adsorbent for the removal of pharmaceuticals from water and wastewater is activated carbon (AC) [160,161]. However, the best available data in the literature refers to the laboratory – scale investigation. For example, carbamazepine, metronidazole, paracetamol and caffeine were almost fully adsorbed from water by two kinds of AC after four hours of contact [161]. In other studies, the adsorption of amoxicillin, dimetridazole, metronidazole or sulfamethoxazole was investigated, which resulted in adsorption capacity on AC between 100 and 300 mg/g [162]. Nevertheless, there are some reports on implementing additional adsorption process on AC in wastewater treatment. In the large, pilot-scale investigation in the working WWTP it was found that the removal of various pharmaceuticals and their TPs by the adsorption onto powdered AC combined with ultra-filtration was between 20 and 99 % [163]. Even though it reduced the amount of pollutants significantly, they were still in the effluent, some with concentrations over 100 ng/L and few over 1 μg/L. It has to be highlighted that AC is not an ultimate material for water purification from pharmaceuticals. The microporous morphology of AC can cause size-exclusion effect for bigger molecules, long equilibrium time, pore clogging or biofilm growth [121,164,165]. Therefore, many other adsorbents are evaluated for this purpose, such as clays, zeolites, charcoal, various biosorbents, mesoporous silica, but also more sophisticated materials, like graphene nanoplateletes, carbon nanofibers, carbon xerogel or carbon nanotubes [16,121,166–169]. There are many studies on this topic, reviewing various materials and pollutants. Experiments regarding adsorption take a lot of time and work force, various factors have an impact on the adsorption efficiency and even comparison of the results is not easy because of that. Therefore, there is ongoing search for an efficient adsorbent for water purification.

2.3.1 Carbon nanotubes as promising adsorbent for the removal of pollutants from water

Carbon nanotubes (CNTs) as one of many carbon-based materials has been found as a very good adsorbent. They were discovered in 1991 by Iijima [170]. Thanks to their properties, such as mechanical strength, thermal and chemical tolerance, as well as accessible surface, possible to introduce modifications, it was quickly adopted as a promising adsorbent [171].

2.3.1.1 The characteristic of the material

CNTs can be characterised as rolled graphene sheets in a cylindrical form [172]. Carbon building this structure is sp^2 hybridization, each bonded with three other carbon atoms, creating a honeycomb structure [173]. If it is a single sheet they are called single-walled carbon nanotubes

(SWCNTs), if two double-walled carbon nanotubes (DWCNTs) and if multiple graphene sheets form this structure it is called multi-walled carbon nanotubes (MWCNTs) [174]. The structures are presented in **Figure 4**.

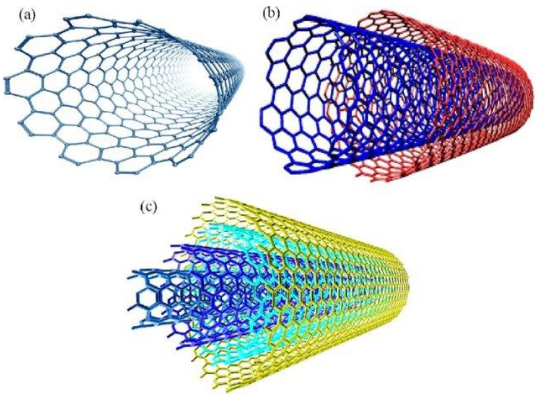


Figure 4 The visualization of structures of SWCNTs (a), DWCNTs (b) and MWCNTs (c). The figure was published by Rafique et al., 2015 [175]

Depending on the way of graphene sheet rolling, different chiral version of CNTs structures are created, namely zig-zag, armchair or chiral [176]. The surface of CNTs is hydrophobic. The diameters of CNTs are between 1 nm and 200 nm, while length from few hundred nm to few μm [177]. The ends of CNTs can be open or closed with fullerene caps [173]. However, current manufacturing methods do not provide perfectly made CNTs and different defects occur, such as incomplete bonding, missing atoms, carbon with sp^3 hybridization, rings with 5 or 7 carbon atoms (instead of regular 6) and functional groups attached to their surface. Moreover, CNTs may contain amorphous carbon, metals, which were used as catalysts during their production and other impurities [176,178]. Nevertheless, some defects allow modification of the CNTs surface, by introducing functional groups, such as hydroxyl, carboxyl or epoxy groups, which are much easier bonded where the structure is corrupted [15,179]. Changing the structure of CNTs can make them more dispersive in water or create additional interactions with molecules to adsorb. It should be highlighted that CNTs are also investigated as hybrids with other materials for various reasons, for example to enhance their sorption potential, change their form from powder into more solid state, create a composite with photocatalytic properties or use them as sensor for detection of pharmaceuticals [14,180,181]. They can be combined with iron oxide, chitosan, *β*-cyclodextrin, poly(acrylic) acid, ionic liquids and so on, as well as be transformed into specific forms, like fix-bed columns, tablets, SPE columns or sponges [182–187]. It is worth mentioning, that CNTs have several characteristics that make them promising and efficient adsorbent towards various pollutants. Their small size and high aspect ratio can result in large surface area [188,189]. The surface area to weight ratio is also high [189]. They offer several sorption sites, namely the internal

hollow space (if the caps on the ends of CNTs are removed and the entry is available), interstitial spaces between single CNTs, external groove site, which is where two nanotubes meet and the external surface [171]. The large external surface contributes to the fast adsorption kinetics, due to its availability. In fully porous materials, which adsorption relies on pore diffusion (such as AC), the sorption equilibrium is predominantly reached in longer time. Moreover, oxidation of CNTs and possibility to introduce functional groups onto CNTs surface can enhance the adsorption of pollutants, mostly those which adsorb through ionic interactions [190]. CNTs provide various interactions with molecules, such as electrostatic and $\pi - \pi$ interactions, hydrophobic interactions or hydrogen bonding [171,191].

2.3.1.2 Adsorption of pharmaceuticals onto carbon nanotubes

The adsorption of various pharmaceuticals onto CNTs have been already investigated, for example for norfloxacin [192], tetracycline [193], sulfadimethoxine, sulfapyridine [194], ciprofloxacin [168], amoxicillin [195]. Therefore, only selected detailed information on the application of CNTs as adsorbent for the removal of pharmaceuticals of concern in this thesis are presented in the **Table 6**. In general, the state of the knowledge is based on laboratory-scale investigations, in most cases involving basic research of CNTs as adsorbents. These studies usually involve investigation of the equilibrium time, efficiency of removal (often as a percentage of the removed amount of a compound from water), fitting of isotherm models to describe the adsorption process and determining the maximum capacity of the adsorbent towards an analyte. In addition, the influence of pH, ionic strength and presence of co-pollutants are also frequently verified. In general, the adsorption onto CNTs occurs quickly and it is suitable for wide range of pharmaceuticals. The most commonly fitted isotherm models are Freundlich and Langmuir. However, there are many others proposed, like Polanyi-Manes or Sips [184]. Moreover, linear function of concentration adsorbed versus concentration remaining in the solution at different concentration levels is often calculated. The influence of various factors depends on the analytes; pH is the most commonly tested variable, which influences the adsorption process mostly for the compounds that change ionic forms with the pH change, which results in stronger or weaker (or even repulsive) interactions.

Based on the data presented in the **Table 6** it might be concluded that there is very limited data on the adsorption of TPs onto CNTs. To the best of my knowledge, there was only one study performed concerning the evaluation of dispersive SPE based on CNTs adsorbent to the extraction of selected PTPs [196]. Most of the already performed adsorption studies onto CNTs refer to native forms of pharmaceuticals such as: NSAIDs, antibiotics and carbamazepine.

Table 6 Data on the adsorption of selected pharmaceuticals onto carbon nanotubes (numbers by the CNTs are diameters, MWCNTs-COOH mean modification with this functional group)

Type of CNTs	Compounds	Sorption data	Comments	Lit.
MWCNT-COOH	Carbamazepine	Freundlich isotherm fitting: $R^2 = 0.98$ n = 0.48 $K_F = 13900$.	K_F/surface area ratio 2 orders of magnitude bigger than for AC.	[165]
MWCNTs with diameters 10 – 100 nm	Carbamazepine	Polanyi-Manes isotherm model: $R^2 = 0.98$-0.99 $Q^0_p = 40 – 8\,000$.	Fast adsorption, surface area plays key role in adsorption capacity.	[197]
CNTs/Al_2O_3 composite	Carbamazepine Diclofenac	Langmuir isotherm: maximum capacity for CBZ 157.4 μmol/g, for DIC 106.5 μmol/g. No pH influence for CBZ, for DIC the more acidic the bigger sorption.	There were only little differences in adsorption between CNTs and CNTs/Al_2O_3.	[198]
Magnetic-CNTs (various dimensions and magnetite-CNTs ratio)	Ketoprofen Diclofenac Ibuprofen	The best performance was 1:1 ratio, 60-100 nm of diameter, longer CNTs, pH 5.	Magnetic-CNTs were used for extraction, with efficiency in water 70-100 %, in wastewater 49-100 %.	[199]
SWCNTs MWCNTs Oxidized-MWCNTs	Ibuprofen	Good fitting with Polanyi-Manes model. $R^2 = 0.99$ $Q^0_p = 40 – 500$ Q^0_p/surface area = 0.07-0.7. pH-dependent, better below pKa (acidic).	Highest sorption for SWCNTs, lowest for O-MWCNTs, but after normalization with surface area MWCNTs the best. Fulvic acid decreased sorption.	[200]
MWCNTs MCNTs-COOH MWCNTs-octadecyl amine groups	Ibuprofen	Good Freundlich isotherm fitting: $R^2 = 0.97$-0.99 n = 0.61-0.85 q_e = 229-389.	Application in micro-SPE.	[201]
MWCNTs-8 nm MWCNTs-50 nm MWCNTs-COOH-50 nm Helical MWCNTs	Cyclophosphamide Ifosfamide 5-fluorouracil	MWCNTs-8 nm with highest surface area has the highest capacity. K_d (MWCNTs-8 nm) for CP: 1600, IF: 450, 5-FU: 720. Freundlich isotherm fitted better than Langmuir.	No influence of ionic strength. Not clear pH influence, but more or less similar sorption at pH 4-10 for CP and IF, clear decrease for 5-FU at pH 10.	[202]
MWCNTs-8 nm MWCNTs-50 nm MWCNTs-OH-50 nm MWCNTs-COOH-50 nm Helical MWCNTs	Metronidazole Sulfamethoxazole *N*^{4}-acetylsulfamethoxazole Diclofenac 4-hydroxydiclofenac 5-hydroxydiclofenac Naproxen *O*-desmethylnaproxen	Used in dispersive SPE. Quick removal (20 – 30 min). Surface area has big impact on the performance. Functional groups did not enhance the adsorption.	4 mg of MWCNTs-8 nm was enough to remove all but SMX (0.5 mg/L, 10 mL) in 30 minutes. MWCNTs-Helical adsorbed all of them.	[196]
Poly(ethylene glycol) grafted MWCNTs	Ibuprofen Diclofenac Naproxen	Thermally stable (to 320 °C), much better performance at acidic pH. 60 min is optimal for extraction.	Good analytical method.	[203]
Diethylene glycolated-MWCNTs	Imatinib	3 mg of MWCNTs-2EG removed 8.8 mg out of 9 mg in the solution.	70 – 85 % of IMT was recycled at different pH.	[204]

Q^0_p – maximum sorption capacity in Polanyi-Manes isotherm model; K_F – Freundlich adsorption constant, representing sorption capacity; n – adsorption intensity, regarding relative distribution of the energy and heterogeneity of the adsorption sites; q_e – amount of a compounds adsorbed at equilibrium (mg/g or g/kg)

2.3.1.3 The possibilities of regeneration and reuse

CNTs besides many advantages are still recognized as very expensive alternative to other popular adsorbents, like AC or biosorbents. This results mainly from the fact that their production requires high temperatures and suitable equipment, which generate costs. Nevertheless, CNTs production is getting cheaper with increasing demand on this material and more cost-effective production techniques are developed to reduce costs [205,206]. Therefore, multiple use and possibility of the regeneration are mandatory for this kind of materials to be applied in bulk quantity. Moreover, regeneration is supposed to restore the ultimate performance of the sorbent, which reaches saturation at some point [207] or even enhance sorption capacity. Even regeneration at small scale, for example in the case of household water purification or for SPE adsorbents etc. could prolong the usage of the adsorbent. There are at least several CNTs regeneration methods proposed, which are based either on the providing energy (temperature, ultrasounds, light etc.) for the degradation of pollutants and unblocking active sorption sites or using solutions of different solvents to flush out the adsorbed entities (**Figure 5**).

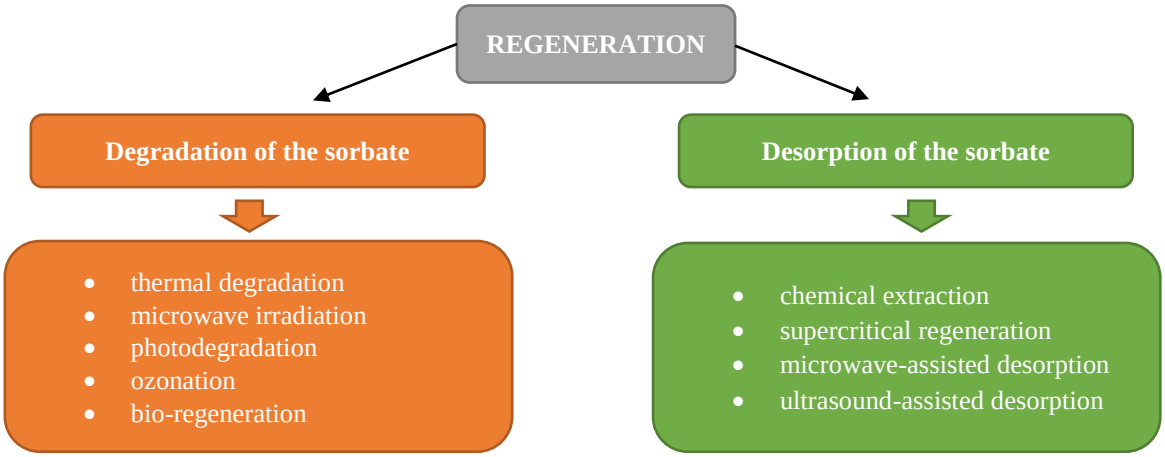


Figure 5 Possible regeneration methods for CNTs

Depending on the purpose of the adsorption and the adsorbate itself, the regeneration can be targeted to remove the adsorbed matter without retrieving it or to reclaim it. Recovering the adsorbate is an objective when specific species are adsorbed, for example metals (rather valuable), single compounds or group of compounds etc. It suggests that the matrix that was purified with the adsorbent has known pollutants and the adsorption process is aimed at recovering it. With more complex matrix, like wastewater, where there are many different compounds and the composition may vary, the regeneration of adsorbent is aimed at recovering the adsorbent and restoration of its original properties, not keeping the adsorbed molecules. In the case of pharmaceuticals, the degradation of adsorbed matter is preferential, due to the fact, that wastewaters or other types of potentially polluted matrix, even drinking water, is the main matrix that CNTs are used for.

Nevertheless, CNTs are also used in SPE or SPME (solid-phase microextraction), so in this case desorption of the retained compounds is desired. Unfortunately, the regeneration and reuse of CNTs is not fully investigated. The large part of the knowledge on this topic is based on flushing CNTs after the adsorption process and desorbing whatever they adsorbed. This approach was presented mostly for the metals. For example, a PTFE (polytetrafluoroethylene) column filled with MWCNTs was used as adsorbents for Cd, Mn, and Ni and regenerated with 10 mL of 1 M HNO_3 and 20 mL of H_2O [208]. Moreover, the column maintained its capacity for 50 cycles. On the other hand, the study on the Zn^{2+} desorption from SWCNTs and MWCNTs showed, that it is pH-dependent and the best results were obtained at radically low pH of 1 (90 % recovery). The maximum value of the recovery was achieved after 4 hours. After 10 cycles of adsorption and regeneration, the recoveries were down to 20 %. Moreover, the adsorption capacity was also decreasing. Nevertheless, the powdered AC was also studied, and recovery dropped about 60 % after just one regeneration [209]. Other authors presented similar conclusions, that metals can be flushed with high recovery by pH-controlled conditions [17]. Moreover, desorbing of dyes was also tested with ethanol and various pH [210]. However, only around 40 % was removed, which is only an average performance. Other study investigating reactive red M-2BE dye confirmed that it is not easy to desorb dyes from CNTs [211]. Pure ethanol, MeOH, propanol or heptane gave poor results. Aqueous solutions of NaOH gave similar effect. However, the combination of MeOH and 4 M NaOH resulted in 88 % recovery. Additionally, the adsorption of the dye after 4 cycles had 78 % efficiency in comparison to the first cycle. Nevertheless, none of the tested solutions was able to desorb this dye from PAC. Interesting study on the removal of *E. coli*, health-threatening bacteria, showed that the filter made of CNTs can sufficiently clean the drinking water from that pathogen [212]. It should be highlighted, that this kind of filter can be easily regenerated with ultrasonication and autoclaving, which creates advantage over conventional filters. Another proposed form of saturated CNTs restoration was ozone regeneration [213]. It was found that ozone treatment alone was not sufficient to regenerate the adsorbent, which showed worse performance in each adsorption/desorption cycle of atrazine. However, flushing the CNTs with ethanol and water restored its initial adsorption capacity. Therefore, ozonation was only partially degrading the pollutant and probably creating TPs, which were not removed.

In the case of adsorption of the pharmaceuticals and reusing the adsorbent, the new approach of combining CNTs with ionic liquids to prepare a tablet was proposed to remove tetracyclines from water [182]. The desorption with 5 M NaOH was efficient. However, after 3 cycles of such treatment the adsorption dropped to less than 80 %, which was caused by mechanical degradation of the tablet. Nonetheless, the adsorbent was recovered and formed into another tablet, which

resulted in similar performance as the first one. Other approach for removal of tetracycline, in this case by degradation, was proposed as microwave-ultraviolet regeneration [193]. Saturated CNTs with tetracycline were submitted to various conditions, like power of the microwave, time etc. In general, up to 40 % of total mineralization was achieved and 100 % of regeneration, understood as the adsorption rate of tetracycline after the regeneration process. Other experiments included chemical regeneration and comparison of MeOH, ethanol, HCl, NaOH and H_2O_2 was performed [214]. The effectiveness was evaluated by the removal efficiency of several compounds; *inter alia* ketoprofen, in 5 cycles of regeneration. MeOH flushing kept the performance close to 100 % of removal, while other liquids were clearly worse. Interestingly, regeneration of fixed-bed column with CNTs and adsorbed sulfamethoxazole and sulfapyridine by NaCl solution at pH 12 was quite efficient (around 90 % of removal), but in each cycle the performance of the column was decreasing, suggesting that more and more pharmaceuticals retain on the adsorbent [215]. On the contrary, the comparison of chemical, ultrasonic and thermal regeneration showed, that heating is the best approach to regenerate and keep the good performance of CNTs [216]. The regeneration with MeOH and ethanol for ibuprofen was very ineffective. NaCl showed much better performance. Even though the assist of the microwaves increased the process output, the thermal regeneration in 380 °C kept the adsorption on similar level during 4 cycles. Other studies seem to confirm that observation. Regenerable granular CNTs used for adsorption of carbamazepine, tetracycline and diclofenac was used in 5 cycles of adsorption/regeneration and the adsorbed amount remained the same each time [217]. Additionally, carbamazepine and diclofenac were fully degraded, while tetracycline was only partially oxidized.

As it was proved, there are many methods for the regeneration of used CNTs. Selection of a proper one should be based on the efficiency of the method, but also on the costs, simplicity, required equipment, the limitations of the amount that can be regenerated at once and the waste produced by the method. Chemical desorption is good for metals recovery and other adsorbed species. However, it can be useful only when water is purified from known contaminant and it is desired to recover it. In the case of treating wastewaters or other complex mixtures, the methods with the degradation or at least transformation of pollutants are more needed. Therefore, the thermal treatment seems to be promising approach.

3. OBJECTIVES OF THE WORK

The main objective of this PhD thesis was the cross – sectional investigation of pharmaceuticals belonging to different therapeutic groups and their selected transformation products in terms of their presence in the water samples, their hydrolytic stability and possibility to remove them from water with repeatedly regenerated multi-walled carbon nanotubes (MWCNTs) as well as MWCNTs/chitosan based membranes.

The detailed objectives include:

- development and validation of the analytical methods for single compounds using HPLC-UV/Vis for the determination of the analytes in model experiments of hydrolysis and/or adsorption,

- development and validation of the analytical methods using LC-MS/MS systems with different equipment to select the best SPE conditions for the extraction of the pharmaceuticals and their transformation products and to investigate their presence in the environmental water samples as well as in model experiments with mixtures of these analytes for the adsorption experiments,

- evaluation of hydrolytic stability of the analytes according to the OECD Guideline 111,

- assessment of the adsorption performance of MWCNTs subjected to the thermal and chemical regeneration process,

- evaluation of the usefulness of the MWCNTs/chitosan based membranes for the quick removal of the investigated analytes.

4. MATERIALS AND METHODS

4.1 List of reagents

Chemicals used during the experimental part are listed in **Table 7**.

Table 7 The list of analytical standards, solvents and other chemicals used in this study

Chemical standards of the analytes		
Cyclophosphamide monohydrate (CAS: 6055-19-2, Sigma Aldrich, Germany)	Ifosfamide (CAS: 2778-73-2, Sigma-Aldrich, Germany)	5-fluorouracil (CAS: 51-21-8, Sigma-Aldrich, Germany)
Imatinib mesylate (CAS: 220127-57-1, Santa Cruz Biotechnology)	Methotrexate (CAS: 59-05-2, Sigma-Aldrich, Germany)	7-hydroxymethotrexate (CAS: 5939-37-7, TRC, Canada)
Carbamazepine (CAS: 298-46-4 Sigma Aldrich, Germany)	10,11-dihydro-10-hydroxycarbamazepine (CAS: 29331-92-8, TRC, Canada)	10,11-dihydro-2-hydroxycarbamazepine (CAS: 68011-66-5, TRC, Canada)
Carbamazepine-10,11-epoxide (CAS: 36507-30-9, TRC, Canada)	Ibuprofen (CAS: 15687-27-1, Sigma-Aldrich, Germany)	2-hydroxyibuprofen (CAS: 51146-55-5, Sigma-Aldrich, Germany)
Ibuprofen carboxylic acid (CAS: 15935-54-3, TRC, Canada)	Tramadol hydrochloride (CAS: 36282-470, Sigma-Aldrich, Germany)	*O*-desmethyltramadol hydrochloride (CAS: 185453-02-5, Sigma-Aldrich, Germany)
Hydroxymetronidazole (CAS: 15935-54-3, TRC, Canada)	N^4-acetylsulfamethoxazole (CAS: 21312-10-7, Sigma-Aldrich, Germany)	4'-hydroxydiclofenac (CAS: 64118-84-9, Sigma-Aldrich, Germany)
Naproxen (CAS: 22204-53-1, Sigma-Aldrich, Germany)	*O*-desmethylnaproxen (CAS: 52079- 10-4, Sigma-Aldrich, Germany)	
Other chemicals and solvents		
Acetonitrile (HPLC grade, POCH, Poland)	Acetonitrile (LC-MS grade, Sigma-Aldrich, Germany)	Methanol (HPLC grade, POCH, Poland)
Methanol (LC-MS grade, Sigma-Aldrich, Germany)	Hexane (HPLC grade, J.T. Baker, Netherlands)	Monopotassium phosphate (analytical grade, POCH, Poland)
Dipotassium phosphate (analytical grade, POCH, Poland)	Potassium chloride (analytical grade, POCH, Poland)	Boric acid (analytical grade, POCH, Poland)
Citric acid (analytical grade, Stanlab, Poland)	Sodium hydroxide (analytical grade, Stanlab, Poland)	Calcium chloride (analytical grade, POCH, Poland)
Hydrochloric acid (min. 36 %, Stanlab, Poland)	Formic acid (99 %, LC-MS grade, Sigma-Aldrich, Germany)	Ammonium acetate (LC-MS grade, Sigma-Aldrich, Germany)
Nitric acid (LC-MS grade, Sigma-Aldrich, Germany)		

4.2 Equipment

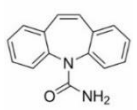
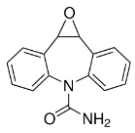
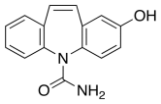
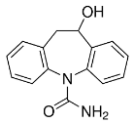
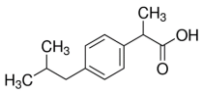
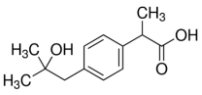
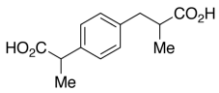
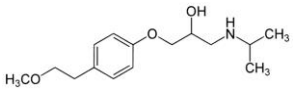
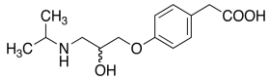
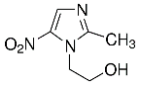
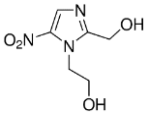
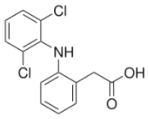
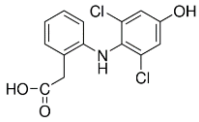
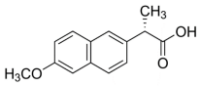
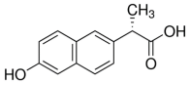
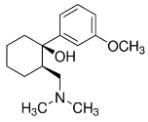
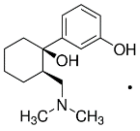
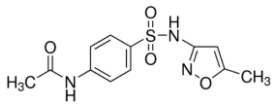
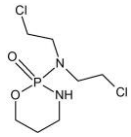
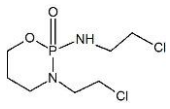
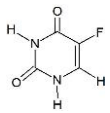
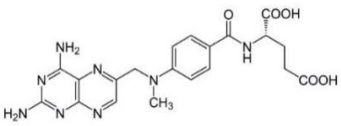
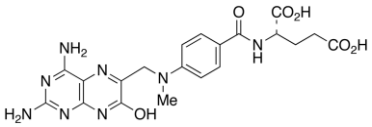
Table 8 The list of the equipment used in this study

Electronic devices		
Series 200 HPLC with UV-Vis detector (Perkin Elmer, USA)	HPLC with LC-20AD pump, SPD-M20A DAD detector and SIL-20AHT autosampler (Shimadzu, Japan)	Agilent 1200 Series LC system (Agilent Technologies Inc., USA) and HCT Ultra ion trap mass spectrometer (Brucker Daltonics, Germany)
UHPLC-MS 1290 Infinity with 6550 iFunnel Q-TOF (Agilent Technologies, USA)	LC-MS-8050 Triple Quadrupole (Shimadzu, Japan)	Magnetic stirrer Colour Squid S000 (IKA, Germany)
Laboratory shaker RS 10 Control (IKA, Germany)	Laboratory dryer SUP-4 (Nysa, Poland)	Ultrasonic bath Sonic-14 (Polsonic, Poland)
Autoclave HV-50 (HMC-Europe, Germany)	Ultrasonic bath Sonorex super RK 106 (Bandelin, Germany)	ICP-OES Optima 2000 (Perkin Elmer, USA)
muffle furnace FCF 5SM (Czylok, Poland)	HLP 5 system for ultrapure water (Hydrolab, Poland)	analytical balance XA 110/2X d = 0.01 mg (RADWAG, Poland)
Analytical balance XA 82/200 d = 0.01 mg (RADWAG, Poland)	pH - meter HI 9125 (HANNA, Poland)	Automatic pipettes 0.1 ml, 0.5 ml, 1 ml, 10 ml (Eppendorf, Germany)
Automatic pipettes Acura 825 (0.2 – 1 ml) and Acura 835 (1 – 10 ml) (Swiss Made, Switzerland)	Automatic pipettes 0.1 – 10 ml (HTL, Poland)	Incubator ILW 115 STD (Pol-eko-Aparatura, Poland)
Utilities and other laboratory equipment		
Luna Omega Polar C18 3 µm 100 Å, 50 mm × 3 mm (Phenomenex, USA)	Gemini C-18 chromatographic column 5 µm 110 Å, 4.6 mm × 150 mm (Phenomenex, USA)	Gemini C6-Phenyl chromatographic column 5 µm 110 Å, 4.6 mm × 150 mm (Phenomenex, USA)
Oasis® HLB SPE cartridges, 200 mg, 6 ml	Paper filters 5 cm diameter	PE syringe filters, 2.5 cm diameter, 20 µm pore size
Nylon membrane filters, pore size 0.22 µm, diameter 4.7 cm (Membrane Solutions, USA)	MWCNTs (Cheap Tubes, USA)	Luna Omega Polar C18 3 µm 100 Å, 100 mm × 3 mm (Phenomenex, USA)

4.3 Analytes

Various pharmaceuticals and their TPs were included in this research (**Table 9**). Stock solutions of each analyte were prepared in MeOH in concentration of 500 mg/L. If needed, they were diluted to 50 mg/L in MeOH in order to prepare the less concentrated working solutions. MeOH stocks were kept in the freezer at -19 °C. The working solutions for all experiments were prepared by taking adequate volumes of stock solutions, evaporating the MeOH and dissolving the precipitate in desired solvent.

Table 9 The list of the analytes included in the research

Carbamazepine **CBZ** M = 236.2 g/mol 	Carbamazepine-10,11-epoxide **CBZ-ep** M = 252.1 g/mol 	10,11-dihydro-2-hydroxycarbamazepine **2-OH-CBZ** M = 252.3 g/mol 	10,11-dihydro-10-hydroxycarbamazepine **10-OH-CBZ** M = 254.3 g/mol 
Ibuprofen **IBU** M = 206.3 g/mol 	2-hydroxyibuprofen **2-OH-IBU** M = 222.3 g/mol 	Ibuprofen carboxylic acid **cx-IBU** M = 236.3 g/mol 	Metoprolol **MTP** M = 267.4 g/mol 
Metoprolol acid **MTPA** M = 267.3 g/mol 	Metronidazole **MTZ** M = 171.2 g/mol 	Hydroxymetronidazole **MTZ-OH** M = 187.2 g/mol 	Diclofenac **DIC** M = 296.1 g/mol 
4'-hydroxydiclofenac **4-OH-DIC** M = 312.1 g/mol 	Naproxen **NPX** M = 230.3 g/mol 	*O*-desmethylnaproxen **des-NPX** M = 216.2 g/mol 	Tramadol **TRA** M = 263.4 g/mol 
O-desmethyltramadol **O-DMTRA** M = 249.4 g/mol 	N^4-acetylsulfamethoxazole **ac-SMX** M = 295.3 g/mol 	Cyclophosphamide **CP** M = 261.1 g/mol 	Ifosfamide **IF** M = 261.1 g/mol 
5-fluorouracil **5-FU** M = 130.1 g/mol 	Methotrexate **MTX** M = 454.4 g/mol 	7-hydroxymethotrexate **7-OH-MTX** M = 470.4 g/mol 	

4.4. Experimental design

The conceptual approach of the performed experiments is presented in the **Figure 6.**

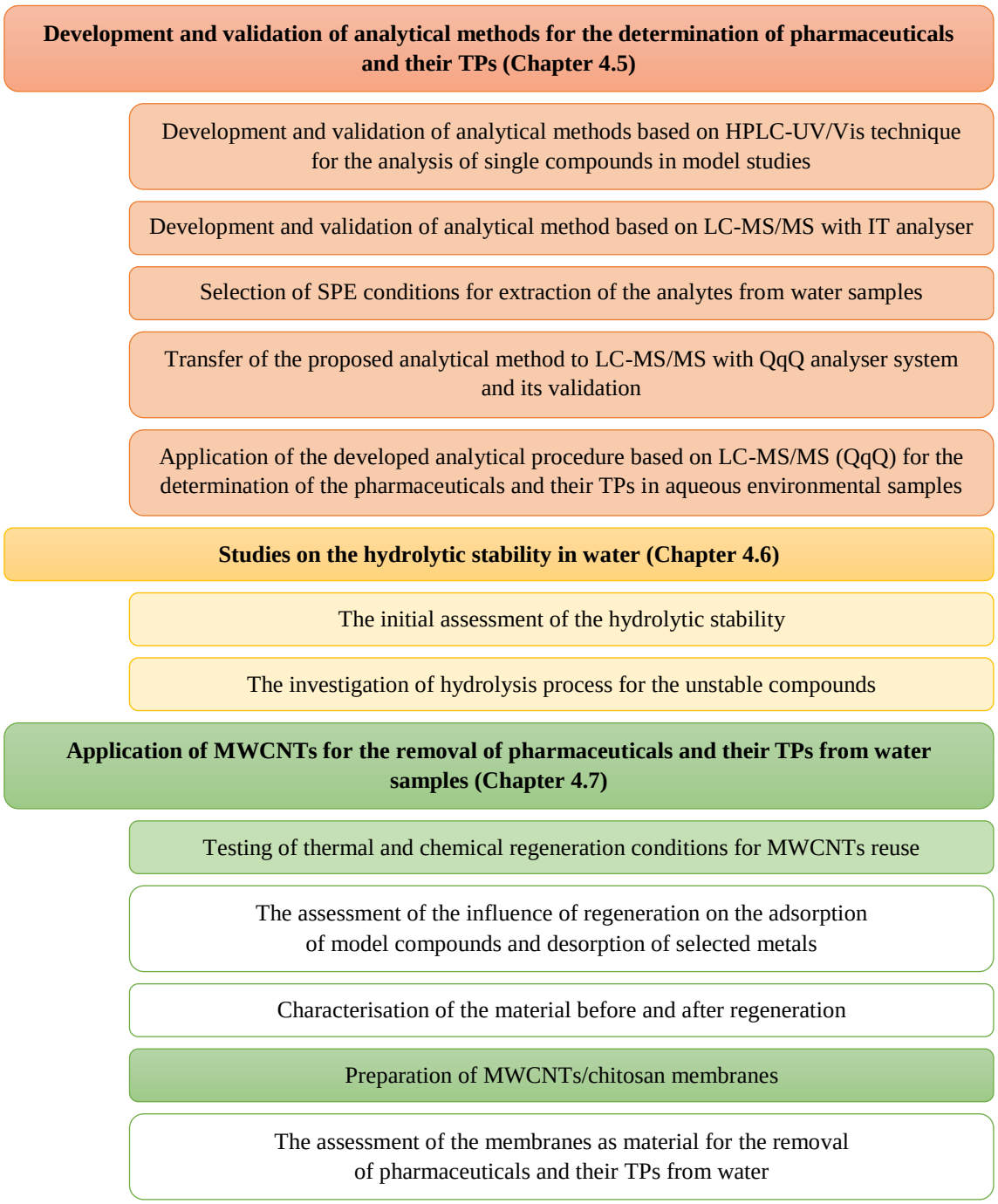


Figure 6 The general outline of the experiments conducted within this PhD thesis

4.5 Development and validation of analytical methods for the determination of pharmaceuticals and their TPs

In this work, several instruments were used for the analytical purposes. First, methods for single compounds were developed based on HPLC-UV/Vis technique, which were then used in model experiments for the hydrolytic stability assessment as well as the adsorption studies. Furthermore, the analytical methods for the determination of mixtures of the analytes used for their detection in the environmental samples and in the experiments with MWCNTs/chitosan based membranes were developed. In general, this was a multistep research objective, which included the realization of different tasks presented in the **Figure 7.**

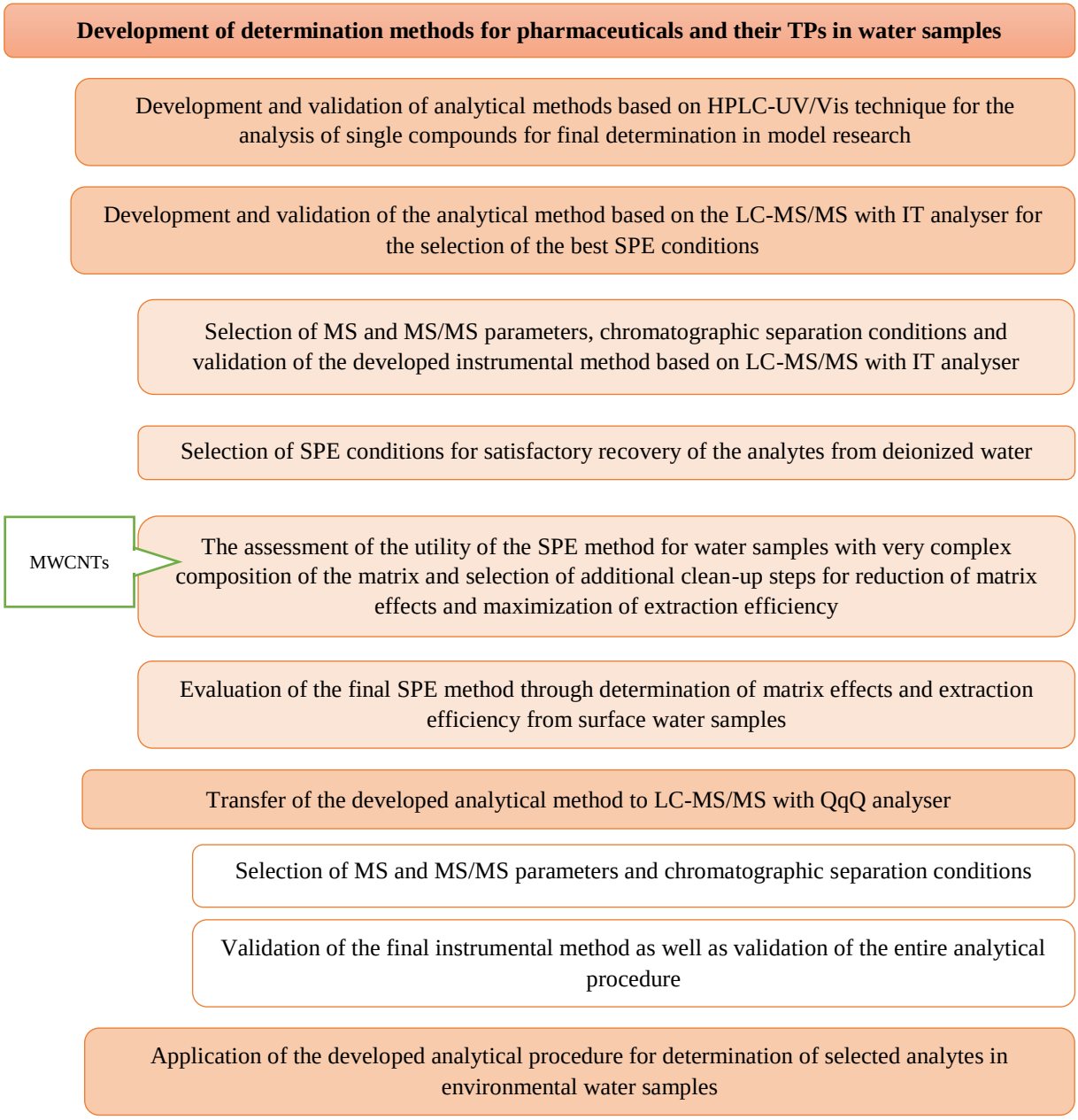


Figure 7 The outline of the experiments presented in this section

4.5.1 Development and validation of analytical methods based on HPLC-UV/Vis technique

Analytical methods based on the application of HPLC-UV/Vis technique were developed for single compounds as they were afterwards used in the model hydrolytic and/or adsorption studies. The developed methods were supposed to be relatively short, easy to employ and useful for samples with different pH. Therefore, the main aspect of developing these methods was the choice of the mobile phase composition. The testing of the mobile phase for the analysis of each analyte included ACN as an organic phase. Testing of the water phase always started with HPLC-grade H_2O, if it was not appropriate H_2O + 0.1 % HCOOH was tested, and eventually, if both phases were not suitable, H_2O + 5 mM ammonium acetate buffer (pH 3.8) was used. The detection wavelength was chosen based on the UV spectra of the compounds. In most cases, Gemini C18 (5 µm 110 Å, 150 mm × 4.6 mm, Phenomenex, USA) was used, but in case of MTX and 7-OH-MTX Gemini C6-Phenyl (5 µm 110 Å, 150 mm × 4.6 mm, Phenomenex, USA) was more suitable. Moreover, for the analysis of CP and IF Luna Omega Polar C18 (3 µm 100 Å, 50 mm × 3 mm, Phenomenex, USA) was employed. Furthermore, all of these methods were fully validated in terms of the determination of its: precision expressed in the coefficient of variation (CV), accuracy, IQL (instrumental quantification limit) and IDL (instrumental detection limit) in a given concentration range. The validation was performed by preparing solutions of each compound of different concentrations and analysing them with the developed analytical method. The number of concentration levels were various for the analytes, but there were minimum 9 calibration points per analyte. Solution at each concentration level was analysed 6 times. The least squares method was used to determine the range of linearity and described parameters. CV was calculated by determination of standard deviation (SD) (**Eq. 1**), then relative standard deviation (RSD) (**Eq. 2**) and multiplying it by 100 % (**Eq. 3**).

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x - \bar{x})^2}{n - 1}} \qquad (1)$$

where:

x – single concentration value calculated at a certain concentration level from the linear regression formula (**Eq. 4**);

$\bar{x}$ – sample mean average of concentration calculated at a certain concentration level from the linear regression formula (**Eq. 4**);

n – sample size.

$$\mathrm{RSD} = \frac{\mathrm{SD}}{\bar{x}} \quad (2)$$

$$\mathrm{CV} = \mathrm{RSD} \cdot 100\ \% \quad (3)$$

Accuracy (**Eq. 5**) is an estimation of compliance between the result determined from the linear regression formula (**Eq. 4**) and the expected value, determined by calculating the concentration at each concentration level and comparing to the expected value. If the calculated and expected concentrations are the same, the accuracy is 100 %.

$$y = ax + b \quad (4)$$

$$\mathrm{Accuracy} = \frac{c_{cal}}{c_{exp}}\ 100\ \% \quad (5)$$

where:

c_{cal} – concentration calculated;

c_{exp} – concentration expected.

IQL was assumed as the lowest concentration of the investigated range during validation for which CV was below 15 % and accuracy in the range 75 – 125 %. IDL was calculated as IQL divided by 3. Finally, instrumental methods for 17 different analytes were developed and validated, namely for CBZ, CBZ-ep, 2-OH-CBZ, 10-OH-CBZ, MTZ-OH, 5-FU, CP, IF, IBU, 2-OH-IBU, cx-IBU, TRA, O-DMTRA, MTX, 7-OH-MTX, 4-OH-DIC, ac-SMX.

4.5.2 Development and validation of the analytical method based on LC-MS/MS with IT analyser for the selection of the best SPE conditions

4.5.2.1 Selection of MS and MS/MS parameters, chromatographic separation conditions and validation of the developed instrumental method based on LC-MS/MS with IT analyser

Initially, for the purpose of SPE method development, the HPLC-MS system Agilent 1200 Series LC system (Agilent Technologies Inc., USA) equipped with HCT Ultra IT mass spectrometer (Brucker Daltonics, Germany) was used. For this reason, single solutions of 18 analytes (CBZ, CBZ-ep, 2-OH-CBZ, 10-OH-CBZ, MTZ, MTZ-OH, IBU, 2-OH-IBU, cx-IBU, TRA, O-DMTRA, DIC, 4-OH-DIC, NPX, des-NPX, MTP, MTPA, ac-SMX) were separately analysed through direct infusion into the MS system to observe pseudo-molecular ions of each compound in positive or negative ionization mode. Then, different fragmentation amplitudes (0.2 – 1.0 V) were tested to

obtain the most intensive signals of the fragmentation ions of each parent compound. Two fragmentation ions were desired, but if the fragmentation was difficult to achieve or ionization of a compound was weak, only one fragmentation ion was chosen in the final method. Afterwards, 0.5 mg/L solution in water of these analytes was used to build full LC-MS/MS method. Gradient composition of the mobile phase was tested in various combinations to achieve separation of all compounds included in the method. The mobile phase consisted of A: H_2O with 1 mM CH_3COONH_4 + 10 % ACN and B: ACN. Two chromatographic columns were tested, Gemini C18 (5 μm, 150 mm × 4.6 mm) and Luna Omega Polar C18 (3 μm, 100 mm × 3 mm) both supplied from Phenomenex (USA). Flow of the mobile phase was 0.4 mL/min, injection volume 50 μL, column temperature 25 °C. ESI was an ion source, with drying gas at 50 psi, 10 L/min, 365 °C, capillary voltage 4 kV, ICC target 50000, MaxAccuTime 200 ms, average 3 scans. Then, the time segments were applied to divide the analytical run into parts where only given MRM transitions were monitored. Afterwards, the instrumental method was validated in terms of the determination its: precision (CV), accuracy, IQL and IDL in given linearity range (accordingly to the description presented above in the **Section 4.5.1**) by analysing the solutions of the analytes on 11 levels of concentration in a range of 0.5 – 500 μg/L.

4.5.2.2 Selection of SPE conditions for the extraction of the analytes from water samples

The selection of optimal conditions for the SPE of the selected pharmaceuticals and their TPs from water samples was based on the best results regarding absolute recovery (AR), extraction efficiency (EE) and matrix effects (ME). These parameters were calculated from these equations:

$$\mathrm{AR}\ (\%) = \left(\frac{\mathrm{C}-\mathrm{D}}{\mathrm{A}}\right)\cdot 100 \tag{6}$$

$$\mathrm{EE}\ (\%) = \left(\frac{\mathrm{C}-\mathrm{D}}{\mathrm{B}-\mathrm{D}}\right)\cdot 100 \tag{7}$$

$$\mathrm{ME}\ (\%) = \left(\frac{\mathrm{B}-\mathrm{D}}{\mathrm{A}} - 1\right)\cdot 100 \tag{8}$$

where:

A – surface are of the analyte's signal detected for the standard solution;

B – surface are of the analyte's signal detected for the test sample spiked after extraction;

C – surface are of the analyte's signal detected for the test sample spiked before extraction;

D – surface area the analyte's signal detected for the test sample (without spiking).

The SPE was performed with the Oasis® HLB 200 mg as one of the most popular sorbents used for such purpose based on the presented literature review in the theoretical part. Initial SPE method had several steps to isolate and concentrate the 18 analytes (initially those which were included in the LC-MS/MS (IT) method, **Chapter 4.5.2.1**) along with the selecting the SPE conditions, some steps were modified or added, which is shown in the outline in **Figure 8**.

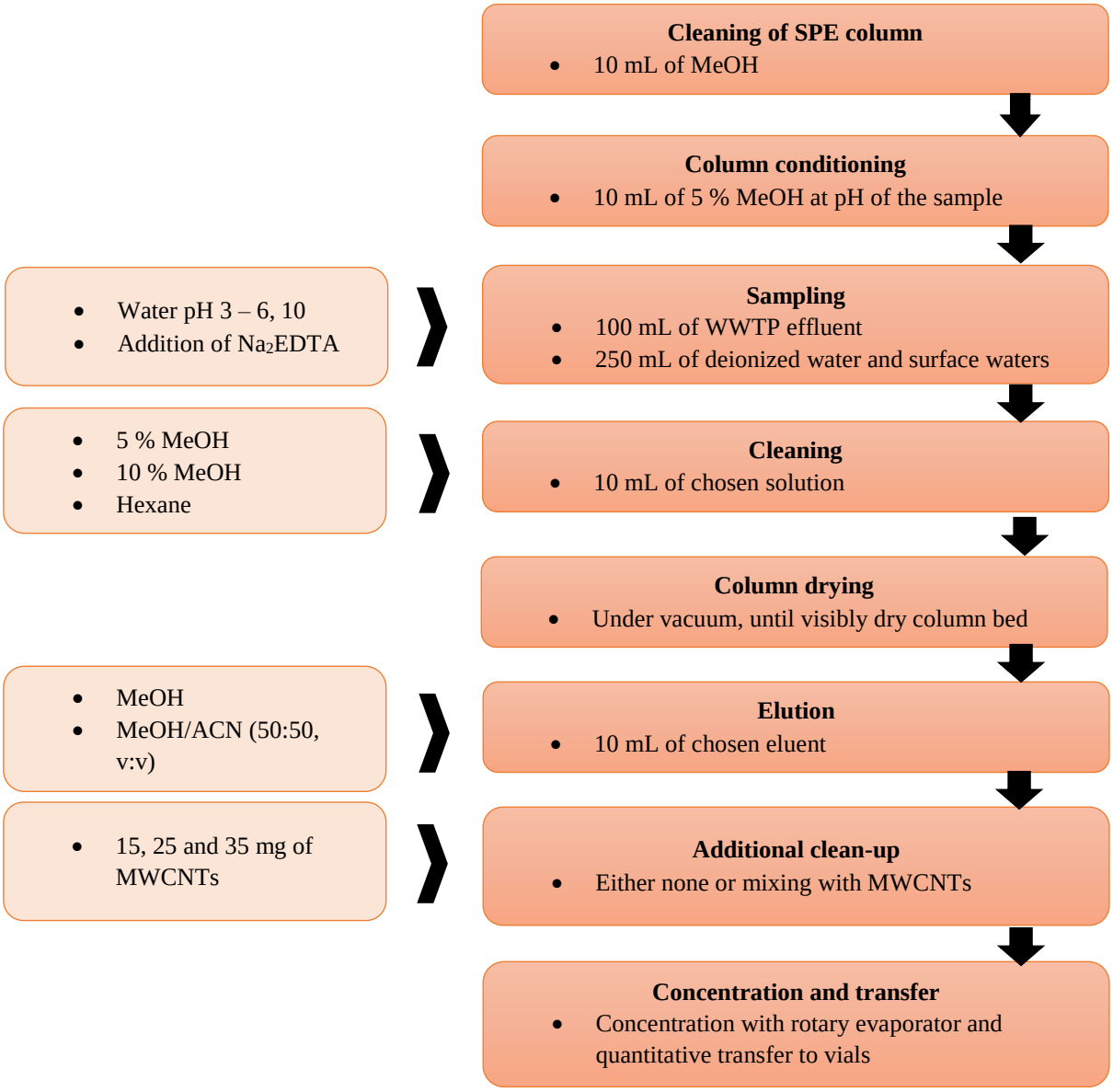


Figure 8 The outline of the tested SPE procedure (on the left side tested different conditions of a certain SPE step are presented)

As a first step, the AR was evaluated for the analytes in simple matrix, namely deionized water (250 mL) spiked with analytes at a concentration of 2 µg/L. At this stage, pure water at pH 3, 6 and 10 was prepared. Two parallel samples were prepared for each pH. Each water sample was spiked with the analytes, put through the SPE procedure and the obtained samples were analysed, each in triplicate. The absolute recovery was calculated for each compound. Additionally, another

study was conducted to verify the effectiveness of the procedure at sample pH 4, 5 and 6 as well as with comparison of MeOH and ACN/MeOH (1:1, v:v) as eluent. Afterwards, the most suitable conditions were tested for the extraction of the analytes from much more complicated matrix, namely WWTP effluent (100 mL per sample, with concentration of the analytes at 2.5 µg/L). Simultaneously, several different clean-up options were verified to assess the influence of such process on matrix effects and extraction efficiency. Besides flushing with 5 % MeOH after sample loading, variant of cleaning with 10 mL of 10 % MeOH and after that 10 mL of hexane as well as variant with addition of 0.5 g Na_2EDTA to the water sample before sample loading and cleaning with 10 mL of 10 % MeOH and after that 10 mL of hexane were tested. Finally, additional step of clean-up was investigated, in which the MeOH eluate was shaken with the addition of 15, 25 and 35 mg of MWCNTs.

Cleaning of the SPE column was performed to remove any impurities left after the manufacturing, then sorbent was conditioned before sample application. The sample was put through the column under vacuum *via* SPE vacuum manifold at the flow rate of around 5 mL/min. After the sampling, step of removal of the interferents was applied with cleaning solution put through by gravity. Next, drying to the visibly dry adsorbent was performed and gravitational elution with pure methanol into the heart-shaped 25 mL flask, which were then connected to the automatic rotary evaporator. However, when the additional clean-up step was added, the eluate was transferred to the glass 10 mL vials with MWCNTs. The vials were shaken for 30 minutes in the laboratory shaker at 400 rpm. Then, the eluate was filtered with syringe filter into the heart-shaped 25 mL flask and concentrated. The volume of the solution was reduced to around 0.5 mL and transferred to the 1.5 mL chromatographic vial. The heart-shaped flask was then flushed with 1 mL of methanol, which was then transferred to the same vial, which was performed twice. Meanwhile, the solution in the vial was kept under constant stream of nitrogen. Finally, entire solvent was evaporated and the precipitate was dissolved in initial mobile phase of the analytical method prior to the analysis. Until that time, the samples were kept in the freezer (at -19 °C). When the selection of conditions was finished, the analytical procedure was evaluated through determining ME, EE and AR in different surface water samples (listed in the **Table 10**), and then fully validated in terms of the determination of its parameters such as: precision (CV), accuracy, limit of quantification (LOQ) and limit of detection (LOD) of the whole analytical procedure. LOD and LOQ were quantified the same as IDL and IQL as described in **Chapter 4.5.1.**

Table 10 Samples used for the development and evaluation of the analytical procedure for the determination of the selected analytes with the application of LC-MS/MS with IT analyser

Type of a sample	Date of the collection	Location	Purpose
WWTP effluent	November 2018	WWTP "Wschod" in Gdansk.	Selection of the parameters of the SPE procedure.
	December 2018	WWTP "Wschod" in Gdansk.	Selection of the parameters of the SPE procedure.
Surface water	February 2019	Potok Oliwski Stream, 100 m before the mouth of the stream.	Method evaluation and validation.
	March 2019	Radunia River, by the Biskupia street in Gdansk.	Method evaluation and determination of the analytes.
		Motlawa River, at the spot where it crosses Elblaska street in Gdansk.	
		Strzyza River, by the Reymonta street in Gdansk.	
		Mlynski Pond, in Oliwa-Gdansk.	
		rainwater from the collector "Kolobrzeska" in Brzezno-Gdansk.	

4.5.3 Transfer of the developed method for the determination of selected pharmaceuticals and their TPs to LC-MS/MS with QqQ analyser

The proposed methodology for the determination of selected pharmaceuticals and their TPs was transferred to new and more sensitive LCMS-8050 system equipped with QqQ analyzer (Shimadzu, Japan) and LabSolutions software. The development of this method started with the automated selection of the MRM transitions for each compound, which involved searching for pseudo–molecular ions, selection of the best voltage for each quadrupole and of the most stable and intensive signals representing fragmentation ions. It was performed with two different mobile phases; ACN/H_2O + 1 mM ammonium acetate buffer (pH 3.8) (10:90, v:v) and ACN/H_2O + 0.1 % HCOOH (10:90, v:v). Next, the chromatographic separation was performed with a column Luna Omega Polar C18 (3 µm 100 Å, 50 mm × 3 mm, Phenomenex, USA). The MS conditions were as follows: interface voltage -4 kV, source temperature 300 ºC, desolvation temperature 526 ºC, heating gas flow 10 L/min, drying gas flow 10 L/min, nebulizing gas flow 3 L/min. Various gradient elution programs were tested for the most suitable separation. Flow of the mobile phase was 0.4 mL/min and injection volume 2 µl. After the instrumental method was established, it was validated in terms of its: precision (CV), accuracy, IQL and IDL (the description and calculation methods are presented in **Chapter 4.5.1**). For the validation purposes, the solutions of the analytes were prepared in the range of 0.05 µg/L to 200.0 µg/L with eleven levels of concentrations. Afterwards, this instrumental method was combined with previously described SPE procedure for the determination of pharmaceuticals and their TPs in water samples. Its performance was evaluated by determining the ME, AR and EE for several samples collected during 2 campaigns

in December 2021 and February 2022 in the area of Rybno village (Masuria region, Poland). In the area there is a Wel River, which passes through the Zarybinek Lake and which is a recipient of treated wastewater from local MBR reactor. Additionally, in this area there is a fish farming facility and ponds surrounding it. Hence, samples were taken from one of the ponds, Zarybinek Lake, Wel River before and after the wastewaters discharge and the WWTP effluent. For each sample, 3 repetitions of the blank sample were prepared. The analyzed volume of the WWTP effluent samples was 100 mL and 250 mL for the other samples (surface waters). For the ME and EE calculation, one additional sample was always spiked with the analytes before the SPE extraction and one after entire SPE procedure, so the final concentration of each analyte in the vial was 10 μg/L. Also, during these analyses there was always a sample of 10 μg/L of the analytes prepared in pure solvent. Each sample was analyzed 4 times.

Afterwards, the developed analytical procedure was fully validated using two different matrices: the WWTP effluent from the area of Rybno village and the Zarybinek Lake. The range of concentrations of spiked lake water samples was from 0.2 to 200.0 ng/L on 6 levels of concentration and the range concentrations of spiked WWTP effluent was 5.0 to 2 000.0 ng/L on 6 levels of concentration. Samples at each level of concentration were prepared in 3 aliquots. Based on the performed validation, such parameters as: precision (CV), accuracy, LOQ and LOD were determined (accordingly to the description in the **Chapter 4.5.1**). LOQ and LOD were calculated the same as IQL and IDL.

Finally, this validated procedure was used to determine the presence and concentrations of the analytes in various environmental water samples (listed in **Table 11**).

Table 11 Samples used for the evaluation of the developed analytical procedure for the determination of the selected analytes with the application of LC-MS/MS with QqQ analyser

Type of a sample	Date of the collection	Location	Purpose
WWTP effluent	December 2021	Rybno.	Method evaluation, validation and determination of the analytes.
	February 2022		
	November 2021	WWTP in Gniewino.	Determination of the analytes.
		WWTP "Wschod" in Gdansk.	
WWTP influent		WWTP in Gniewino.	
		WWTP "Wschod" in Gdansk.	
Surface water	November 2021	Zagorska Struga River, 100 m downstream the fish farm.	Determination of the analytes.
		Zagorska Struga River, 1000 m downstream the fish farm.	Method evaluation and determination of the analytes.
	January 2022	Motlawa River, on the Wyspa Spichrzów Island in Gdansk, next to the Jaglana street.	Determination of the analytes.
	December 2021	Wel River, before WWTP effluent discharge.	Method evaluation and determination of the analytes.
	February 2022		
	December 2021	Wel River after WWTP discharge.	
	February 2022		
	December 2021	Zarybinek Lake.	Method validation.
	February 2022		Method evaluation and determination of the analytes.
	December 2021	Pond next to the Zarybinek Lake.	
	February 2022		

4.6 Studies on the hydrolytic stability of pharmaceuticals and their TPs in water

The experiments were based on the OECD Guideline 111, which refers to the hydrolysis as a function of pH [218]. The general outline of the experiments is presented in **Figure 9**.

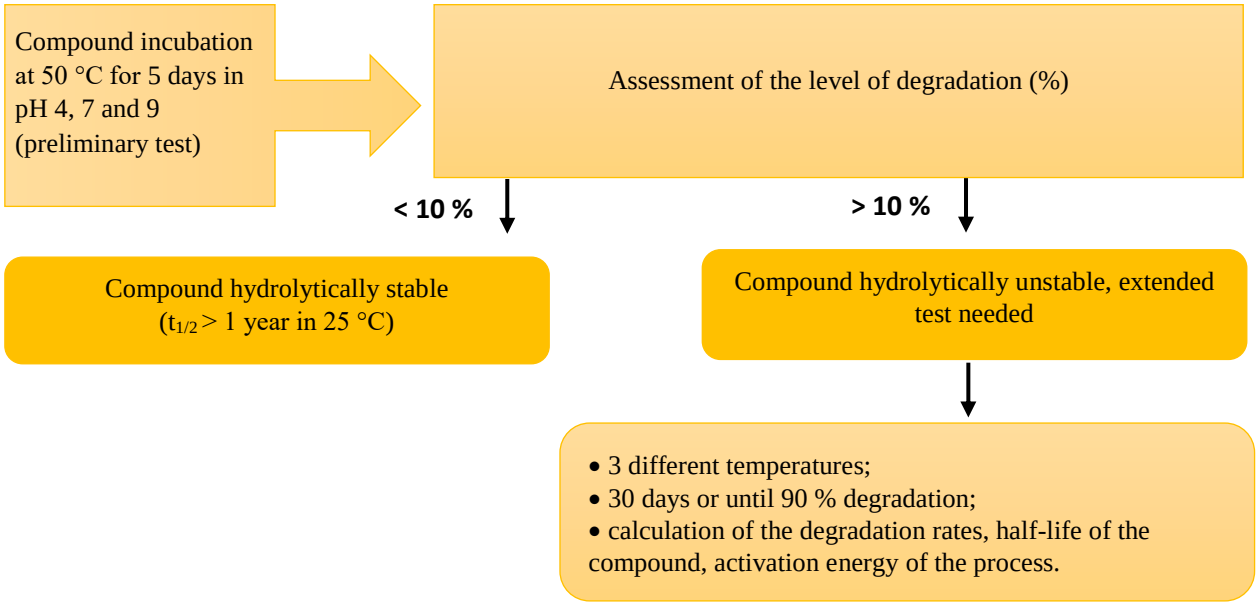


Figure 9 The outline of the hydrolytic stability tests

The initial concentration of each compound in the tests was 10 mg/L. The pH 4, 7 and 9 were buffers prepared in autoclaved ultrapure water. Buffer pH 4 consistent of 0.2 M dipotassium phosphate and 0.1 M citric acid, buffer pH 7 of 0.1 M sodium hydroxide, 0.1 M dipotassium phosphate and water, buffer pH 9 of 0.1 M sodium hydroxide, 0.1 M boric acid and water.

4.6.1 The initial assessment of hydrolytic stability

The preliminary assessment of hydrolytic stability was performed at pH 4, 7 and 9. The 2 mL aliquots of the solutions of each compound at three different pH were added to the 4 mL headspace glass vials in triplicates. The vials were put under nitrogen stream for 30 seconds to remove the air from above the solutions and closed with sealed caps with a capper. Such samples were incubated for 5 days at 50 °C in the dark. At the same time, each solution was analysed immediately after preparation and was incubated at 4 °C for 5 days. After 5 days, samples were analysed and the comparison of peak areas responding to the analytes in control sample (kept at 4 °C) and test sample (incubated at 50 °C) were used to calculate the level of degradation (%). Each preliminary test was conducted at least two times. If the degradation was less than 10 %, the compound was recognized as hydrolytically stable, with $t_{1/2}$ > 1 year in 25 °C. If it was more than 10 %, this analyte was recognized as hydrolytically unstable and was directed to the extended tests (only at the pH in which degradation occurred).

4.6.2 The investigation of hydrolysis process for the unstable compounds

Investigation of hydrolysis process of the unstable compounds regarding solutions, glassware etc. was the same as in preliminary test. However, the solutions were incubated at 20, 50 and 70 °C for 30 days or until 90 % of degradation. Therefore, many aliquots (at least 20) of each solution were prepared to observe the degradation. Two aliquots were always taken out of incubation in time intervals and analysed. At least 7 measuring points were achieved for each compound. The obtained results were used to calculate the parameters of hydrolytic degradation, such as rate constant k, half-life of the reaction $t_{1/2}$ and activation energy E_a. Rate constant was calculated by plotting ln c_t/c_0 with time t (**Eq. 9**).

$$\ln \frac{c_t}{c_0} = kt \tag{9}$$

where:

c_t – concentration at the time interval;

c_0 – concentration at time 0;

t – time.

From the linear regression equation (y=ax, b=0), rate constant k is equal the intercept a. Half-life $t_{1/2}$ was calculated after obtaining the rate constants from the **Eq. 10**:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \tag{10}$$

Additionally, from the Arrhenius equation (**Eq. 11**) it is possible to calculate activation energy E_a and extrapolate the results to other temperatures.

$$k = A \times e^{\frac{-E}{R \times T}} \quad \text{or} \quad \ln k = \frac{-E}{R \times T} + \ln A \tag{11}$$

4.6.2.1 The assessment of the presence of potential degradation products after hydrolysis experiments

The assessment if there are any degradation products in the sample after the hydrolysis study was performed by analysing the samples of unstable compounds after the test at 70 °C. They were analysed with the LC-MS equipment with IT analyser (Brucker Daltonics, Germany). For this purpose, three different samples were always analysed, test sample (with analyte, subjected to the test), blank sample (without addition of an analyte, but incubated in the same conditions as test sample) and control sample, which was the solution of the analyte not subjected to the test (kept in 4 °C during tests). Mass spectra in TIC mode were collected for each sample and the obtained ions compared between the samples. If the *m/z* of a signal was found in the test sample, but not in the blank and control samples, it was considered as a potential degradation product. The analytical methods utilised for this purpose consisted of mobile phase A: 1 mM CH_3COONH_4 in H_2O + 10 % ACN and B: ACN and HPLC column Gemini C18 (5 μm, 150 mm × 4.6 mm, Phenomenex, USA). The injection volume was 50 μL and the parameters of MS were: drying gas 50 psi, 11 L/min, 360 °C, capillary voltage 4 kV and range of the scanning 50 – 400 *m/z*. The analysis were performed in positive and negative ionization mode. The isocratic elution program was established for the samples with 4-OH-DIC (A 60 % : B 40 %, v:v), MTZ-OH (A 95 % : B 5 %, v:v) and CBZ-ep (A 95 % : B 5 %, v:v). Samples with CP and IF were analysed with gradient elution program, with initial composition of mobile phase 80 % A and 20 % B, which changed within 20 minutes to 35 % A and 65 % B, and within 10 minutes back to the initial composition.

4.7 Application of MWCNTs for the removal of pharmaceuticals and their TPs from water samples

In the initial phase of this research, the attention was focused on the evaluation of regeneration possibilities of the MWCNTs and their performance as adsorbents after the regeneration process. For this purpose, 3 anticancer drugs, CP IF and 5-FU were chosen as model compounds in these experiments for the evaluation of their adsorption level during regeneration experiments. These pharmaceuticals are of special importance, because due to their mutagenic and cancerogenic properties, their presence in the environment is especially dangerous [3,219,220]. Therefore, their removal from water matrices should be investigated. Moreover, this investigation is a continuation of model studies on the adsorption of these compounds onto MWCNTs [202], where it was observed that one type, with the highest surface area, had the biggest sorption capacity. Therefore, these MWCNTs were chosen for the experiments in this PhD thesis. Furthermore, the topic of

water purification by MWCNTs was expanded on the removal of the mixture of pharmaceuticals and their TPs by the MWCNTs/chitosan membranes. This mixture includes pharmaceuticals and PTPs frequently determined in various surface water and wastewater samples, which prompts to look for methods of removing them from the water. Therefore, the conducted study was based on the assessment of thermal and chemical regeneration process of MWCNTs influence on the adsorption of selected analytes as well as preparation of MWCNTs/chitosan membranes and evaluation of their removal potential of pharmaceuticals and their TPs.

The MWCNTs used in this study are characterised by the manufacturer (Cheap Tubes, USA) with outer diameter <8 nm, length 10 – 30 μm, ash content <1.5 %, purity <95 % and surface area 500 m^2/g. For clarification, the term "pristine MWCNTs" used in this thesis refers to the material that was not subjected to any regeneration process.

4.7.1 Study on the regeneration of MWCNTs

The performed regeneration studies included several tasks, which are schematically presented in the **Figure 10**. In general, the studies involved experiments with thermal and chemical regeneration. The main goal was to assess the effectiveness of the proposed regeneration conditions (both thermal and chemical) by the evaluation of adsorption potential of selected model pharmaceuticals. Additionally, metals concentration in the collected fractions of acid solutions were additional step of evaluation of the performance of the method. It must be also explained that during these studies two different batches (called as 1^{st} and 2^{nd} batch) of MWCNTs were tested, which were bought from the same supplier/manufacturer, but at different time.

First task of the regeneration study was to select the conditions of the thermal regeneration in terms of the maximum temperature of the process that could be used without degradation of the MWCNTs. Therefore, MWCNTs were incubated for 2 h at temperature of 400 °C, 350 °C and 300 °C and their mass was verified before and after the process. Furthermore, cycles of pollution and thermal regeneration of MWCNTs, in which 1 g of MWCNTs was mixed with previously filtered wastewater effluent from WWTP in Gdańsk, Poland (collected in the November 2018), to arrange hard working conditions of the adsorbent. The mixing continued for 16 h, then most of the water was removed and MWCNTs were dried in a laboratory drier for visibly dry material. Subsequently, the MWCNTs were transferred to the porcelain crucible and thermally regenerated in a chosen temperature program. After the process, the material was cooled down and grated to obtain homogeneous powder. Then, a few micrograms were taken for the adsorption experiments, while the rest of MWCNTs were mixed with wastewater, dried, regenerated etc. There were five such cycles, after each the adsorption levels of CP, IF and 5-FU as model pharmaceuticals were

determined. Additionally, before the adsorption experiments, the equilibrium time of the adsorption process was determined with the MWCNTs after 5 cycles of contamination/regeneration. Moreover, Freundlich and Langmuir isotherm models were fitted for thermally regenerated and not regenerated MWCNTs.

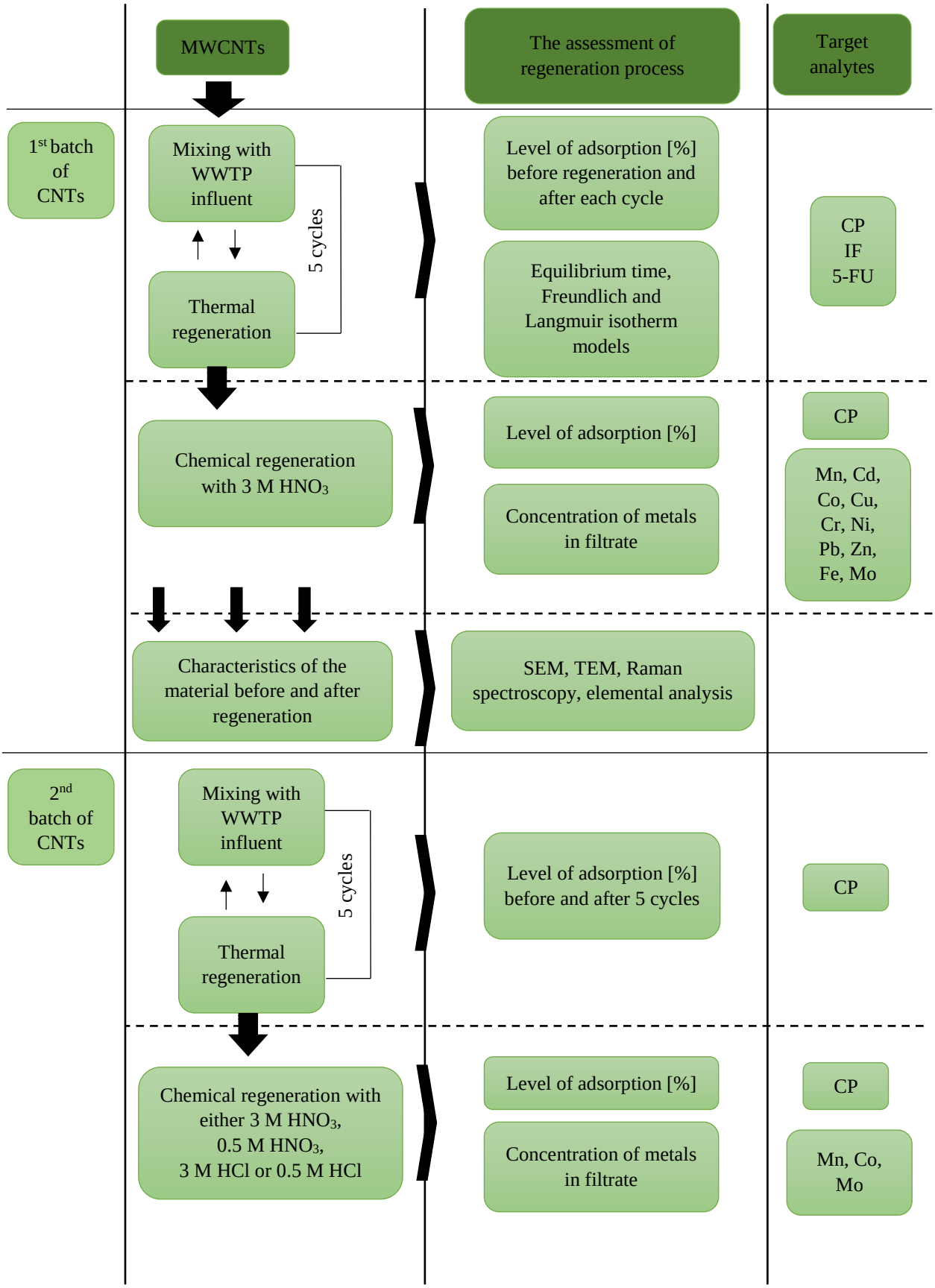


Figure 10 The outline of the performed regeneration studies of MWCNTs

Then, the chemical regeneration was performed. The MWCNTs (10 mg) were packed into empty polypropylene column and flushed with 3 portions (3 × 10 mL) of 3 M HNO_3. MWCNTs were then flushed with water and methanol, dried, and the sorption experiments were performed for model compound - CP. Moreover, each collected fraction of acid was then analysed for presence of typical metals, which occur among CNTs after their manufacturing as well in wastewaters, such as Cu, Cr, Cd, Ni, Pb, Zn, Fe, Mn, Co and Mo. Additionally, pristine MWCNTs were flushed with acids exactly the same as the MWCNTs after full cycles, to compare if there are significant differences in metals composition. The determination of selected metals was performed by Dr. Aleksandra Bielicka-Giełdoń (previously Department of Environmental Technology, now Department of General and Inorganic Chemistry, Faculty of Chemistry, University of Gdansk) with the ICP-OES Optima 2000 (Perkin Elmer, USA) equipment.

Moreover, to verify if there were any structural changes to the adsorbent SEM (Scanning Electron Microscope), TEM (Transmission Electron Microscopy) imaging as well as Raman spectra and elemental analysis were made by an external institution, Laboratory for Instrumental Analysis at Nicolaus Copernicus University in Torun (Poland). SEM/FIB Quanta 3D FEG (Fei, USA) operating at 30 kV was used for SEM images and Tecnai F20 X-Twin (FEI, USA) for TEM. CHN analyser Vario MACRO no. 11.45-0000 (Elementar-Analysesysteme, Germany) equipped with a thermal conductivity detector was used for determining the content of nitrogen, hydrogen and carbon. The content of oxygen was measured with the organic elemental analysis (Flash 2000, Thermo Scientific, USA) technique plus thermal conductivity detector. Raman dispersive spectroscope Senterra (Bruker, USA), with high-resolution video camera, automated continuous calibration and fluorescence removal was applied to obtain Raman spectra at an excitation wavelength of 532 nm, a spectral range of 50 – 4400 1/cm and at a power of 2 mW. These experiments were conducted for pristine (non-regenerated) MWCNTs, as well as thermally and chemically regenerated.

Briefly, the assessment of the influence of regeneration process on the adsorption of model compounds was determined by the evaluation of the sorption level of selected compounds onto regenerated MWCNTs in comparison to non-regenerated MWCNTs in standard batch adsorption studies. They were conducted in 10 mL glass vials. Always, 1 mg of MWCNTs was put into the vial and 5 mL of the solution of each of the investigated compounds was added (the tests were conducted separately for each compound). The concentration of each compound was 10 mg/L, prepared in 0.01 M $CaCl_2$. The vials were closed and shaken on the laboratory shaker for 2 h. Then, the samples were filtered and closed in the chromatographic vials. The HPLC-UV/Vis equipment was used to analyse the samples (**Chapter 4.5.1**). Comparison between test samples

(solution of a compound mixed with MWCNTs) and control samples (only the solution of a compound, not subjected to any process) gave the information of the adsorption level. Each test sample was prepared in four replicates. Blank samples were also made (the solution of a matrix without the analyte mixed with MWCNTs). The ratio between MWCNTs and the amount of the analyte was chosen based on the previous work [202] to reach the adsorption level between around 20 and 50 %, to observe any changes that could occur after various MWCNTs treatment. Moreover, the equilibrium time was investigated by preparing mixtures of MWCNTs and the analytes' solution as in adsorption experiments above, but with different time contact, such as 0.5, 1, 1.5, 2, 4, 6, 8, 16 and 24 h. For each time interval, there were two replicates. Moreover, for the adsorption isotherm models determination, solutions of different concentrations of the investigated pharmaceuticals were prepared (0.625, 1.25, 2.5, 5, 10, 20, 40, 60, 80 mg/L). Linear, Freundlich and Langmuir models were fitted. Linear isotherm represents the relationship between concentration of the analyte adsorbed and left in the solution. The distribution coefficient K_d [L/kg] is calculated from the equation (**Eq. 12**):

$$K_d = \frac{c_s}{c_w} \tag{12}$$

where:

c_s – concentration adsorbed [mg/kg];

c_w – concentration in aqueous phase [mg/L];

It can be also calculated from the slope of the linear regression function.

Freundlich isotherm is described by equation (**Eq. 13**):

$$c_s = K_F \cdot {c_w}^{1/n} \tag{13}$$

where:

K_F – Freundlich equilibrium constant [$mg^{1-1/n}\, kg^{-1}\, l^{1/n}$];

1/n – arbitrary constant, indicator of linearity of the equation.

By plotting $\log c_s$ and $\log c_w$, the parameters can be calculated from the linear form of Freundlich isotherm:

$$\log c_s = \frac{1}{n}\log c_w + \log K_F \tag{14}$$

The Langmuir model is described by the equation (**Eq. 15**):

$$c_s = \frac{c_{max} \cdot K_L \cdot c_w}{1 + K_L \cdot c_w} \quad (15)$$

where:

c_{max} – maximum amount of the analyte that can be adsorbed;

K_L – Langmuir constant.

By plotting $1/c_s$ and $1/c_w$, the parameters can be calculated from the linear form of Langmuir isotherm:

$$\frac{1}{c_s} = \frac{1}{K_L c_{max}} \times \frac{1}{c_w} + \frac{1}{c_{max}} \quad (16)$$

Moreover, to confirm obtained results, 2nd batch of the same type MWCNTs was purchased and the same 5 cycles of pollution and regeneration were performed. This time, only the adsorption level of CP was verified before and after 5 cycles. However, the chemical regeneration was expanded to flushing with 3 M HNO_3, 0.5 M HNO_3, 3 M HCl and 0.5 M HCl; each acid was used separately for an individual portion of thermally regenerated MWCNTs. In this case, 30 mg of MWCNTs was flushed with 3 × 30 mL of acid solution. The adsorption level was investigated, as well as each fraction was also analysed to determine to concentration of metals.

4.7.2 The preparation and assessment of the MWCNTs/chitosan based membranes as material for the removal of pharmaceuticals and their TPs from water

The outline of these experiments aimed at preparation and application of MWCNTs/chitosan based membranes for the removal of selected pharmaceuticals and their TPs from water samples is presented in the **Figure 11**. First, to three beakers filled with 500 mL 0.1 M NaCl, 500 mg of low molecular weight chitosan was added. Next, 5 mL of concentrated HCl was added to each beaker and all of them were subjected to ultrasounds for 30 minutes. When chitosan was dissolved, 100 mg of pristine MWCNTs from 2nd batch, 100 mg of thermally regenerated MWCNTs form 2nd batch and 100 mg of MWCNTs from 3rd batch were added to the solutions. All solutions were mixed on the magnetic stirrer for 20 h (400 rpm). Then, 20 mL aliquots were taken from each solution and were filtered under reduced pressure through the nylon membranes, which caused deposition of the MWCNTs/chitosan hybrid on the membrane. From each kind of MWCNTs, 20 membranes were prepared. Such membranes were tested for the removal of the selected

pharmaceuticals and their TPs from water. For this purpose a mixture of 12 compounds (CBZ, CBZ-ep, 2-OH-CBZ, 10-OH-CBZ, MTZ, MTZ-OH, O-DMTRA, DIC, 4-OH-DIC, MTP, MTPA, ac-SMX) was prepared in pure water at concentration of 10 µg/L. The determination of the adsorption level (%) was performed by comparison of the surface areas of signals representing analytes in samples before and after experiments. The developed method based on the application LC-MS/MS with QqQ analyser (described in **Chapter 4.5.3)** was used for this purpose. All experiments were performed with vacuum filtration apparatus with glass funnel for vacuum filtration on top, where the working solution was always poured. First, aliquots of 100 mL of working solution were passed under vacuum through each kind of membrane in 5 repetitions (5 membranes, each 100 mL of solution). The speed of the process was controlled so the samples were passed through from 9 to 11 minutes.

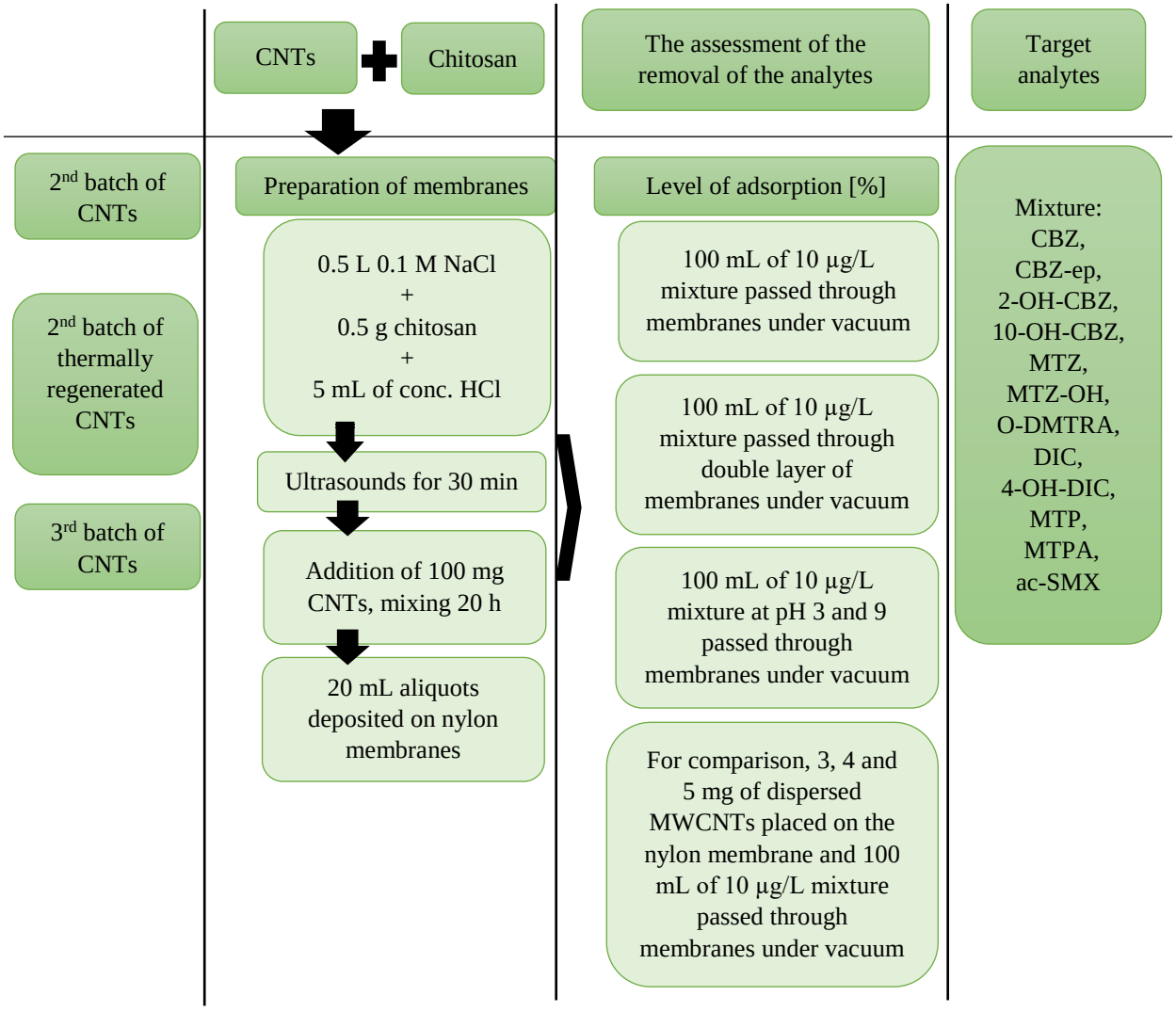


Figure 11 The outline of the preparation of MWCNTs/chitosan membranes and their application

Moreover, double membranes (2 membranes on top of each other) were tested under the same conditions to verify the increase in removal efficiency. Additionally, the influence of different pH values (pH 3 and 9) of the solution on the removal efficiency were also tested, but only for regenerated 2nd batch of MWCNTs and 3rd batch of pristine MWCNTs. Furthermore, in order to compare the obtained results and the performance of the membranes, dispersive adsorption of pharmaceuticals and their TPs onto investigated MWCNTs not mixed with chitosan was studied. For this purpose 3, 4 or 5 mg of each kind of MWCNTs (2nd batch, regenerated 2nd batch and 3rd batch) were put on top of the clean nylon membrane in the glass funnel for vacuum filtration. Then, the aliquot of 100 mL of working solution was added to each MWCNTs and it was filtered under vacuum. Each experiment was performed in triplicate.

5. RESULTS AND DISCUSSION

Various pharmaceuticals and their TPs were included in this research (**Table 9**). They belong to different therapeutic groups, such as antiepileptic, non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers, antibiotics, opioid analgesics and anticancer drugs. They were chosen for several reasons; firstly, many of them are consumed in high quantities, especially those sold over-the-counter (for example, consumption of DIC is estimated for around 1000 t worldwide [221]). Secondly, they are being often found in the environmental samples at level from ng/L to μg/L [82,84,109]. Thirdly, they cause adverse effects once administered (like anti-cancer drugs). Moreover, the chosen PTPs are the metabolites created during metabolic processes in the largest quantities, possess biological activity (like CBZ-ep, MTZ-OH or 7-OH-MTX) and/or have already been detected in the environmental samples [97,100,222]. Among this group of chemicals different analytes were selected depending on the research objective.

5.1 The characteristics of the developed analytical methods

Firstly, simple analytical methods based on the application of HPLC-UV/Vis systems were developed for the analysis of 17 single compounds as a final determination step that was further used in the model studies on hydrolytic stability and adsorption onto MWCNTs. Then, methods for the determination of the mixture of pharmaceuticals and their selected TPs by using different LC-MS/MS systems were also developed. Initially, development of such method for the determination of 18 selected pharmaceuticals and their TPs was performed with the LC-MS/MS system equipped with ion trap (IT) analyser. This method was used for testing various conditions of the SPE process for selected analytes to propose their effective extraction procedure from different water samples. This was evaluated based on the assessed appropriate parameters such as: matrix effects (ME), extraction efficiency (EE) as well as absolute recovery (AR). Next, the proposed method was validated and afterwards used for preliminary assessment of the presence of the selected analytes in the environmental samples. Finally, this method was transferred to more sensitive and suitable for trace analysis of such analytes LC-MS/MS system equipped with triple quadrupole (QqQ) analyser. After the reassessment of its quality and performance characteristic in terms of the AR, EE and ME, it was fully validated and applied to the analysis of selected in environmental water samples.

5.1.1 The characteristics of the developed analytical methods based on the application of HPLC-UV/Vis technique

Final, selected conditions of the analysis with HPLC-UV/Vis technique of each analyte are presented in **Table 12**, whereas determined validation parameters are presented in the **Table 11**.

Table 12 Analytical methods applied for the analysis of single compounds based on the HPLC UV-Vis technique

Analyte	HPLC column	Detection wavelength [nm]	Phase A	Phase B	Composition of mobile phase (v:v)	Injection volume [µL]	Flow rate [mL/min]
CBZ	Gemini C18 5 µm 110 Å, 150 mm x 4.6 mm	285	ACN	H_2O	35 : 65	50	1
CBZ-Ep		209			30 : 70		
10-OH-CBZ		237			25 : 75		
MTZ-OH		315			8 : 92		
5-FU		266			5 : 95	10	0.7
CP	Luna Omega Polar C18 3 µm 100 Å, 50 mm x 3 mm	200			20 : 80	50	0.6
IF		200			20 : 80		
2-OH-CBZ	Gemini C18 5 µm 110 Å, 150 mm x 4.6 mm	284		H_2O + 0.1 % HCOOH	25 : 75		1
IBU		219			55 : 45		
2-OH-IBU		218			30 : 70		
CX-IBU		219			30 : 70		
TRA		271			15 : 85		
O-DMTRA		272			8 : 92		
MTX	Gemini C6-Phenyl 5 µm 110 Å, 150 x 4.6 mm,	305			10 : 90		
7-OH-MTX		302			15 : 85		
4-OH-DIC	Gemini C18 5 µm 110 Å, 150 mm x 4.6 mm	221		H_2O + 5 mM CH_3COONH_4 + 5 % ACN	35 : 65		
ac-SMX		263			20 : 80		

The developed methods were satisfying and suitable for the purpose they were created. The recommended validation criteria in international guidelines state [223], that the level of variation should not exceed 15 – 20 %, which means that CV not to exceed 20 % and accuracy should be in the range of 80 – 120 %, which was fulfilled for all analytes for intended research purpose (**Table 13**).

Table 13 Basic validation parameters of the developed analytical methods presented in Table 10

Analyte	Linearity range [mg/L]	R^2	Precision (CV) [%]	Accuracy [%]	IQL [mg/L]	IDL [mg/L]
CBZ	0.031 – 5 10 – 80	1.000 0.999	0.1 – 5.0 0.1 – 0.4	99.0 – 103.3 99.1 – 100.9	0.031	0.010
CBZ-ep	0.016 – 0.300 0.500 – 20	0.999 1.000	1.1 – 6.4 0.2 – 1.2	80.2 – 105.7 81.1 – 102.9	0.016	0.005
2-OH-CBZ	0.008 – 0.125 0.500 – 20	1.000 1.000	1.2 – 3.5 0.1 – 0.5	98.2 – 103.3 94.0 – 100.8	0.008	0.003
10-OH-CBZ	0.016 – 20	1.000	0.2 – 7.0	84.0 – 101.5	0.016	0.005
MTZ-OH	0.031 – 2.5 5 – 80	1.000 0.998	0.1 – 3.0 0.1 – 0.2	99.1 – 105.4 97.5 – 113.4	0.031	0.010
5-FU	0.063 – 80	1.000	0.1 – 5.4	99.5 – 114.2	0.063	0.021
CP	0.300 – 80	0.999	0.2 – 5.6	96.8 – 118.6	0.250	0.080
IF	0.125 – 80	1.000	0.1 – 5.0	96.3 – 108.9	0.125	0.042
IBU	0.125 – 20	1.000	0.2 – 8.6	95.6 – 101.0	0.125	0.042
2-OH-IBU	0.250 – 2.5 1.3 – 20	0.999 1.000	1.0 – 8.3 0.3 – 8.3	94.9 – 103.8 97.5 – 100.7	0.250	0.083
CX-IBU	0.125 – 20	1.000	0.8 – 7.7	98.1 – 116.4	0.125	0.042
TRA	0.063 – 20	0.999	0.1 – 9.0	93.2 – 102.0	0.063	0.021
O-DMTRA	0.500 – 10 2.5 – 80	0.999 0.999	1.5 – 10.6 0.4 – 2.8	96.5 – 104.7 97.1 – 116.2	0.500	0.167
MTX	0.063 – 10	0.999	1.2 – 9.1	87.9 – 112.6	0.063	0.021
7-OH-MTX	0.125 – 10	0.999	0.4 – 2.6	93.3 – 97.7	0.125	0.042
4-OH-DIC	0.063 – 20	0.999	0.6 – 4.8	99.0 – 108.5	0.063	0.021
ac-SMX	0.063 – 20	0.999	0.2 – 3.7	88.4 – 104.5	0.063	0.021

5.1.2 The characteristics of the developed analytical method based on LC-MS/MS with IT analyser

Initially, the working conditions of MS with ESI were established, with drying gas at 50 psi, 10 L/min, 365 °C, capillary voltage 4 kV, ICC target 50000, MaxAccuTime 200 ms, average 3 scans. Then, selection of MRM transitions (pseudo–molecular ion → fragmentation ions) was performed for each analyte. Finally, the MRM transitions have been established for all 18 analytes (**Table 14**). A minimum of 2 transitions were found for 10 compounds, while for the other only one transition was selected mainly due to poor ionization (in case of most NSAIDs).

Table 14 The ionization mode and MRM transitions selected for the analysis of investigated pharmaceuticals and their transformation products (the fragment ion presented in bold states for the ion chosen for quantification)

Compound	Ionization mode	MRM transition (*m/z*)	Fragmentation amplitude [V]
CBZ	Positive $[M+H]^+$	**237 → 194**	0.6
		237 → 220	0.6
CBZ-ep	Positive $[M+H]^+$	**253 → 210**	0.6
		253 → 236	0.6
10-OH-CBZ	Positive $[M+H]^+$	**255 → 237**	0.6
2-OH-CBZ	Positive $[M+H]^+$	**253 → 210**	0.6
		253 → 236	0.6
IBU	Negative $[M-H]^-$	**205 → 159**	0.4
2-OH-IBU	Negative $[M-H]^-$	221 → 133	0.8
		221 → 177	0.8
cx-IBU	Negative $[M-H]^-$	**235 → 191**	0.8
TRA	Positive $[M+H]^+$	**264 → 246**	0.4
O-DMTRA	Positive $[M+H]^+$	**250 → 232**	0.6
MTZ	Positive $[M+H]^+$	**172 → 128**	0.5
MTZ-OH	Positive $[M+H]^+$	**188 → 123**	0.4
		188 → 144	0.4
Ac-SMX	Positive $[M+H]^+$	296 → 136	0.4
		296 → 188	0.4
		296 → 236	0.4
DIC	Negative $[M-H]^-$	**295 → 250**	1.0
4-OH-DIC	Negative $[M-H]^-$	310 → 194	1.0
		310 → 230	0.8
		310 → 266	0.8
NPX	Negative $[M-H]^-$	**229 → 185**	0.8
des-NPX	Negative $[M-H]^-$	215 → 171	0.8
		215 → 185	0.8
MTP	Positive $[M+H]^+$	**268 → 116**	0.6
		268 → 159	0.6
		268 → 191	0.6
MTPA	Positive $[M+H]^+$	268 → 145	0.6
		268 → 191	0.4
		268 → 226	0.6

Furthermore, the chromatographic separation of selected analytes has been tested. The mobile phase was consisted of A: 1 mM CH_3COONH_4 in H_2O + 10 % ACN and B: ACN, flow of the mobile phase was 0.4 mL/min, injection volume of the sample 50 μL and column temperature 25 °C. The separation of the analytes was tested using two chromatographic columns. However, the retention times of the signals, when Luna Omega Polar C18 (3 μm 100 Å, 100 mm × 3 mm, Phenomenex, USA) was used, were very unstable and could not be stabilized. Therefore, the Gemini C18 (5 μm 110 Å, 150 mm × 4.6 mm, Phenomenex, USA), which provided much more stable analyses was finally selected for further analysis. Several gradient elution programs were tested to obtain the best separation of the analytes. This separation was crucial for two reasons; on one hand, PCs and their TPs often have similar fragmentation ions, which would disrupt the determination of the surface areas of the signals recorded for the analytes. Utmost example are CBZ-ep and 2-OH-CBZ, which have the same molecular mass (the same pseudo–molecular ions,

$[M+H]^+$), exactly the same fragmentation ions and almost the same retention time (**Figure 12**). Their separation was the most difficult to obtain.

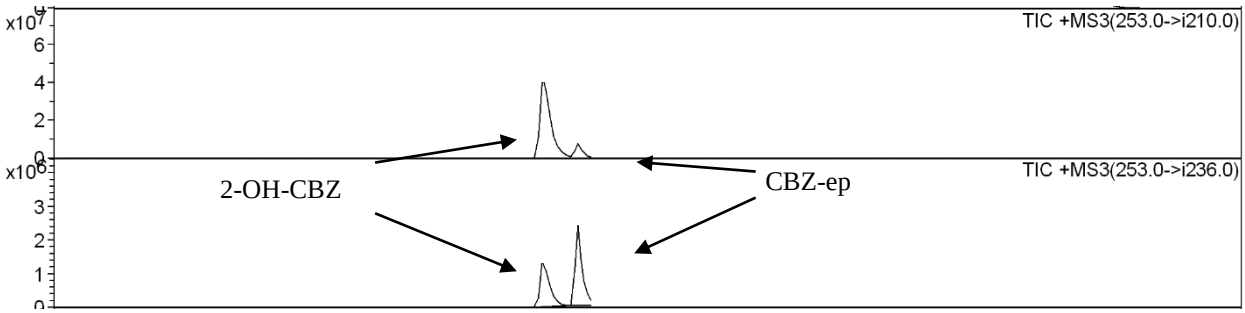


Figure 12 The chromatogram obtained with LC-MS/MS technique in MRM mode with signals of two CBZ metabolites: 2-OH-CBZ and CBZ-ep

It should be highlighted that applied equipment had a big limitation; it was possible to observe only 12 MRM transitions in a run, and the more there were the less scans it was able to make. To overcome this drawback, the time segments (sometimes called time windows) were set, to divide the entire chromatographic run into sections. In each segment only given transitions were searched for. Thanks to that, much more transitions could be observed in the entire analysis, therefore, more compounds could be determined in one run. For this purpose, the final gradient elution program has been established (**Table 15**).

Table 15 The gradient elution program of the developed LC-MS/MS with IT analyser method with the time windows including specific MRM transitions

RT [min]	**5-8**	**8-13**	**13-15**	**15-18**	**18-20**	**20-22**	**22-28**	**28-37**	**37-38**
ionisation	+	+	+	+	-	+	-	-	+
MRM transitions	MTPA **268** → 226 → 191 → 145	MTP **268** → 191 → 159 → 116	10-OH-CBZ 255 → 237	2-OH-CBZ **253** → 236 → 210	2-OH-IBU **221** → 133 → 177	CBZ **237** → 194 → 220	4-OH-DIC **310** → 194 → 230 → 266	DIC **294** → 250	Waste
	MTZ-OH **188** → 144 → 123	TRA **264** → 246		CBZ-ep **253** → 236 → 210	cx-IBU **235** → 191		NPX **229** → 185	IBU **205** → 159	
	O-DMTRA **250** → 232	MTZ **172** → 128		Ac-SMX **296** → 236 → 188 → 136	des-NPX **215** → 171 → 185				
Phase B (ACN) [%]	10 – 44			44 – 70			70 – 80	80 – 10	10
Time [min]	0 – 15			15 – 26			26 – 30	30 – 35	35 – 38

This method for the final determination of selected pharmaceuticals and their TPs was validated through the analysing of the solutions of the analytes' mixtures at different concentration levels (in the range from 0.5 μg/L to 500.0 μg/L) prepared in pure solvent, which was the mobile phase from the initial chromatographic conditions. Determined validation parameters have been presented in the **Table 16**. Despite the fact that the analytical method from chromatographic point of view (the chromatographic separation) was possible to apply for the analysis of 18 analytes, eventually, after validation of the method, it has been recognized that it meets the requirements for 15 analytes. Cx-IBU and NPX were omitted due to their poor results in the terms validation parameters. On the other hand, the ionization of IBU was so weak, that it was impossible to calculate any parameters. Nevertheless, this method, based on the results of validation in pure solvent, is suitable for determination of 5 pharmaceuticals and 10 PTPs included in this study. The precision (CV) was considered acceptable below 15 – 20 % as the international guidelines recommend [223] and very good when it was below 12 %, which is accomplished for most of the analytes (however, the precision at low concentration levels form O-DMTRA was slightly over 20 %), whereas the accuracy was satisfactory if between 80 – 120 %, which also was accomplished for most of the compounds (however, in four cases the bottom range of 80 % was slightly exceeded).

Table 16 Basic parameters of the instrumental validation with the developed method based on the LC-MS/MS with IT analyzer

Compound	Linearity range [μg/L]	Precision (CV) [%]	Accuracy [%]	IQL [μg/L]	IDL [μg/L]
CBZ	0.5 – 10.0 10.0 – 100.0 75.0 – 500.0	2.7 – 11.0 3.1 – 7.7 2.8 – 6.3	90.2 – 104.5 96.1 – 99.7 86.4 – 105.4	0.5	0.2
CBZ-ep	3.0 – 75.0 10.0 – 500.0	5.5 – 10.5 4.9 – 14.9	96.0 – 109.3 93.7 – 107.4	3.0	1.0
10-OH-CBZ	50.0 – 500.0	2.9 – 8.0	94.7 – 107.0	50.0	16.7
2-OH-CBZ	3.0 – 25.0 25.0 – 500.0	3.3 – 8.7 4.6 – 9.1	91.0 – 104.3 71.8 – 105.6	3.0	1.0
2-OH-IBU	10.0 – 500.0	4.0 – 10.8	96.3 – 101.2	10.0	3.3
TRA	6.0 – 50.0	1.4 – 10.0	74.4 – 111.2	6.0	2.0
O-DMTRA	6.0 – 25.0	11.3 – 21.0	71.9 – 92.0	6.0	2.0
MTZ	25.0 – 500.0	3.3 – 5.2	97.4 – 111.3	25.0	8.3
MTZ-OH	50.0 – 500.0	5.9 – 10.4	97.3 – 103.8	50.0	16.7
Ac-SMX	3.0 – 50.0 50.0 – 500.0	3.4 – 10.9 2.6 – 9.9	85.9 – 110.2 75.1 – 107.9	3.0	1.0
DIC	25.0 – 250.0	4.5 – 12.1	91.2 – 108.7	25.0	8.3
4-OH-DIC	6.0 – 125.0	0.9 – 9.9	88.0 – 114.0	6.0	2.0
des-NPX	10.0 – 500.0	4.2 – 9.9	97.7 – 103.3	10.0	3.3
MTP	3.0 – 75.0 50.0 – 500.0	2.9 – 9.8 2.9 – 12.2	89.8 – 120.1 82.0 – 104.8	3.0	1.0
MTPA	3.0 – 125.0	4.9 – 10.6	94.8 – 118.8	3.0	1.0

There are some determination methods for pharmaceuticals in environmental samples with application of LC-MS/MS system with IT analyser available in the literature. These methods contain from 4 to around 20 analytes and last from a dozen up to nearly 40 minutes, which is in accordance with this study. However, the validation parameters are always given for the entire procedure of determination, containing spiking deionized water or environmental water samples with analytes, performing extraction and final analysis with the LC-MS/MS equipment. Therefore, there is lack of data for only instrumental validation [224–230]. However, for example Białk-Bielińska et al. presented results using the same equipment where IQL values for sulfonamides were 0.62 – 5.48 μg/L, the accuracy between 76.2 and 125.6 % and maximum precision 13.5 % [231], which is in accordance with the results obtained in this study. Therefore, the developed method could be used to evaluate the extraction efficiency of selected analytes and to choose the most suitable extraction conditions for most of the compounds from environmental water samples, such as WWTP effluent and surface waters to determine their presence in these samples.

5.1.3 The results of the development of SPE method through testing different extraction conditions

The SPE was performed with Oasis® HLB 200 mg, due to its popularity and proven efficiency in numerous determination methods available in the literature [232,233]. As a first step, the selection of pH of the water sample and the eluting solution was performed, which was done using 250 mL of the spiked deionized water with the mixture of the analytical standards of the analytes at the level of 2 μg/L in the water samples. After establishing the most suitable conditions, the influence of different approaches of sample clean up on the matrix effects and extraction efficiency of selected analytes from samples with more complex matrices was investigated by using 100 mL of the spiked WWTP effluent samples with the analytical standards of the analytes at the level of 2.5 μg/L in the samples. Finally, developed procedure was tested in the analysis of selected pharmaceuticals and their TPs in different natural water samples.

5.1.3.1 The influence of pH and eluting solvent

Initially, broad range of pH of the water samples was tested to observe the general changes on the absolute recovery (AR) of the analytes (**Figure 13**). The obtained results clearly showed, that for most of the analytes pH 10 was much less suitable for the efficient recovery. However, AR at pH 3 and pH 6 was similar for many analytes. Better AR at lower pH can result from the fact, that compounds in their neutral form (not ionized) have bigger affinity to the applied adsorbent. In this situation the hydrophobic interactions as well as $\pi - \pi$ interactions of aromatic rings are probably

main adsorbing force [134,234]. Taking into account the pKa values of the analytes, like 2-OH-IBU (4.6), cx-IBU (4.0) or IBU (4.9), at pH below their pKa they are dominantly in the neutral form. Therefore, in acidic conditions they were recovered very well. However, at pH 10 their AR was close to zero. On the other hand, MTPA with pKa 3.5 was recovered in only around 30 % at pH 3, probably due to dominance of ionized form in these conditions. On the other hand, AR obtained for compounds like MTP (pKa 9.6) or CBZ (pKa 16.0) was quite similar and high across entire tested pH range due to their dominantly neutral form throughout the entire range of tested pH.

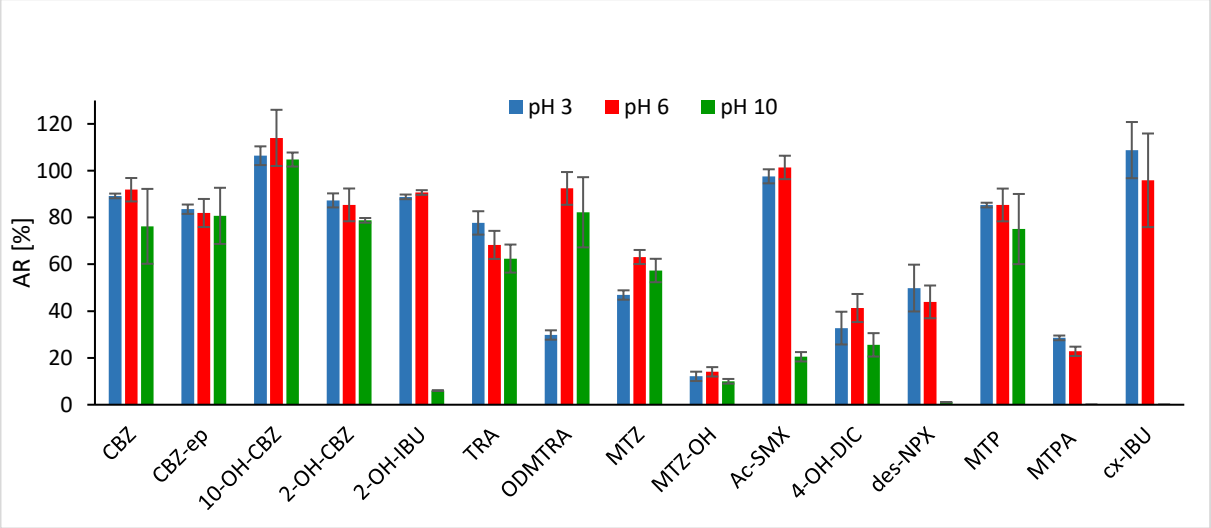


Figure 13 AR of the selected analytes from deionized water determined for the tested SPE procedure at 3 different pH (error bars represent standard deviation)

Therefore, in order to investigate further the influence of acidic conditions on AR of the analytes, spiked water at pH 4, 5 and 6 was additionally tested. At the same time, change of the eluting solvent from MeOH to ACN/MeOH was also investigated (**Table 17**).

Table 17 AR values [%] obtained through SPE procedure from deionized water spiked with analytes at 3 different acidic pH and with 2 different eluting solvents (SD was below 7 % for each analyte; n.d. – not determined)

Analyte	pH 4		pH 5		pH 6	
	MeOH	ACN/MeOH	MeOH	ACN/MeOH	MeOH	ACN/MeOH
CBZ	101	89	97	91	87	86
CBZ-ep	93	79	91	73	11	74
10-OH-CBZ	73	71	77	72	43	60
2-OH-CBZ	87	90	89	89	80	82
IBU	31	20	19	19	15	14
2-OH-IBU	83	82	85	2	71	2
TRA	99	106	85	81	109	75
MTZ	58	54	50	45	49	42
MTZ-OH	12	3	6	0	3	3
ac-SMX	89	86	86	89	63	88
DIC	65	45	48	22	12	18
4-OH-DIC	46	44	50	54	39	36
NPX	70	n.d.	2	22	n.d.	n.d.
des-NPX	71	63	60	68	66	58
MTP	94	95	94	95	91	104
MTPA	30	31	29	19	13	8
cx-IBU	70	61	44	62	43	29

Based on the obtained results it can be concluded that pure MeOH provides at least similar, but in many cases better AR of most of the investigated analytes than the mixture MeOH/ACN. Moreover, pH 4 was selected as the most suitable for extraction of the analytes. The obtained results are in agreement with other studies on SPE of pharmaceuticals available in the literature, where in many cases the sample pH is either acidic or at least neutral [80,82,110,235]. The absolute recoveries obtained through the extraction of pharmaceuticals and their TPs from deionized water found in the literature also vary, for example the recoveries for CBZ and its TPs were between 50 and 120 %, while in the multi-class determination method the AR for 95 analytes was between 10.5 and 170 % [109,236]. Therefore, it might be concluded that results obtained in this study are in accordance with those in the literature. Unfortunately, the recovery of MTZ-OH was poor at all tested conditions. It is small and polar molecule, which may be not sufficiently retained by the Oasis® HLB column, as it was observed for highly polar morphine and gabapentin in other study [104]. Different SPE column or even more acidic conditions could improve the recovery of that analyte, however, it could negatively influence the recoveries of other compounds.

5.1.3.2 The selection of additional clean-up step in the SPE method

As the sample pH and the eluting solution were established for the effective isolation and preconcentration of selected analytes from deionized water samples, the proposed SPE procedure was used for verification of its efficiency in much more complex matrices, which were WWTP effluents. The evaluation of the method performance was also based on the matrix effects (ME), extraction efficiency (EE) and absolute recovery (AR). Results close to 0 % for ME mean they are not observed, negative values indicate the suppression of the signal and positive values the enhancement of the analytes ionization. Therefore, the ME close to 0 % are desirable. Whereas in the case of EE or AR, the results close to 100 % mean that there are is no loss of the analytes observed during the determination procedure. It was expected that the load of interferents will be of a great number, so the sample volume was decreased to 100 mL. Moreover, additional clean-up procedures were tested besides flushing with 10 mL of 5 % MeOH after sample loading (in the standard, described above SPE procedure), namely clean-up after sample loading with 10 % MeOH and after that 10 mL of hexane as well as addition of 0.5 g Na_2EDTA to the water sample before extraction and clean-up after sample loading with 10 % MeOH and after that 10 mL of hexane. The obtained results are presented in the **Figure 14**.

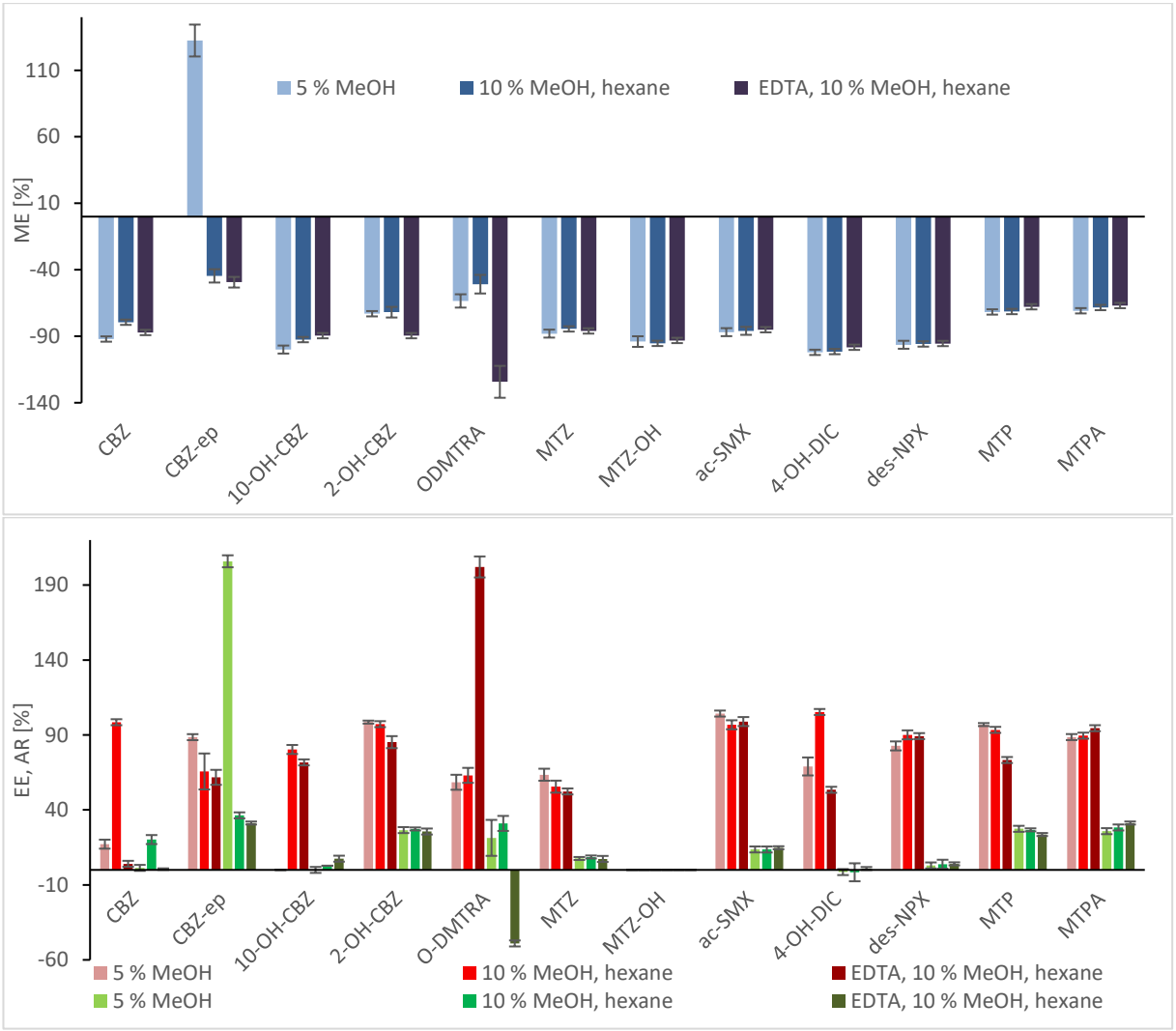


Figure 14 The parameters of SPE procedure with different clean-up variants; shades of blue represent ME, shades of red EE and shades of green AR (error bars represent SD)

It must be highlighted, that ME in such matrix were very high in every tested option (from -45 % to 132 %). It shows how huge impact other components of the water sample have on the signal intensity of target analytes. Nevertheless, judging by the ME, the results are quite similar for most of the compounds. However, the variant with 10 % MeOH and hexane, but without addition of Na_2EDTA seemed to provide the best results in terms of ME (values from -45 % to -102 %) in comparison to other tested options (from -63 % to 132 % for 5 % MeOH and from -49 % to -124 % for EDTA, 10 % MeOH and hexane). Moreover, evaluation of EE shows that addition of Na_2EDTA provided worse results in most cases (EE in the range 4 – 202 %, without including MTZ-OH, which was not extracted at all), whereas variant with 10 % MeOH and hexane was the most efficient (EE in the range of 56 – 105 %, without including MTZ-OH). Therefore, this procedure was chosen for further studies. It must be also highlighted that the problems with high ME in the methods based on LC-MS/MS techniques are in general a common and troublesome issue, which were for example also observed in other studies in complex matrix, like WWTP

effluent, where observed ME were from -10 to 99 % [11,76,237]. However, they can be much lower [127,137]; therefore, due to the observed high ME, further attempts for their reduction were made. Additional clean-up step to determine if more interferents can be removed from the eluate was introduced. For this reason, a step with mixing MeOH eluate with MWCNTs was further evaluated by using different masses of MWCNTs (15, 25 and 35 mg). In this case, the efficiency of the proposed extraction and purification procedure was performed by determining ME, EE and AR (**Figure 15**). Additionally, the results obtained previously without additional step of purification are presented in this figure as well for comparison. Unfortunately, due to LC-MS/MS equipment malfunctions, the results were obtained only for 7 compounds. In general, this step was helpful in reducing ME in comparison with the data obtained before. ME for CBZ before mixing with MWCNTs were close to -80 %, while after it is less than -50 %. For MTP there was a reduction from around -70 % to around -40 %. For ac-SMX there was about 15 % reduction and for des-NPX 20 % reduction. However, based on these results it was observed, that in general the increasing amount of MWCNTs did not reduce the ME efficiently. At the same time EE was maintained at high level for 15 mg of MWCNTs, which was between 80 and 100 % for most of the compounds (only for DIC it was very low, but it was probably due to the analytical issues of NSAIDs ionization; results for IBU were only obtained for one variant, while other analytes and their metabolites belonging to this group were completely omitted). However, larger quantities of MWCNTs caused decrease in EE, which suggests that too strong adsorption forces were affecting the analytes and they were not eluted as sufficiently as for smaller quantities. Additionally, the results confirm that the AR in environmental samples is strongly affected by the ME, which results in much lower values of the recoveries than they really are. Therefore, it is crucial to evaluate all three parameters to obtain more reliable data.

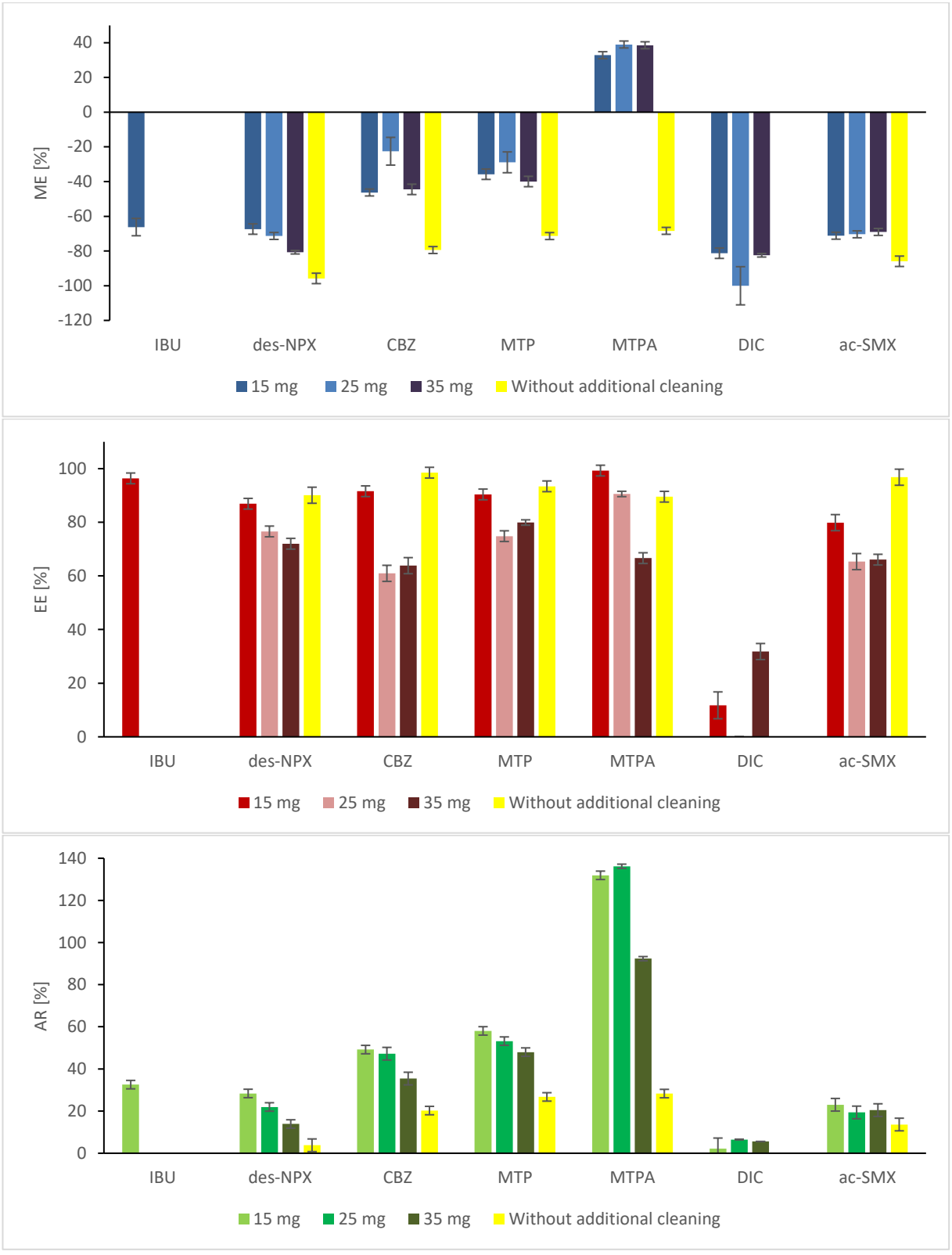


Figure 15 The ME, EE and AR values obtained for chosen analytes with different masses of MWCNTs (15 mg, 25 mg, 35 mg) applied as additional clean-up step in the SPE procedure and without this step (error bars represent SD)

To the best of my knowledge, such SPE procedure with the introduction of a post – elution clean-up step with carbon nanotubes has never been performed. MWCNTs have been used in SPE or in dispersive extraction as a single sorbent or a mixture with other materials for the extraction of the analytes, but not in such way as presented in this thesis [238,239].

Finally, the selected conditions of the SPE procedure are presented in **Figure 16**. Therefore, this procedure was evaluated in terms of its efficiency for the surface water samples and then validated for such samples.

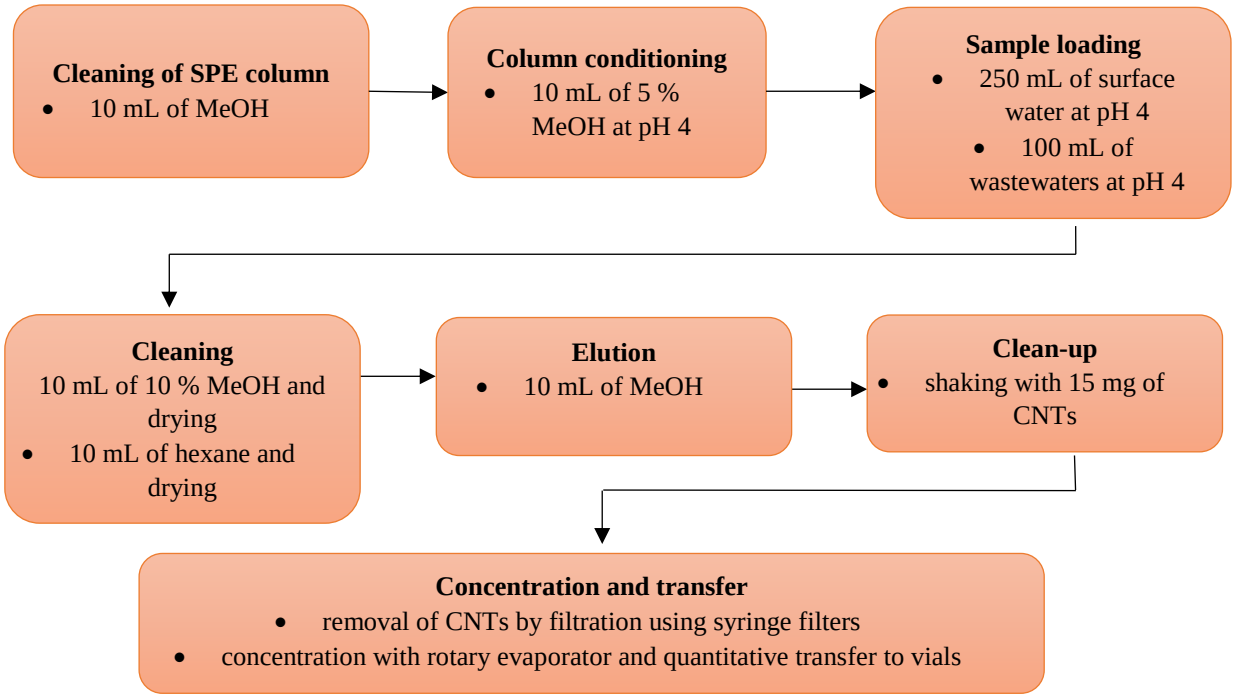


Figure 16 Final conditions of the developed SPE procedure

The evaluation was performed in two ways; first, surface water sample was collected from the Potok Oliwski stream a 100 m before the mouth of the stream, which runs through the Kaszuby region, including significant part of the city of Gdansk. It was spiked with the analytes on 5 levels of concentration, from 0.5 to 5 µg/L. The determined ME, EE and AR with SD for each concentration level and analyte are presented in the table below.

Table 18 The determined ME, EE and AR for the analytes in spiked surface water at five different concentration levels (from 0.5 to 5 µg/L) assessed using the developed LC-MS/MS with IT analyser method

Analyte/	**ME [%]**							**EE [%]**							**AR [%]**						
Concentration [µg/L]	**0.5**	**1**	**2**	**3**	**5**	**Av.**	**SD**	**0.5**	**1**	**2**	**3**	**5**	**Av.**	**SD**	**0.5**	**1**	**2**	**3**	**5**	**Av.**	**SD**
CBZ	-37	-43	-33	-40	-37	**-38**	3	89	97	95	89	96	**93**	3	56	55	64	53	60	**58**	4
CBZ-ep	-11	-19	-10	-17	-18	**-15**	4	112	111	105	93	108	**106**	7	100	90	94	77	89	**90**	7
10-OH-CBZ	-8	-24	-1	-11	-13	**-11**	8	108	105	77	91	104	**97**	12	80	80	76	82	90	**81**	5
2-OH-CBZ	-24	-10	0	-7	-11	**-11**	8	98	99	90	84	104	**95**	7	74	74	90	78	92	**82**	8
IBU	-54	-75	-49	-67	-34	**-56**	14	86	91	112	183	155	**126**	38	40	23	58	61	102	**57**	27
2-OH-IBU	-70	-61	-50	-52	-50	**-56**	8	68	84	89	84	88	**82**	8	21	33	44	40	44	**36**	9
MTZ	-60	-62	-56	-56	-50	**-57**	4	41	45	48	41	38	**43**	4	17	17	21	18	19	**18**	2
MTZ-OH	-20	-18	-30	-8	-19	**-19**	7	4	3	6	5	6	**5**	1	3	3	4	4	5	**4**	1
ac-SMX	-30	-40	-34	-49	-41	**-39**	6	102	98	94	91	93	**96**	4	71	59	62	47	55	**59**	8
4-OH-DIC	-95	-95	-96	-92	-91	**-94**	2	30	38	37	22	19	**29**	8	1	2	2	2	2	**2**	0
des-NPX	-62	-55	-47	-52	-46	**-52**	6	71	69	72	70	70	**71**	1	27	31	39	34	38	**34**	4
MTP	-43	-39	-39	-35	-30	**-37**	5	101	107	98	88	105	**100**	7	57	65	60	57	74	**62**	6
MTPA	-28	-23	-9	n.d.	-34	**-19**	13	61	75	85	n.d.	88	**62**	32	43	58	78	n.d.	58	**47**	26
cx-IBU	-	-	-89	-92	-93	**-91**	2	-	-	66	92	121	**93**	22	-	-	8	7	9	**8**	1

Av. – average; n.d. – not determined

As presented, even though ME varied quite strongly depending on the analyte (from -11 % up to -94 %), these values were at the same level regardless the analyte concentration in the investigated water (SD from 5 different points of concentration were below 10 % in many cases). Moreover, for most of the compounds the values were close or lower than -50 %, which is also very satisfactory. Furthermore, EE for most of the compounds was also satisfying (between 70 and 130 %) and repeatable among different concentration levels, which also resulted in similar results for AR. Only for MTZ-OH and 4-OH-DIC these values were very low. However, these results confirm that developed method was effective and reproducible in the wide range of selected analytes concentrations.

Second step of the evaluation of the method performance on surface water samples was its application to analysis of environmental samples to determine the ME, EE and AR for several surface water samples collected on the area of Gdansk, spiked with the analytes. It included three rivers (Motlawa, Radunia and Strzyza), one pond (Mlynski) and rainwater from city collector. The results are presented in the figures below.

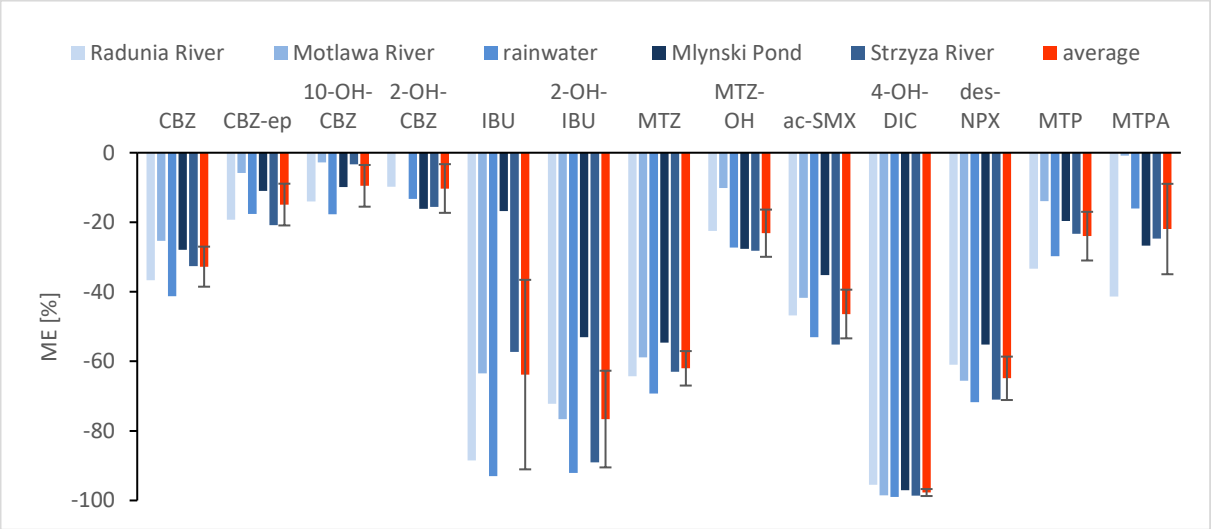


Figure 17 ME obtained for 5 different surface water samples and the average ME with SD as error bars

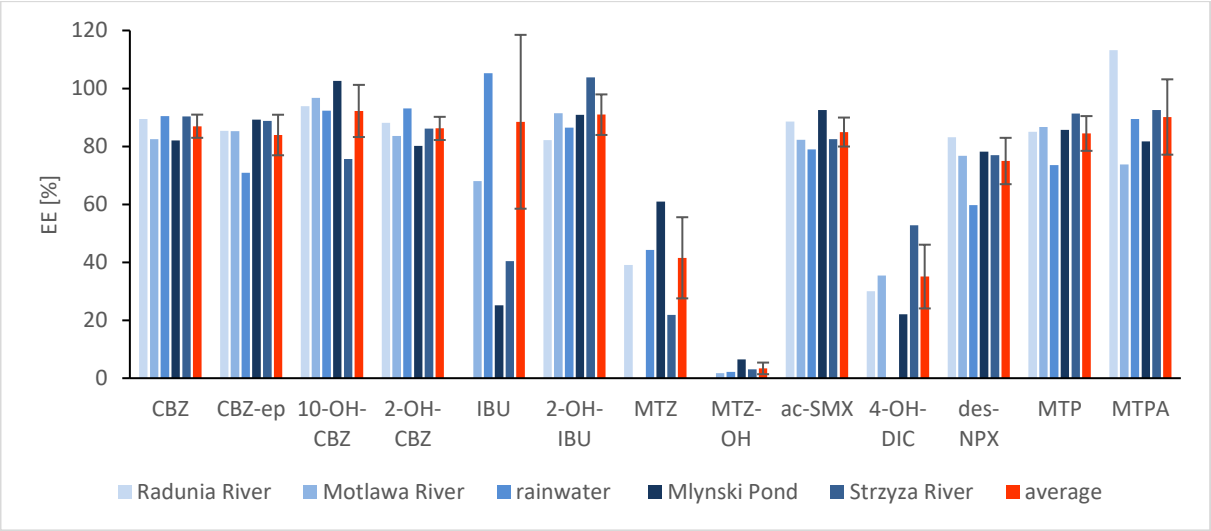


Figure 18 EE obtained for 5 different surface water samples and the average EE with SD as error bars

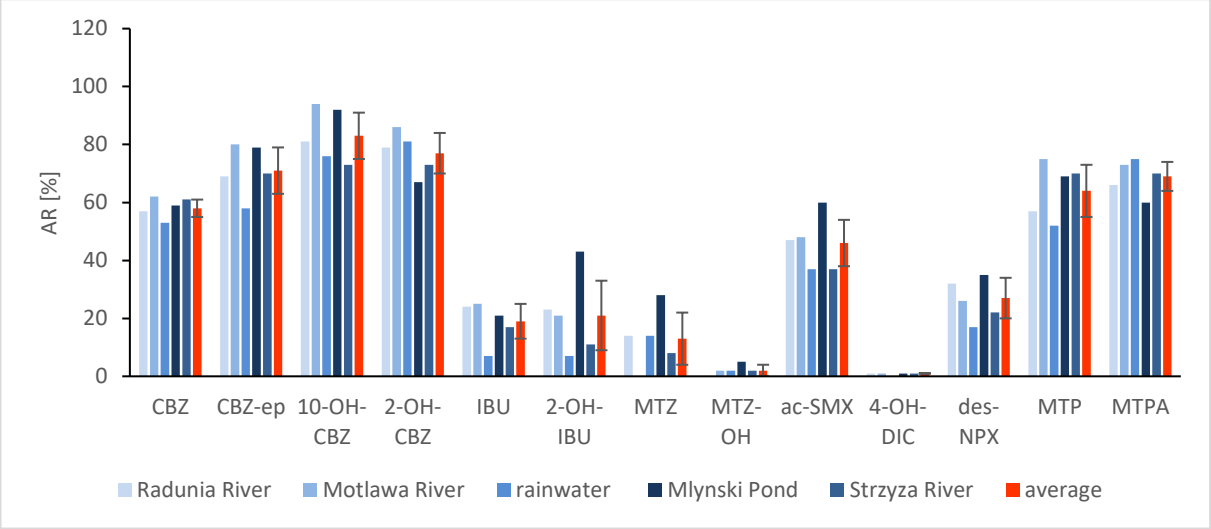


Figure 19 AR obtained for 5 different surface water samples and the average AR with SD as error bars

The presented results are very satisfying in terms of repeatability of obtained ME and EE (the SD of the results did not exceed 10 %) regardless the changes in the composition of the tested surface water samples including relatively clean River Strzyza or Mlynski Pond and stagnant rainwater, which by organoleptic evaluation was much more turbid, darker and with distinctive smell. Moreover, the average results of ME and EE overlap with previously presented results from the samples collected from the Potok Oliwski Stream (**Table 18**), which suggests that the developed method leads to repeatable and reliable results in these terms. It has been proven that calculation of ME is important to obtain reliable results. There are different analytical data available in the literature, which present different approach to present such results. Some papers do not include any sort of information regarding ME [236], other mention that ME were taken into consideration or that were observed, but there is no data presented on that [224,228,240,241]. These papers, which include ME values calculated during SPE-LC-MS/MS determination of the analytes,

provide very diversified numbers, like from -81 % to 1 % in WWTP influent and effluent [79], from -55 % to 19 % in storm water and WWTP effluent [196], from -27 % to -59 % for seawater [242]. These values are in accordance with results obtained in this study.

5.1.3.3 Validation characteristics of the developed analytical procedure based on LC-MS/MS with IT analyser for the determination of selected pharmaceuticals and their TPs

Finally, validation of the developed procedure was performed using water samples collected from Potok Oliwski Stream, which was spiked with the analytes in the range 0.5 – 5.0 μg/L (**Table 19**). The main validation parameters were acceptable only for some analytes (CV below 20 %, accuracy between 80 and 120 %). Those compounds which were poorly ionized like IBU or cx-IBU were characterised with low precision (CV) at lower concentration levels. Moreover, very poor validation parameters were obtained for MTZ-OH, which mainly results from its not efficient extraction from samples.

Table 19 Validation parameters of the developed analytical procedure based on the application of LC-MS/MS system with IT analyser for the determination of pharmaceuticals and their TPs in surface waters

Analyte	R^2	Linearity range [μg/L]	Precision (CV) [%]	Accuracy [%]	LOQ [μg/L]	LOD [μg/L]
CBZ	0.983	0.5-3.0	1.5-7.8	88.3 - 111.8	0.5	0.2
CBZ-ep	0.995	0.5-5.0	4.4- 10.6	82.3 - 111.2	0.5	0.2
10-OH-CBZ	0.997	0.5-5.0	2.9-10.4	74.0 - 107.8	0.5	0.2
2-OH-CBZ	0.995	0.5-5.0	2.3-10.8	70.4 - 110.3	0.5	0.2
IBU	0.995	0.5-5.0	0.9-21.0	75.2 - 110.1	0.5	0.2
2-OH-IBU	0.998	0.5-5.0	2.7-7.7	95.0 - 105.4	0.5	0.2
MTZ	0.990	0.5-5.0	4.4-23.3	84.8 - 113.1	0.5	0.2
MTZ-OH	0.941	2.0-5.0	4.9-28.2	63.1 - 131.0	2.0	0.7
ac-SMX	0.993	0.5-5.0	1.8-7.8	70.3 - 112.3	0.5	0.2
4-OH-DIC	0.998	1.0-5.0	6.5-10.4	97.1 - 104.0	1.0	0.3
des-NPX	0.999	0.5-5.0	1.8-5.4	93.7 - 107.7	0.5	0.2
MTP	0.994	0.5-5.0	1.6-8.5	69.4 - 106.7	0.5	0.2
MTPA	0.966	1.0-5.0	3.5-10.7	75.2 - 122.3	1.0	0.3
cx-IBU	0.992	2.0-5.0	11.8-29.0	95.0 - 105.1	2.0	0.7

During the development of the entire SPE method, at each stage real WWTP effluent water samples were used as a matrix (as blank samples). Therefore, during their analysis some of the investigated analytes were detected. The frequency of detection is presented in the figure below.

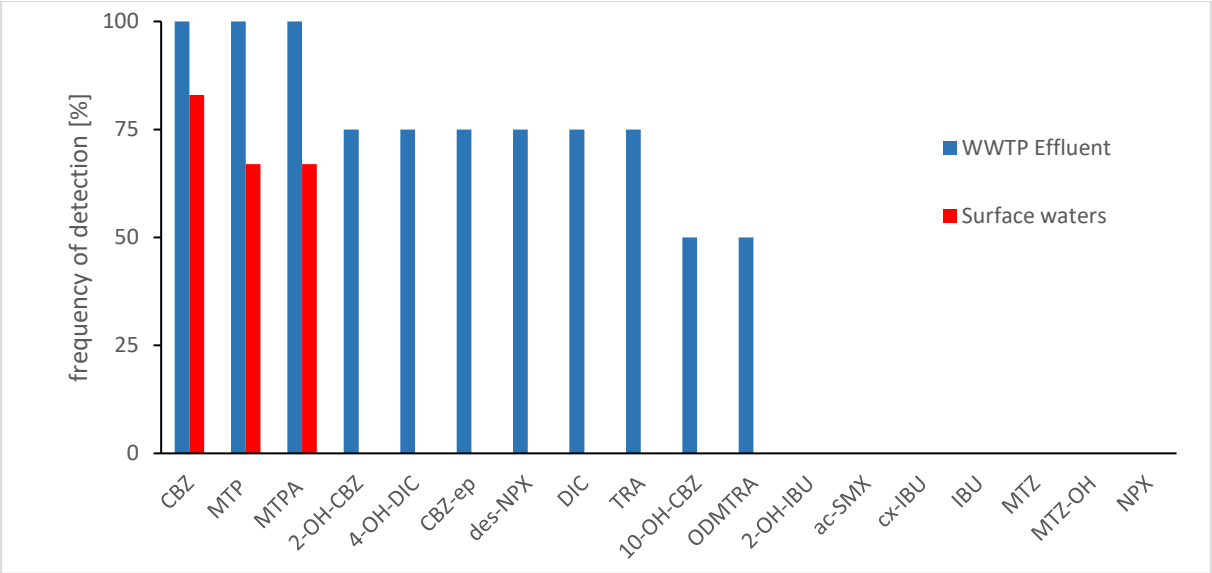


Figure 20 The frequency of detection (n=4) of the investigated analytes in WWTP effluent samples during SPE procedure development as well as for surface waters (n=6) during method evaluation

Such detections confirm, that most of the investigated analytes can be found in real water samples. High frequency of detection for CBZ and its TPs in wastewaters are in accordance with the literature data, which provides large number of reports confirming their presence in such matrix [71,76,222]. Nevertheless, MTP, DIC and their TPs are also quite frequently detected [86,95]. On the other hand, O-DMTRA is far less investigated regarding its presence in wastewaters, but for example it was found in Croatia [97]. Moreover, three analytes were found in the investigated surface water samples, namely CBZ, MTP and MTPA. However, their concentrations were below LOQ.

Taking into account the obtained results from the validation and observed problems during the analysis of different environmental samples in terms of: precision for selected analytes; segment/time regime to monitor all selected MRM transitions (any slight time shift could cause the loss of some analytes) and hence the necessity to perform one analysis for 38 minutes; slow scanning and in consequence only several determination points for drawing the peaks influencing the precision and repeatability; it became obvious that faster, more sensitive and selective technique should be applied for this purpose. Therefore, it was decided to transfer this method to another, brand new (that became available) LC-MS/MS system with the most often utilised analyser in pharmaceutical analysis – triple quadrupole (QqQ), which is described in detail below.

5.1.4 The developed analytical method based on LC-MS/MS with QqQ analyser

5.1.4.1 The characteristics of the developed analytical method based on LC-MS/MS with QqQ analyser

At the beginning, solutions of single analytes were prepared and automated process of the selection of each quadrupole voltage (including collision energy) as well as fragmentation ions for each analyte that give the most intensive and well-shaped signals was processed. It was done with two different mobile phases: ACN/H_2O + 1 mM ammonium acetate buffer (pH 3.8) and ACN/H_2O + 0.1 % HCOOH. However, the intensities of most of the analytes were higher with ACN/H_2O + 0.1 % HCOOH phase, therefore, this one was chosen for further development. Various gradient eluent programs were tested to obtain the shortest method which included all analytes, from the most polar MTZ-OH to DIC, which elutes as a last one. The most challenging task which occurred again, was to separate 2-OH-CBZ and CBZ-ep from each other, due to their identical precursor ion *m/z* and the fragmentation ions. Even though 2 out of 3 fragmentation ions chosen for them were different in final method, the same ions are found for both of them, there is just a difference of intensities. Due to the mass spectrometer operational process, all analytes don not have to be separated chromatographically from each other, which allows to prepare short methods for the mixture of the compounds. The equipment used in this study provides very fast scanning, which is necessary in such case. Moreover, the method was developed with a mobile phase flow of 0.4 mL/min. Slower flow, 0.3 mL/min was also investigated, but the shapes of the peaks, surface areas as well as overall time of the analysis was worse, so the initial flow of 0.4 mL/min was selected. Initial compositions of mobile phase was 10 % of ACN + 0.1 % HCOOH (phase B) and 90 % H_2O + 0.1 % HCOOH (phase A). There was a steady upward slope for 6.5 min up to 70 % of phase B, constant conditions until 7^{th} min and then comeback to initial conditions for one minute. Next, additional 2 minutes were sacrificed for column conditioning, which all resulted in 10 min of entire method. In total, 14 analytes were transferred and included in this method. The parameters of the method are presented in the table below, whereas the exemplary chromatogram in the **Figure 21**.

Table 20 The parameters of the analytical method developed on the LC-MS/MS with QqQ analyzer

Analyte	Polarity	Precursor *m/z*	Products *m/z*	Q1 [eV]	CE [eV]	Q3 [eV]	Dwell time [ms]	RT [min]
CBZ	+	237.0	194.0 193.0 220.0	-12 -11 -11	-20 -34 -24	-20 -20 -19	21 21 21	4.46
CBZ-ep	+	253.1	180.0 236.0 210.1	-12 -12 -28	-27 -12 -16	-12 -16 -22	21 21 21	3.74
10-OH-CBZ	+	255.0	194.1 237.1 193.1	-20 -24 -12	-21 -11 -37	-22 -24 -20	21 21 21	3.32
2-OH-CBZ	+	253.1	210.1 208.0 167.1	-12 -12 -12	-20 -24 -38	-21 -21 -17	21 21 21	3.52
cx-IBU	-	257.0	213.3 189.4 211.3	10 13 14	14 26 19	14 12 13	21 21 21	4.11
TRA	+	264.0	58.0 246.0	-13 -13	-22 -11	-23 -23	34 34	2.92
O-DMTRA	+	250.1	58.0 232.0	-12 -12	-16 -16	-22 -22	34 34	2.12
MTZ	+	172.1	128.1 82.1 42.1	-12 -19 -19	-16 -25 -45	-13 -15 -16	21 21 21	1.64
MTZ-OH	+	188.1	123.0 126.2 144.0	-15 -14 -15	-3 -18 -6	-30 -22 -30	21 21 21	1.33
ac-SMX	+	295.9	134.2 198.0 65.1	-14 -14 -15	-25 -19 -42	-13 -20 -27	21 21 21	3.65
DIC	+	296.0	214.0 214.9 249.9	-22 -22 -22	-33 -19 -14	-14 -21 -25	21 21 21	6.40
4-OH-DIC	+	312.0	230.0 231.0 265.9	-23 -12 -24	-33 -21 -14	-15 -16 -18	21 21 21	5.34
MTP	+	268.1	116.1 133.1 74.2	-13 -13 -13	-20 -25 -22	-11 -13 -14	21 21 21	2.85
MTPA	+	268.0	145.1 191.0 226.1	-13 -13 -13	-24 -19 -17	-14 -19 -15	21 21 21	1.99

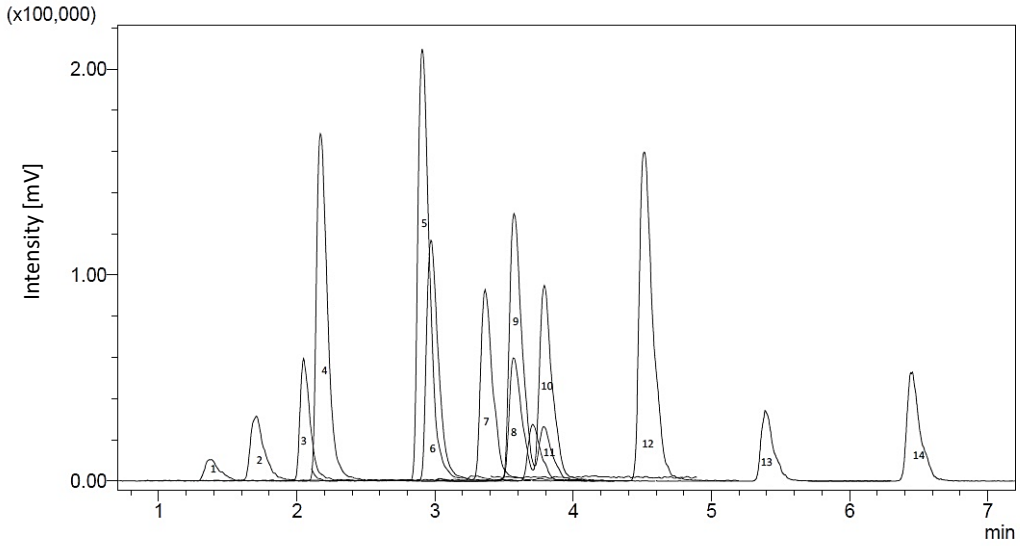


Figure 21 Chromatogram obtained for pharmaceuticals and their TPs included in the analytical method at the concentration of 10 µg/L with the application of LC-MS/MS technique with QqQ analyzer (1 – MTZ-OH, 2 – MTZ, 3 – MTPA, 4 – O-DMTRA, 5 – MTP, 6 – TRA, 7 – 10-OH-CBZ, 8 – 2-OH-CBZ, 9 – ac-SMX, 10 – CBZ-ep, 11 – cx-IBU, 12 – CBZ, 13 – 4-OH-DIC, 14 – DIC)

It should be highlighted, that the developed method is short (total of 10 minutes), especially in comparison with the previously described method with the LC-MS/MS equipment with IT (38 minutes). Moreover, minimum two fragmentation ions were obtained for each analyte, which is in accordance with the general standards in analytical chemistry [243]. Fast scanning ensured the good quality of the peaks and repeatability of their surface areas.

5.1.4.2 Instrumental validation and evaluation of method performance on the environmental samples

The proposed new instrumental method based on the LC-MS/MS with QqQ analyser was also fully validated (**Table 21**). In general, the method is characterized with low IQL levels and very satisfying precision (below 5 %). It proves reliability of the equipment in terms of repeatability of the injections volume, stable ionization and efficient work of the detector, even at low concentration levels. Moreover, the IDL and IQL values are much better (for most analytes below 1.00 µg/L) than those obtained with LC-MS/MS with IT (**Chapter 5.1.2**), which were from 0.5 µg/L (only CBZ) up to 50.0 µg/L.

Table 21 Basic parameters of the instrumental validation with the developed method based on the LC-MS/MS with QqQ analyzer

Analyte	R^2	Linearity range [µg/L]	Precision (CV) [%]	Accuracy [%]	IQL [µg/L]	IDL [µg/L]
CBZ	1.000 1.000	0.10-2.00 5.00-50.00	0.6-3.7 0.6-1.6	83.7-103.4 90.5-102.8	0.10	0.03
CBZ-ep	0.999 0.999	0.10-2.00 5.00-100.00	2.0-4.1 1.2-2.7	80.5-103.4 81.0-102.5	0.10	0.03
10-OH-CBZ	1.000 0.999	0.10-2.00 5.00-100.00	2.5-4.5 1.0-1.4	88.0-104.6 82.4-105.3	0.10	0.03
2-OH-CBZ	1.000 1.000	0.10-2.00 5.00-50.00	1.4-4.3 0.9-1.2	85.7-104.6 91.5-103.2	0.10	0.03
cx-IBU	1.000	20.00-200.00	2.2-2.7	92.7-103.1	20.00	6.67
TRA	1.000 0.999	0.10-5.00 10.00-200.00	1.4-3.2 0.5-0.9	82.0-103.1 82.1-130.8	0.10	0.03
O-DMTRA	0.999 1.000	0.05-1.00 2.00-50.00	1.3-3.3 1.2-2.0	85.9-108.9 83.9-103.1	0.05	0.02
MTZ	1.000 1.000	0.50-10.00 10.00-200.00	1.1-4.3 0.5-1.7	84.1-105.5 82.7-103.6	0.50	0.17
MTZ-OH	1.000	1.00-100.00	0.9-4.4	86.0-102.7	1.00	0.33
ac-SMX	1.000	0.50-50.00	0.6-4.7	82.3-104.6	0.50	0.17
DIC	1.000 1.000	0.50-20.00 10.00-200.00	0.6-3.3 0.3-1.3	96.0-114.7 97.4-114.5	0.50	0.17
4-OH-DIC	1.000	1.00-200.00	0.5-1.7	98.3-112.2	1.00	0.33
MTP	1.000 0.999	0.10-2.00 5.00-100.00	1.6-2.8 0.4-1.1	87.5-104.2 84.1-104.2	0.10	0.03
MTPA	1.000	0.50-100.00	0.5-4.9	81.3 - 103.9	0.50	0.17

Afterwards, the determination method was evaluated in terms of the ME, EE and AR for selected pharmaceuticals and their TPs in five different water samples collected during 2 campaigns in December 2021 and February 2022 in the area of village Rybno (samples from Zarybinek Lake collected only in December) as described in **Chapter 4.5.3**. Moreover, sample collected from Zagorska Struga River, located near the town Rumia in the Pomerania region was also included. The obtained results are presented in the **Table 22**.

Table 22 The values of ME, EE and AR obtained for the analytes in different spiked environmental water samples using LC-MS/MS with QqQ analyser method

Analyte	WWTP Effluent		Wel River before WWTP discharge		Wel River after WWTP discharge		Pond next to fish farm		Zarybinek Lake	Zagorska Struga River
	Dec.	Feb.	Dec.	Feb.	Dec.	Feb.	Dec.	Feb.	Dec.	
ME [%]										
MTPA	55	85	9	4	12	14	41	30	19	1
MTZ-OH	-20	-10	-14	-12	0	-1	-5	2	1	-13
MTZ	-31	-18	-25	-23	-14	-15	-19	-8	-14	-17
O-DMTRA	91	109	2	-6	3	0	31	16	12	-29
MTP	-23	5	-7	-7	-1	5	15	7	7	-6
TRA	93	183	-24	-15	-20	-8	-2	-7	-11	-18
10-OH-CBZ	2	-25	-4	3	6	12	1	17	15	5
2-OH-CBZ	-38	-7	-37	-28	-29	-21	-33	-16	-32	-17
ac-SMX	-32	-31	-23	-19	-10	-9	-27	-18	-9	-14
CBZ-ep	-13	40	4	30	22	37	18	42	27	6
CBZ	100	57	-13	-7	1	12	-24	-1	-1	-2
4-OH-DIC	29	13	-50	-28	-45	-19	-54	-43	-41	-34
DIC	-3	-2	-72	-28	-64	-9	-75	-66	-50	-68
EE [%]										
MTPA	50	42	47	35	31	37	49	41	28	70
MTZ-OH	19	5	8	4	8	6	8	0	6	3
MTZ	99	73	74	58	71	63	59	33	69	60
O-DMTRA	107	75	110	79	83	89	99	67	93	104
MTP	79	62	74	54	67	62	68	58	63	103
TRA	64	79	108	70	n.d.	85	84	71	89	106
10-OH-CBZ	149	104	113	86	106	91	100	73	102	109
2-OH-CBZ	111	79	106	80	102	91	95	71	96	106
ac-SMX	96	73	94	69	89	80	91	66	83	102
CBZ-ep	104	89	101	84	106	89	99	75	101	110
CBZ	118	93	114	59	94	101	111	65	107	92
4-OH-DIC	107	56	40	32	40	33	50	56	27	80
DIC	94	23	54	138	41	21	67	100	40	84
AR [%]										
MTPA	77	78	51	37	34	42	69	53	33	71
MTZ-OH	17	4	7	3	8	6	7	0	6	3
MTZ	68	60	55	44	61	54	48	30	59	50
O-DMTRA	204	156	111	74	86	89	130	78	103	74
MTP	61	65	69	50	67	65	78	62	67	97
TRA	123	224	81	59	n.d.	78	83	66	79	87
10-OH-CBZ	152	78	108	89	113	102	101	86	117	114
2-OH-CBZ	69	73	67	58	72	72	64	60	65	88
ac-SMX	66	50	73	56	80	73	66	54	76	87
CBZ-ep	91	125	108	108	129	122	117	107	129	116
CBZ	237	145	99	55	95	113	84	65	106	98
4-OH-DIC	137	63	20	23	22	27	23	32	16	53
DIC	91	23	15	99	15	19	17	33	20	71

It can be seen that there are some differences in the values between different months of analysing; this can be due to the matrix changes through the 2 months which separate these 2 campaigns. The biggest variability is observed for the wastewaters, which is not surprising. It is the most complex matrix, reach with the interferents and very variable. Moreover, the detector is sensitive and big load of ions can sometimes lead to differential results. It proves that such analyses should be performed for each sample that is a subject of an analytical study. However, at the same time such equipment can provide good sensitivity, which is crucial for detection of trace amounts of the analytes. Nevertheless, the transfer from the LC-MS/MS wit IT analyser was needed and it was made successfully. The analytical procedure still can be utilised for analyses of the environmental samples, especially those with very complex matrix, like WWTP effluent. Due to the way of work of the QqQ analyser, the target ions can be extracted from whole mixture of other ions and presented as single peak, without interfering signals corresponding to the other compounds present in the sample. The ME for many analytes in such matrix were low, for MTZ-OH, MTZ, MTP, 10-OH-CBZ, 2-OH-CBZ, CBZ-ep, ac-SMX, DIC and 4-OH-DIC did not exceed 40 % (the absolute value). Moreover, the ME in surface waters, where much less analytes were observed, were at low level for vast majority of the compounds, with average value of 13 % for all measured analytes in each sample when the enhancement was observed and average 22 % when the suppression was observed. However, even if the ME were high, it was still possible without a problem to observe and calculate the results, due to the high sensitivity of the method, while with the IT analyser, with high ME the it was difficult to observe the signals at lower levels of concentration.

5.1.4.3 Validation of developed analytical procedure

Afterwards, the determination method was validated for two different matrices: the surface water collected from Zarybinek Lake (**Table 23**) and WWTP effluent collected in Rybno (**Table 24**). The range of concentrations for the WWTP effluent was from 5.0 to 2 000.0 ng/L (6 calibration points), whereas for surface water from 0.2 to 200.0 ng/L (6 calibration points).

Table 23 Validation parameters of the developed analytical procedure based on the application of LC-MS/MS system with QqQ analyser for the determination of pharmaceuticals and their TPs in surface waters

Analyte	R^2	Linearity range [ng/L]	Precision (CV) [%]	Accuracy [%]	LOQ [ng/L]	LOD [ng/L]
CBZ	0.999	20.0-200.0	1.5-6.9	98.6-102.4	20.0	6.7
CBZ-ep	0.999	4.0-200.0	1.5-9.5	92.0-102.4	4.0	1.3
10-OH-CBZ	0.999	4.0-200.0	3.7-9.2	89.4-105.6	4.0	1.3
2-OH-CBZ	0.999	4.0-200.0	2.4-9.5	99.4-102.5	4.0	1.3
TRA	0.999	4.0-200.0	1.0-3.4	71.7-104.8	4.0	1.3
O-DMTRA	0.999	4.0-200.0	1.3-4.9	84.7-118.5	4.0	1.3
MTZ	0.999	4.0-200.0	1.5-7.3	82.8-118.9	4.0	1.3
ac-SMX	0.999	20.0-200.0	1.0-5.1	87-1-107.2	20.0	6.7
DIC	0.999	20.0-200.0	1.7-10.9	82.1-110.0	20.0	6.7
4-OH-DIC	0.997	20.0-200.0	4.5-5.9	83.4-109.2	20.0	6.7
MTP	0.999	4.0-200.0	1.2-6.0	76.0-115.3	4.0	1.3
MTPA	0.996	20.0-200.0	0.2-7.8	70.7-116.4	20.0	6.7

Table 24 Validation parameters of the developed analytical procedure based on the application of LC-MS/MS system with IT analyser for the determination of pharmaceuticals and their TPs in wastewaters

Analyte	R^2	Linearity range [ng/L]	Precision (CV) [%]	Accuracy [%]	LOQ [ng/L]	LOD [ng/L]
CBZ	0.998	100.0-2000.0	3.5-9.7	84.6-110.4	100.0	33.3
CBZ-ep	1.000	50.0-2000.0	1.5-4.5	94.8-102.6	50.0	16.7
10-OH-CBZ	1.000	50.0-2000.0	6.9-8.4	94.7-102.7	50.0	16.7
2-OH-CBZ	1.000	50.0-2000.0	0.5-4.9	85.3-107.5	50.0	16.7
TRA	1.000	50.0-500.0	0.30-2.7	96.9-101.7	50.0	16.7
O-DMTRA	0.995	100.0-2000.0	5.8-9.8	87.7-115.8	100.0	33.3
MTZ	1.000	50.0-2000.0	2.7-7.9	88.7-105.7	50.0	16.7
ac-SMX	1.000	50.0-2000.0	2.6-8.8	97.6-101.2	50.0	16.7
DIC	1.000	5.0-100.0	6.5-16.0	98.9-105.3	5.0	1.6
4-OH-DIC	1.000	100.0-2000.0	4.2-18.7	89.2-102.7	100.0	33.3
MTP	1.000	50.0-2000.0	0.0-0.1	90.4-104.9	50.0	16.7
MTPA	1.000	10.0-100.0	3.8-7.4	97.7-119.1	10.0	3.3

The validation parameters in lake water are mostly satisfactory, with precision not exceeding 15-20 % and accuracy around 80 – 120 %. The LOQ at the level 4.0 – 20.0 ng/L are quite low and such method can be definitely applied for trace analysis. The results for the wastewater in terms of accuracy are very satisfactory. The precision is also acceptable. The linearity range in such complex matrix is really broad and the LOQ values around 50.0 ng/L for most of the analytes ensure determination of many of them, since they are frequently detected at such levels in WWTP effluents. Literature provides different values of detection limits of pharmaceuticals obtained with LC-MS/MS technique; for example, LOQ calculated by Grujić et al. for surface water was between 0.5 – 41.5 ng/L [226], by Fick et al. between 10 and 50 ng/L [244] and by Garcia-Galan et al.

between 0.1 to 244 ng/L [76]. It can be concluded that the LOQ values obtained in this study (4.0 – 20.0 ng/L) for surface water is in accordance with the literature data. In the case of more complex matrix, like WWTP effluent, the literature LOQ values are very different. For example, LOQ in wastewater effluent was between 920 and 1 950 ng/L for tetracycline degradation products [245], whereas for various anticancer drugs between 9 and 260 ng/L [246]. The results obtained in this study (LOQ for WWTP effluent between 5.0 and 100.0 ng/L) are either in line with these described above or even better. However, there are some analytical methods which provide much higher LOQ values, like between 1 180 and 4 115 ng/L for statins and fibrates in wastewaters and river [130]. The precision values available in the literature also vary, they are in the range from less than 1 % to almost 40 % [75,76,247], which is in accordance with our results. Therefore, it can be concluded that developed analytical method is satisfactory and can be used with success for the determination of pharmaceuticals and their TPs in different water samples.

5.1.4.4 Presence of the selected pharmaceuticals and their transformation products in the investigated environmental samples

The results obtained from the analysis of various water samples in terms of their contamination by the selected in this doctoral thesis pharmaceuticals and their transformation products are presented in **Table 25** and **Table 26**.

Table 25 The data on presence and concentration of investigated analytes in various water samples collected in two campaigns near the Rybno village in northeast Poland

Analyte	WWTP Effluent		Wel River after WWTP discharge		Wel River before WWTP discharge		Pond next to fish farm		Zarybinek Lake
	Dec.	Feb.	Dec.	Feb.	Dec.	Feb.	Dec.	Feb.	Dec.
MTPA	362 ± 65	997 ± 33	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
MTZ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
O-DMTRA	$7.7 \times 10^2 \pm 1.0 \times 10^2$	715 ± 21	7.3 ± 1.3	8.3 ± 2.1	<LOQ	<LOQ	n.d.	n.d.	<LOQ
MTP	588 ± 17	271.8 ± 3.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ
TRA	$29.2 \times 10^2 \pm 1.4 \times 10^2$	1 467 ± 34	4.8 ± 1.2	21.84 ± 0.91	<LOQ	6.2 ± 1.6	<LOQ	n.d.	<LOQ
10-OH-CBZ	815 ± 59	702.6 ± 6.0	4.1 ± 1.1	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
2-OH-CBZ	243.1 ± 2.1	184.8 ± 8.8	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
ac-SMX	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CBZ-ep	267.5 ± 6.3	503 ± 11	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	<LOQ
CBZ	$12.2 \times 10^2 \pm 1.2 \times 10^2$	1 458 ± 29	90.4 ± 2.1	78.3 ± 2.3	76.6 ± 1.6	152.8 ± 2.9	70.4 ± 3.2	129.9 ± 5.8	66.8 ± 5.8
4-OH-DIC	349 ± 70	618.7 ± 6.4	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
DIC	689 ± 71	3 552 ± 31	21.6 ± 2.5	41 ± 19	n.d.	n.d.	<LOQ	n.d.	n.d.

n.d. – not determined, <LOD

Table 26 The data on presence and concentration of investigated analytes in various water samples

Analyte	Zagorska Struga 100 m downstream fish farm	Zagorska Struga 1000 m downstream fish farm	Motlawa River	WWTP influent Gniewino	WWTP effluent Gniewino	WWTP influent Gdansk "Wschod"	WWTP effluent Gdansk "Wschod"
MTPA	<LOQ	<LOQ	90 ± 13	521.6 ± 2.6	151.9 ± 1.4	3 182 ± 57	373.4 ± 5.2
MTZ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	131.8 ± 3.9
O-DMTRA	<LOQ	<LOQ	11.4 ± 1.3	129.7 ± 1.4	465.5 ± 7.1	320.9 ± 9.4	610.7 ± 7.8
MTP	<LOQ	n.d.	<LOQ	144.8 ± 1.5	238.2 ± 3.4	817 ± 12	813.7 ± 9.3
TRA	<LOQ	<LOQ	108.7 ± 8.1	604 ± 4.6	1 823 ± 11	1 872 ± 34	2 304 ± 16
10-OH-CBZ	<LOQ	n.d.	5.3 ± 2.3	1 122 ± 15	3 661 ± 37	1 186 ± 11	1 861 ± 31
2-OH-CBZ	n.d.	n.d.	<LOQ	163.2 ± 1.6	378.7 ± 4.1	296 ± 17	247.0 ± 2.5
ac-SMX	n.d.	n.d.	n.d.	86.4 ± 4.3	n.d.	690 ± 61	<LOQ
CBZ-ep	n.d.	n.d.	n.d.	119.9 ± 1.8	256.6 ± 2.6	124 ± 73	88.7 ± 1.6
CBZ	<LOQ	<LOQ	64 ± 11	585.2 ± 4.6	2 134.0 ± 4.2	$12.3\times10^2 \pm 1.3\times10^2$	1 564 ± 11
4-OH-DIC	n.d.	n.d.	n.d.	267 ± 20	254.9 ± 1.6	1 185 ± 36	419.4 ± 5.7
DIC	n.d.	<LOQ	n.d.	490 ± 81	1 329 ± 16	$54.9\times10^2 \pm 5.6\times10^2$	2 010 ± 27

n.d. – not determined,<LOD

The investigated compounds have been detected frequently in the wastewaters and in some cases in natural waters. The concentrations of some of them reach the level of μg/L. Samples collected from the Rybno area show a clear pattern from being the most concentrated in the WWTP effluent and then diluted after they get to the river. The further from the discharge point the less number of detected compounds. The highest concentrations in the WWTP effluent from Rybno were found for TRA, CBZ and DIC. However, all of the investigated compounds besides 4-OH-DIC were found and the level of concentration was counted in hundreds of ng/L. After the discharge of the effluent to the river, the most abundant compounds were still found, but the concentrations were lower than 100 ng/L. In waters which do not receive such load, only CBZ concentration was above the LOD and LOQ of the method. Moreover, high concentrations of the analytes in wastewaters in Rybno are not an exception; similar matrix from other WWTP have also high loads of pharmaceuticals and their TPs. It shows how inefficiently purified wastewaters are disposed into environment. The fact that majority of the PTPs have been detected indicates, that the theoretical dispute on their possible presence in the environment due to high rates of metabolic changes of the PTs is confirmed. What is alarming, some analytes were determined in Motlawa River up to around 100 ng/L. The river which is only 65 km long, but runs through large number of towns and finally Gdansk, evidently collects some loads of pollutants throughout its course. In small stream Zagorska Struga none of the analytes were determined above the LOQ. Nevertheless, high concentrations of the analytes in wastewaters are not surprising if compared to literature data (**Table 3**, **Chapter 2.1.4**). In many cases, concentration of pharmaceuticals like CBZ, DIC or even their metabolites were found on the levels of hundreds ng/L or several μg/L [71,75,76,88,89,93]. Therefore, it was confirmed that wastewaters collected in Poland and analysed in this study are also reach in such pollutants and even after wastewater treatment they are still present in the effluent, which is introduced to the environment. Such process provides the environmental waters continuously with pharmaceutically active compounds and anthropogenic markers. CBZ is a fair choice for such marker of anthropogenic pollution, due to the presence in most of the investigated samples. It was also noticed by worldwide analytics, because CBZ is one of the 11 most frequently detected endocrine disrupting compounds in water samples [165]. High amounts of TRA and even its metabolite O-DMTRA are more difficult to explain, because this is strong analgesic, which is mostly prescribed for severe pain. However, it is an opioid drug, which influences the uptake of norepinephrine and serotonin [248]. Therefore, it has been also used in some cases of anxiety and depression [23]. However, organic compounds with these characteristics are also used as a recreational drugs of abuse, which may contribute for the overall presence of this compounds and its TP in wastewaters.

5.2 Evaluated hydrolytic stability of selected pharmaceuticals and their TPs

Initial investigation of hydrolytic stability for CBZ, 10-OH-CBZ, 2-OH-CBZ, CBZ-ep, IBU, 2-OH-IBU, cx-IBU, TRA, O-DMTRA, MTZ-OH, ac-SMX, 4-OH-DIC, CP, IF, 5-FU, MTX and 7-OH-MTX was based on a preliminary test, which was aimed at separating hydrolytically unstable compounds from those resistant to hydrolytic transformation. The determined unstable analytes were then subjected to the longer, extended tests, which resulted in more specific description of their hydrolysis process. These experiments were based on OECD Guideline 111 [218], to perform easily reproducible studies for building the foundations of the database regarding this topic, so the results could be easily compared and verified. Nowadays, the available results in this area for pharmaceuticals are inconsistent; the methodology of hydrolytic stability tests are divergent, which makes them difficult to compare with each other and draw conclusions, especially in terms of stability in the environmental compartments. Moreover, the data for PTPs is very limited (the available data is presented in the **Chapter 2.1.2.3**). Therefore, it is crucial to perform studies with unified methodology to easily determine which compounds are susceptible to hydrolysis and which are stable in water. Such results can be used for the assessment of environmental threat posed by these compounds as well as to gather knowledge on the most persistent pharmaceuticals and their TPs, which may prioritise the determination of the target compounds in environmental samples. Moreover, such studies provide information if the hydrolytic stability of the PTPs is different from their PCs. The obtained results presented in this chapter were published in two publications [119,249].

5.2.1 The assessment of hydrolytic stability in the preliminary test

After 5 days of incubation in the dark at 50 °C at 3 different pH (4, 7 and 9), most of the 17 compounds remained stable (**Figure 22**). The threshold of the instability was established as 10 % (according to the OECD 111 [218]) of the decrease of the initial amount of the compound. All of the analytes, which degradation did not exceed 10 %, were recognized as stable and their $t_{1/2}$ might be estimated as higher than 1 year at 25 °C. Nevertheless, noticeable, but not very high degradation was observed for MTZ-OH at pH 9 (27 %), 4-OH-DIC at pH 4 (13 %) and pH 9 (26 %). Higher degradation levels were found for IF, which was the most susceptible for hydrolysis at pH 4 (50 %), whereas at pH 7 (22 %) and 9 (27 %) it was much more stable. On the other hand, its analogue, CP, was degraded completely at pH 4 and 9 and almost completely (over 90 %) at pH 7. Nevertheless, it confirmed pH-dependency of these compounds regarding hydrolytic

stability. Moreover, equally great degradation was observed for CBZ-ep, but only at acidic conditions.

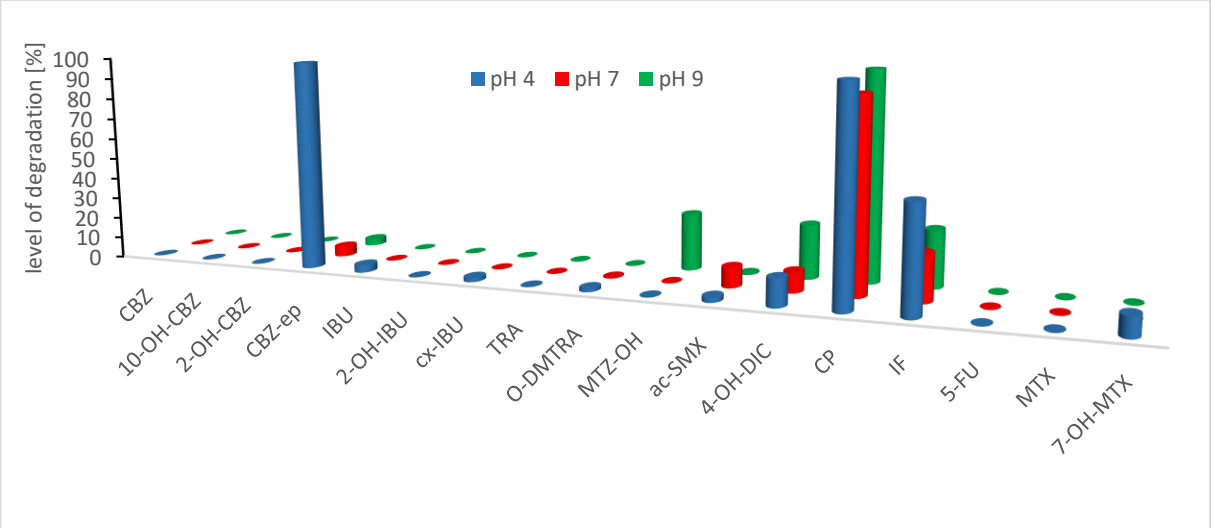


Figure 22 The results of preliminary investigation of the hydrolytic stability

The analytes tested in this study have not yet been subjected to hydrolytic stability studies according to the OECD 111. Nevertheless, there is some data on other pharmaceuticals that have been studied according to the same procedure. Various sulfonamides were found the most stable at pH 9, whereas the degradation to some extent was observed at pH 4 (but only 10 – 20 %) in the preliminary test. Moreover, it might be also concluded that the TPs of sulfamethoxazole, ac-SMX, investigated in this study was more resistant to the hydrolysis than its PC, which was investigated in the previously mentioned study of Białk-Bielińska et al. [54].

The knowledge on the analytes investigated in this study is limited, especially for the TPs. The stability of popular medicaments like IBU, CBZ, TRA, 5-FU, MTX or NPX was confirmed in different variants of temperature, containers, matrix (like saline or 5 % dextrose in water) and concentrations levels [248,250–256]. Nevertheless, all these tests aimed at investigation of their stability as typical dose of the pharmaceutical to be administered for a patient. However, these results confirm the data obtained in this study. Also in the study of Li et al., high stability of CBZ, NPX and TRA was confirmed, which is in accordance with our study [50]. In general, this study provided for the first time standardized data in terms of hydrolytic stability of many pharmaceuticals and especially their TPs, according to the international guideline. Such results can enhance the knowledge on environmental fate of the investigated compounds and attribute to selection of the most persistent compounds.

5.2.2 Extended investigation of the hydrolysis process for the unstable compounds

Each of the analytes that degraded more than 10 % in the preliminary investigation at certain pH was subjected to extended tests. They were conducted at 20, 50 and 70 °C in the dark for 30 days or until 90 % of the degradation observed. First, the function of ln c_t/c_0 of time was drawn (**Figure 23**). From the equation of linear function for each compound, temperature and pH, the degradation rates (k) and half-lives of the compounds ($t_{1/2}$) were calculated. Moreover, obtained degradation rates were used to calculate the activation energy (E_a) of the process (**Table 27**).

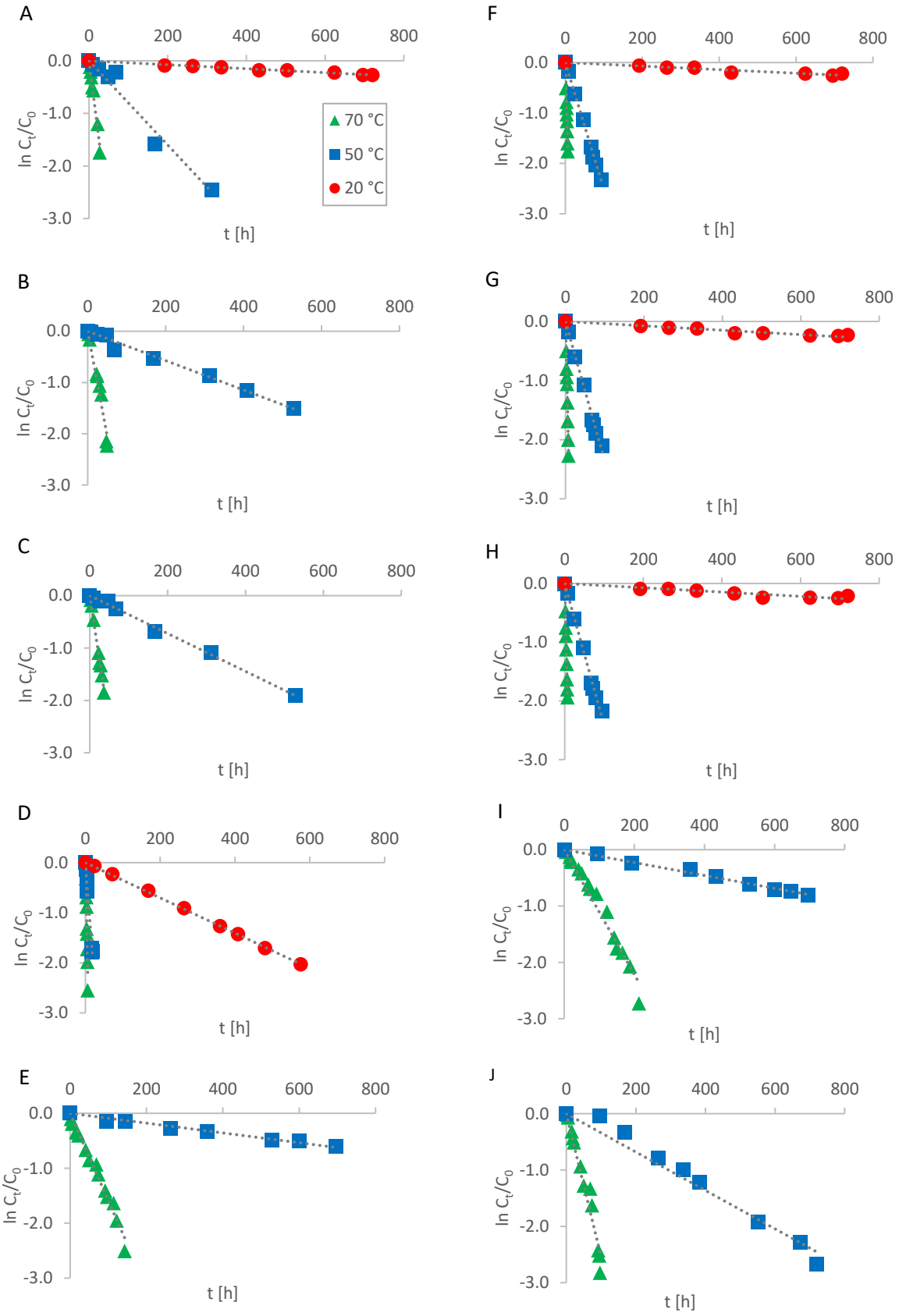


Figure 23 The plot of ln c_t/c_0 with time for (A) IF at pH 4, (B) IF at pH 7, (C) IF at pH 9, (D) CBZ-ep at pH 4, (E) 4-OH-DIC at pH 9, (F) CP at pH 4, (G) CP at pH 7, (H) CP at pH 9, (I) 4-OH-DIC at pH 4 and (J) MTZ-OH at pH 9 (the legend in the plot A refers to all figures; lack of plots for 20 °C for analytes is due to the lack of degradation)

Table 27 The rate constants (k), half-lives ($t_{½}$), coefficient of determination (R^2) and activation energy (E_a) determined for the investigated compounds in the extended test

Analyte	pH	20 °C			50 °C			70 °C			E_a [kJ/mol]
		k [1/h]	$t_{½}$ [h]	R^2	k [1/h]	$t_{½}$ [h]	R^2	k [1/h]	$t_{½}$ [h]	R^2	
CP	**4**	$3.5 \cdot 10^{-4}$	1963.2	0.932	$2.5 \cdot 10^{-2}$	27.6	0.997	$2.7 \cdot 10^{-1}$	2.6	0.998	111.2
	7	$3.8 \cdot 10^{-4}$	1867.9	0.940	$2.4 \cdot 10^{-2}$	29.4	0.995	$2.8 \cdot 10^{-1}$	2.5	0.997	110.6
	9	$3.7 \cdot 10^{-4}$	1893.4	0.889	$2.4 \cdot 10^{-2}$	28.8	0.995	$2.7 \cdot 10^{-1}$	2.5	0.995	110.5
IF	**4**	$3.8 \cdot 10^{-4}$	1843.1	0.984	$8.0 \cdot 10^{-3}$	86.5	0.969	$5.9 \cdot 10^{-2}$	11.7	0.983	84.2
	7	n.d.	n.d.	n.d.	$2.9 \cdot 10^{-3}$	241.9	0.984	$4.1 \cdot 10^{-2}$	16.9	0.965	122.2
	9	n.d.	n.d.	n.d.	$3.6 \cdot 10^{-3}$	191.7	0.996	$4.9 \cdot 10^{-2}$	14.0	0.996	120.1
CBZ-ep	**4**	$3.5 \cdot 10^{-3}$	197.1	0.999	$1.0 \cdot 10^{-1}$	7.0	0.998	$3.6 \cdot 10^{-1}$	1.9	0.951	78.8
4-OH-DIC	**4**	n.d.	n.d.	n.d.	$1.1 \cdot 10^{-3}$	608.0	0.991	$1.1 \cdot 10^{-2}$	62.6	0.970	92.2
	9	n.d.	n.d.	n.d.	$8.9 \cdot 10^{-4}$	778.8	0.974	$1.6 \cdot 10^{-2}$	43.6	0.980	104.4
MTZ-OH	**9**	n.d.	n.d.	n.d.	$3.4 \cdot 10^{-3}$	203.4	0.969	$2.5 \cdot 10^{-2}$	27.3	0.965	132.4

n.d. – not determined (due to the lack of the degradation)

It was confirmed that the temperature has an influence on the degradation rates for all analytes. What is the most interesting from the environmental point of view is that at 20 °C MTZ-OH, 4-OH-DIC and IF at pH 7 and 9 remained completely stable for 30 days. It indicates that at environmentally relevant temperatures these compounds would be persistent in terms of their hydrolytic stability. However, IF at pH 4 and CP in all conditions degraded to some extent. Their estimated half-lives at 20 °C (around 80 days) indicate that this degradation occurred quite slowly. Only CBZ-ep exhibited quite quick disappearance, which half-life was a little more than 8 days. It is in accordance with the chemistry of epoxides, which at acidic environment are highly reactive due to opening of the epoxide ring through protonation, which makes the structure vulnerable to the nucleophilic attack [257]. However, in the terms of environmental fate, such acidic waters occur rather rarely. To confirm the negligible hydrolysis rates at environmental conditions, the obtained results were used to extrapolate them to lower temperatures (**Table 28**). The half-lives even at 15 °C suggest that these compounds would be persistent in terms of hydrolytic stability in water.

Table 28 The extrapolated data for environmentally relevant temperatures

Analyte	pH	4 °C		10 °C		15 °C	
		k [1/h]	t½ [days]	k [1/h]	t½ [days]	k [1/h]	t½ [days]
CP	**4**	$5.1 \cdot 10^{-5}$	568	$7.1 \cdot 10^{-5}$	406	$1.6 \cdot 10^{-4}$	179
	7	$5.3 \cdot 10^{-5}$	549	$7.4 \cdot 10^{-5}$	393	$1.7 \cdot 10^{-4}$	174
	9	$5.3 \cdot 10^{-5}$	550	$7.3 \cdot 10^{-5}$	394	$1.7 \cdot 10^{-4}$	174
IF	**4**	$8.3 \cdot 10^{-5}$	349	$1.1 \cdot 10^{-4}$	271	$2.0 \cdot 10^{-4}$	146
	7	$0.3 \cdot 10^{-5}$	9 219	$0.5 \cdot 10^{-5}$	6 364	$1.1 \cdot 10^{-5}$	2 578
	9	$0.4 \cdot 10^{-5}$	6 509	$0.6 \cdot 10^{-5}$	4 521	$1.6 \cdot 10^{-5}$	1 859
CBZ-ep	**4**	$9.7 \cdot 10^{-4}$	30	$1.2 \cdot 10^{-3}$	24	$2.2 \cdot 10^{-3}$	13
4-OH-DIC	**4**	$3.4 \cdot 10^{-6}$	8592	$4.6 \cdot 10^{-6}$	6260	$1.0 \cdot 10^{-5}$	2 892
	9	$0.6 \cdot 10^{-6}$	52 425	$0.8 \cdot 10^{-6}$	35 090	$2.2 \cdot 10^{-6}$	13 179
MTZ-OH	**9**	$2.0 \cdot 10^{-5}$	1 451	$2.6 \cdot 10^{-5}$	1 097	$5.2 \cdot 10^{-5}$	555

In general, 3 out of 5 unstable compounds were pharmaceuticals' TPs. It has to be highlighted that this is the first time such results are presented. It can be only mentioned, that their PCs were found to be hydrolytically stable, CBZ in this study, while MTZ in other study [127], which suggests that their TPs are slightly less stable. However, the hydrolysis of CP and IF has been investigated before. For example, it was found that CP in water taken from lake incubated at 20 °C in the dark was slowly degrading with $t_{1/2}$ around 80 days, which is a very similar result to what was calculated in this study [258]. Moreover, there was no degradation of IF observed. According to Muñoz et al., the degradation rates of IF depend on the pH of the solution and the slowest degradation occurs at neutral pH [259], which was also observed in this study. Moreover, the $t_{1/2}$ at 25 °C and pH 5 was around 620 days, indicating that the degradation at temperatures close to the environmental values occurs very slowly. There was also interesting observation made, that the ionic strength or the increase in the concentration of acetate buffer has no influence on the degradation rates. The results obtained by Gilard et al. only confirm that IF is quite stable at room temperature, where there was no degradation observed in 30 days at pH 6.8 and 5.5 [59], which supports our results. The results of hydrolytic stability of pharmaceuticals and their TPs indicate, that most of the compounds are not susceptible to hydrolysis in investigated conditions. CP and IF at all pH, 4-OH-DIC at pH 4 and 9 as well as MTZ-OH at pH 9, which were found unstable, at environmentally relevant temperatures (20 °C and lower) either do not degrade or this process is very slow. The only exception is CBZ-ep at pH 4, which degraded quite quickly. These results

contribute to the knowledge of stability of the pharmaceuticals and especially their TPs, which has not been investigated before, which is a significant achievement in that field.

5.2.2.1 Determination of potential degradation products

As a result of the performed preliminary investigation for the determination of potential degradation products of samples collected from the extended tests in the temperature at 70 °C, specific ions were selected that might refer to specific degradation products. For MTZ-OH no ions were chosen as potential degradation products; 3 were initially selected, but running SIM mode for the same samples showed, that such ions with the same mass spectra are obtained at various retention times, which indicates that these are signals from the background noise. However, for 4-OH-DIC there was a spectrum found only in test samples, with signals of *m/z* 128, 172 and 234 in positive mode. The same signals were obtained at pH 4 and pH 9. For CBZ-ep at pH 4 in positive ionization mode *m/z* 234 and 240 were found, whereas in negative ionization mode 208, 251 and 194. For CP and IF there were much more ions found only in the test samples. For CP at pH 4 in positive mode ions with *m/z* 218 and 207 were obtained, at pH 7 ions with *m/z* 277 and 245 and at pH 9 ions with *m/z* 265, 243, 281 225, 263 257. No ions were found in the negative mode for CP. For IF at pH 4 in the positive mode, ion with *m/z* 213 and 231 were obtained, at pH 7 with *m/z* 245, 281 and 231 and at pH 9 with *m/z* 175, 225, 249, 265, 281, 263. In the negative mode, at pH 7 there was an ion with *m/z* 255, whereas at pH 9 ions with *m/z* 237, 293, 207, 251, 173 and 285. The obtained ions were compared with the literature data on the degradation products of these analytes and only the *m/z* 225 found for the CP and IF could be identified as a adduct $[224+H]^+$. Transformation product of CP with mass 224 g/mol was described in the literature as a potential degradation product of IF in alkaline conditions [60]. The authors propose two potential structures, from which the aziridine structure, assessed through observation of the hydrolysis of phosphoramide mustard is more likely to appear.

5.3 Performance of MWCNTs as adsorbents for the removal of pharmaceuticals and their TPs from water

CNTs, including MWCNTs, have been widely investigated as adsorbents for the removal of many water pollutants, including pharmaceuticals [121,260]. However, indisputable part of their application should be the possibility of regeneration and multiple use to decrease operational cost, which are high due to the price of CNTs. Moreover, it is also important to search for the ways of their application in some form, not only in dispersive mode. Therefore, in this chapter the results on the preliminary selection of the optimal conditions of the regeneration method of the specifically selected MWCNTs and its influence on the adsorption effectiveness of selected anticancer drugs was investigated. These results presented in this chapter were already published [261]. Moreover, the preparation and performance of MWCNTs/chitosan membranes is presented. Preparation of such product was dictated by several reasons. First of all, MWCNTs are very good new class of the adsorbent for removal of wide range of pollutants, including pharmaceuticals, which makes researchers around the world search for the ways of their application. The main problems of their wider use in water purification are not only their price and methods for the regeneration of the material, but also introduction of simple, cost efficient and quick technological solution, which would allow to use the MWCNTs as adsorbents for water purification and at the same time would allow to separate the material from water after the process. For all these reasons, in this thesis, new solution, namely the membranes with MWCNTs and chitosan has been proposed and evaluation of the removal of the mixture of pharmaceuticals and their TPs by the membranes was performed.

5.3.1 Thermal and chemical regeneration and its influence on MWCNTs performance

The experiments started with the selection of the maximum temperature for the regeneration. For this reason, 400, 350 and 300 °C were tested. The selection of these temperatures was based on the literature data for MWCNTs and their thermal treatment, which was performed at 400 °C as well the information provided by the manufacturer, that stated they should be thermally stable at 400 °C [198,217]. First of all, the effectiveness of this process was controlled by the mass loss of the selected to the study MWCNTs before and after the regeneration. As a result, at 400 °C after 2 h exposure only 60 % of initial mass of MWCNTs remained. At 350 °C the loss of weight was less drastic, but still only 73 % of MWCNTs remained. Moreover, in both cases there was visible grey coating on the top of the material reminding ash. However, after heating at 300 °C, 93 % of initial mass of MWCNTs remained, thus this temperature was accepted for the thermal

regeneration. It has to be underlined that these losses can partially result of the transferring the adsorbent from crucible to weighing vessel and back, so the influence of the temperature could be even less. Final temperature program used in muffle furnace involved reaching 250 °C in 15 minutes, keeping it for 5 minutes, then reach 300 °C in 30 minutes and keeping it for 120 minutes. When the regeneration program was settled, the same part of MWCNTs was regenerated 5 times in a row and the mass loss was monitored. The obtained results are presented in the **Figure 24**.

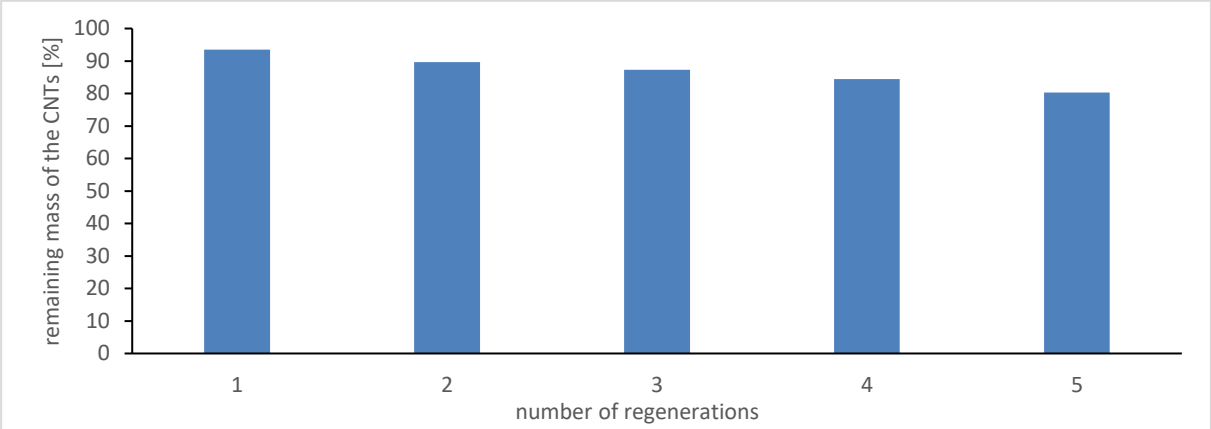


Figure 24 The loss of the MWCNTs mass during each thermal regeneration in 300 °C

The average mass loss was 4.3 ± 1.5 mg per regeneration (initial mass 1000 mg), which was acceptable. Therefore, MWCNTs were mixed with WWTP influent for 16 h to arrange hard working conditions of the adsorbent. Afterwards, they were dried and thermally regenerated in the selected conditions. Then, the adsorption studies were performed to evaluate the influence of the regeneration on the adsorption of model compounds – three selected anticancer drugs (CP, IF and 5-FU), which were already investigated during my master thesis [202], which was focused on the preliminary assessment of the adsorption potential of different CNTs towards these three pharmaceuticals. Based on this first study, it was established that MWCNTs with the highest surface area and smallest dimensions (outer diameter <8 nm, length 10 – 30 μm and surface area 500 m^2/g) had the biggest adsorption capacity towards these compounds, therefore, it was chosen for the studies described in this chapter. There were 5 cycles of contamination/regeneration of the same MWCNTs batch. Before the adsorption tests, the adsorption equilibrium time was investigated after 5 cycles to verify how quickly the equilibrium is reached (**Figure 25**). It was observed, that the process is fast for each analyte. For the purposes of further studies it was established that any sorption studies will be conducted for 2 h. Quick establishing of the equilibrium time is with accordance with other sorption studies on CNTs, where the investigations of contact time resulted in values from several minutes to several hours [121,165,174,189].

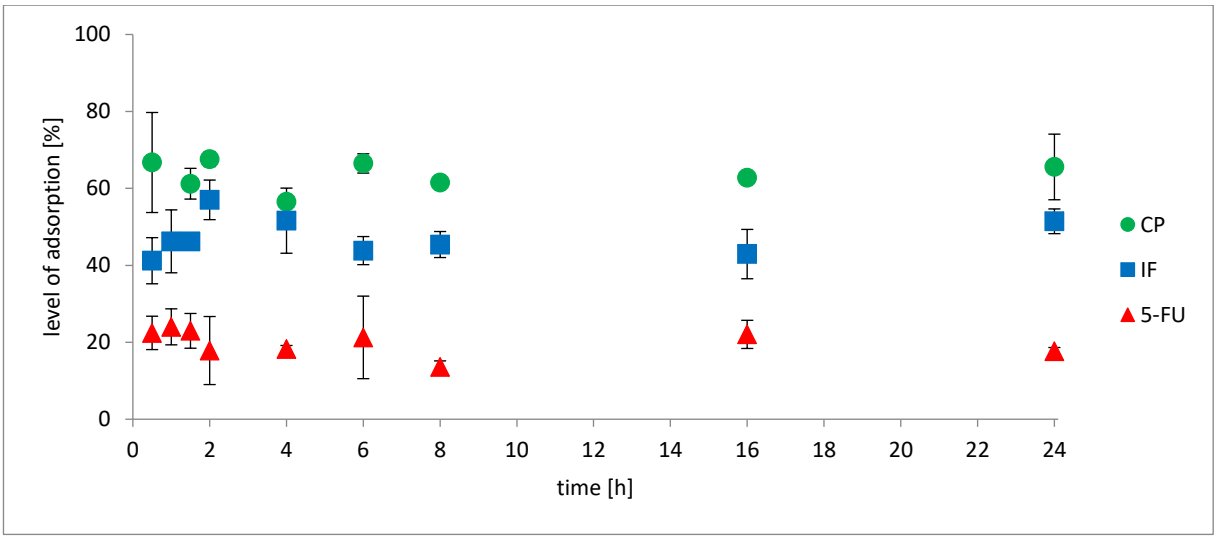


Figure 25 The equilibrium time of the adsorption process on the thermally regenerated MWCNTs for three anticancer drugs

After each thermal regeneration, level of the adsorption of CP, IF and 5-FU was evaluated. Additionally, the same test was performed for MWCNTs before any regeneration process for comparison. The results of that experiment are presented in the figure below.

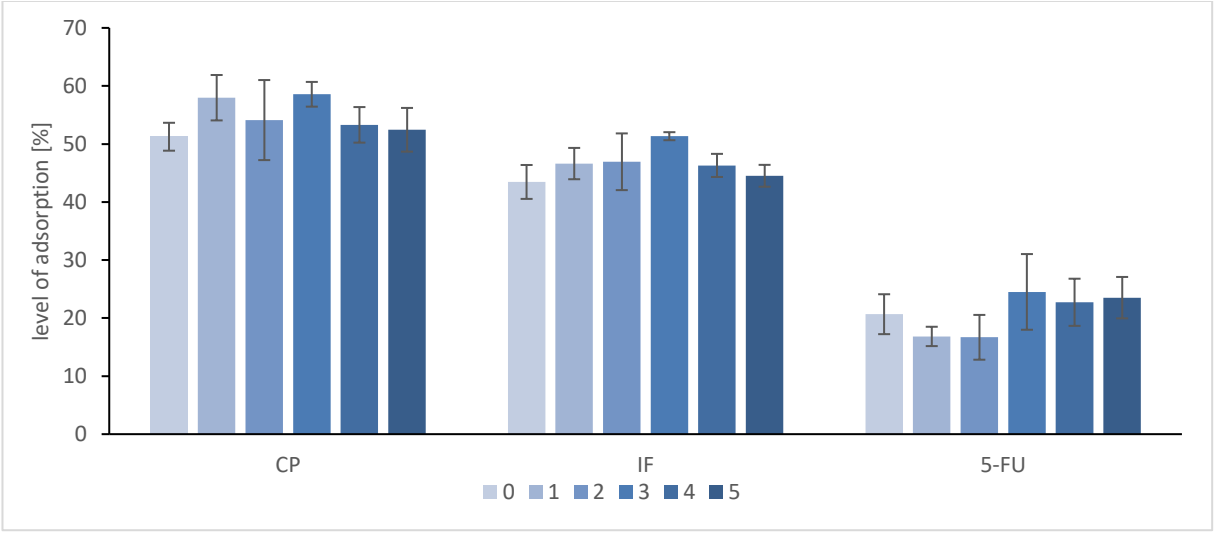


Figure 26 The levels of adsorption for three model pharmaceuticals before (0) and after each cycle (1 – 5) of contamination/thermal regeneration

These results indicate that the adsorption was maintained at similar level for each compound; therefore, there was no negative influence of the contamination and regeneration observed. It is in accordance with some literature data available; for instance, the adsorption capacity of MWCNTs towards ibuprofen and triclosan was maintained during 5 cycles of thermal regeneration, while for acetaminophen it was even higher for regenerated than pristine MWCNTs [216]. In other study, after 5 cycles of thermal regeneration in 400 °C of granular MWCNTs the adsorption capacity towards carbamazepine and diclofenac was also maintained [217]. However, it must be highlighted

that in none of these mentioned studies the adsorbent was additionally contaminated like in this study by using the WWTP influent.

Moreover, for better description of the adsorption process on the MWCNTs, linear, Freundlich and Langmuir isotherm models were fitted (**Figure 27**), which was performed by using the pristine (not thermally regenerated) MWCNTs and regenerated MWCNTs after 5 cycles of pollution/thermal regeneration.

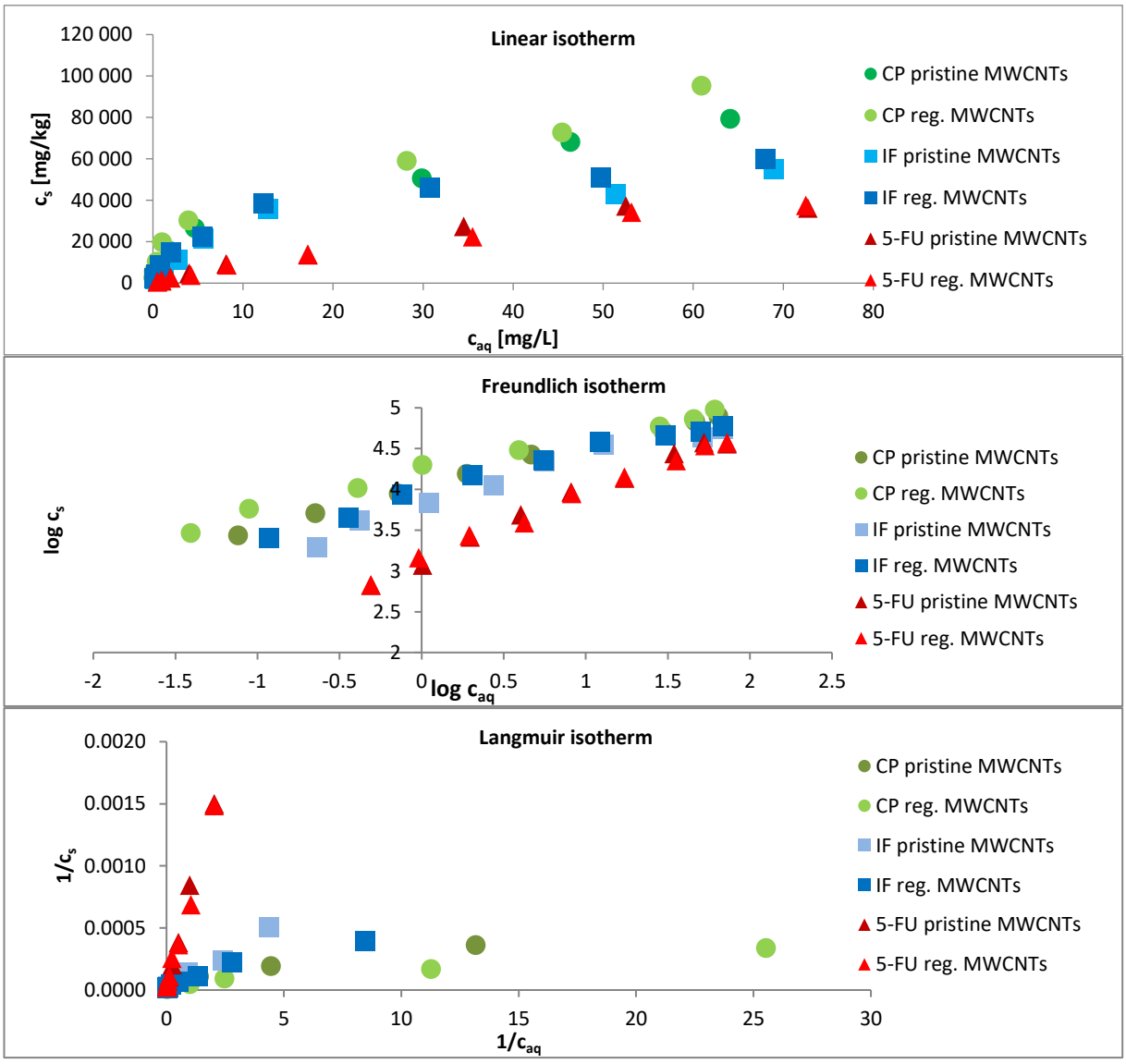


Figure 27 The plots of the linear, Freundlich and Langmuir isotherms

Out of the plotted curves' formulas, basic parameters of the isotherms were calculated (**Table 29**).

Table 29 The parameters calculated for linear, Freundlich and Langmuir isotherms and three anticancer drugs

Isotherm model	Parameter	CP		IF		5-FU	
		Pristine MWCNTs	Reg. MWCNTs	Pristine MWCNTs	Reg. MWCNTs	Pristine MWCNTs	Reg. MWCNTs
Linear	R^2	0.930	0.943	0.794	0.835	0.931	0.973
	K_d	1 149	1 378	712	792	550	534
Langmuir	R^2	0.951	0.978	0.986	0.955	0.995	0.994
	K_L	1.36	2.23	0.24	0.80	0.03	0.04
	C_{max}	27 902	35 829	39 888	27 778	41 982	39 324
Freundlich	R^2	0.995	0.983	0.964	0.978	0.990	0.993
	K_F	10 627	15 143	6 266	8 682	1 347	1 401
	1/n	0.49	0.43	0.56	0.50	0.83	0.79

The obtained values of the equilibrium distribution coefficient K_d indicate, that the ratio between the analyte adsorbed and remaining in the solution is kept on similar level on pristine and regenerated MWCNTs. In the case of CP there is even around 20 % increase of the value, smaller for IF and little decrease was observed for 5-FU. Moreover, the results of the fitting to Freundlich and Langmuir models suggest, that there are very small differences; for CP a little better fitting was observed for Freundlich model for regenerated and not regenerated MWCNTs. On the other hand, for IF and pristine MWCNTs the Langmuir model was fitted slightly better, but after regeneration the trend has reversed. For 5-FU the differences of R^2 are just negligible. Therefore, when evaluating both isotherm constants K_F and K_L, which both are related to the adsorption capacity, for each compound the capacity increases after regeneration. In the case of 5-FU this increase is less noticeable, but for IF and CP it is clear that the adsorption capacity significantly increases. It indicates that thermal regeneration influenced the overall number of active sites for the adsorption of these compounds. It could be due to the removal of pollutants occupying these active sites or removal of amorphous carbon and other morphological abnormalities, which is possible by heating [262].

It has to be highlighted, that thermal regeneration can only be effective to limited number of regenerations (nevertheless, this number can be high). Moreover, this type of treatment does not remove all possible pollutants, such as metals. Therefore, another method of regeneration was explored, which could be complementary to the thermal one. For this reason, a chemical regeneration was performed. For this purpose, MWCNTs before regeneration (10 mg) and after 5 cycles of contamination and thermal regeneration (10 mg) were flushed with 3 portions of 10 mL of 3 M HNO_3 each. These fractions were collected, and the concentration of selected metals was determined in these solutions of acid. At the same time, the chemically regenerated MWCNTs were flushed with water and methanol, dried and adsorption test for the model compound CP was

performed for each MWCNTs. It was observed that the adsorption level of CP on MWCNTs regenerated only chemically was 31.2 % and regenerated thermally and chemically 30.9 %. Compared to the results for pristine or thermally regenerated MWCNTs, on which the adsorption of CP was over 50 %, flushing them with 3 M HNO_3 had negative influence on the adsorbent capacity towards CP. It could be due to the introduction of active groups on the surface of MWCNTs, which reduced hydrophobic interactions between CP and the surface of the adsorbent. In our previous study [202] it was observed that CP had lower affinity to MWCNTs with –COOH groups than to the same adsorbent without these groups. Therefore, introduction of such entities with the acid flushing could be the reason of lowered adsorption level. Nevertheless, the main purpose of this kind of regeneration was evaluated, namely the efficiency to remove metals from MWCNTs. The results are presented in the tables below. These experiments were performed twice in order to check the reproducibility of this process.

Table 30 The concentration of selected metals in three fractions of the filtrates of 3 M HNO_3 after chemical regeneration (1st experiment)

Type of MWCNTs	**Concentration [µg/L]**									
	Cu	**Cd**	**Cr**	**Pb**	**Mn**	**Zn**	**Ni**	**Fe**	**Mo**	**Co**
Pristine fraction 1	n.d.	n.d.	40.8	0.6	10.3	19.3	93.2	859.6	3 349.7	8 661.8
Pristine fraction 2	n.d.	n.d.	4.2	0.1	2.1	3.9	8.3	23.8	953.7	723.5
Pristine fraction 3	n.d.	n.d.	0.3	n.d.	n.d.	3.0	0.3	10.0	245.7	47.9
After 5 cycles of contamination – thermal regeneration fraction 1	17.8	n.d.	18.3	2.5	17.2	56.3	113.1	1 033.4	1 568.5	9 941.7
After 5 cycles of contamination – thermal regeneration fraction 2	1.8	0.1	2.2	0.9	1.0	1.0	10.0	49.8	334.1	1 580.4
After 5 cycles of contamination – thermal regeneration fraction 3	n.d.	n.d.	0.4	n.d.	n.d.	n.d.	1.6	25.5	147.8	224.9

n.d. – not determined, <LOD

Table 31 The concentration of selected metals in three fractions of the filtrates of 3 M HNO_3 after chemical regeneration (2nd experiment)

Type of MWCNTs	Concentration [µg/L]									
	Cu	**Cd**	**Cr**	**Pb**	**Mn**	**Zn**	**Ni**	**Fe**	**Mo**	**Co**
Pristine fraction 1	n.d.	n.d.	41.3	n.d.	10.6	1.7	91.1	890.5	3 698.3	8 969.8
Pristine fraction 2	n.d.	n.d.	1.3	0.2	1.7	4.4	1.7	29.8	584.6	154.3
Pristine fraction 3	n.d.	n.d.	0.3	n.d.	n.d.	4.5	0.6	9.4	217.2	40.6
After 5 cycles of contamination – thermal regeneration fraction 1	n.d.	n.d.	46.9	n.d.	11.6	37.8	100.3	979.6	4 093.1	10 000.3
After 5 cycles of contamination – thermal regeneration fraction 2	n.d.	n.d.	3.1	1.6	0.4	n.d.	5.9	51.3	673.1	918.8
After 5 cycles of contamination – thermal regeneration fraction 3	n.d.	n.d.	0.9	n.d.	n.d.	n.d.	1.4	20.8	363.7	212.1

n.d. – not determined, <LOQ

The determination of metals concentration in 3 fractions allowed to observe how well they are removed from MWCNTs. It is clear that with each fraction the amount of metals was enormously lower. The highest concentrations were found for Co, Mn (several mg/L) and Fe (nearly 1 mg/L). Nevertheless, they were efficiently removed with only several per cent of the initial concentration left in the third fraction. On the other hand, other metals were found on the level up to around 100 µg/L or less. Nonetheless, the chemical regeneration was efficient for every metal investigated in this study. There was a small increase in the concertation in the filtrates between pristine and regenerated MWCNTs, indicating that the metals were adsorbed from the WWTP effluent used for the contamination of MWCNTs. However, the small increase indicates that the MWCNTs can work for a long time with such complex matrix like WWTP effluent without a need to be chemically regenerated. According to the literature, the CNTs remove efficiently metals like Ni, Zn, Cd or Cu from water at pH above 7 [208,263]. Moreover, HNO_3 was used to desorb successfully some metals from CNTs [209,264]. Therefore, these results are in agreement with our observation and the application of acidic solution to remove metals from MWCNTs is advisable.

The changes observed in adsorption capacity of thermally regenerated MWCNTs in isotherm parameters and chemically regenerated MWCNTs from the drop of the adsorption level for CP, must result in the changes of the adsorbent. Therefore, some measurements were performed to characterise the material. Pristine, thermally and chemically regenerated MWCNTs were

examined through elemental analysis, SEM, TEM imaging and Raman spectroscopy. The elemental analysis included determination of the content of oxygen, carbon, hydrogen and nitrogen (**Table 32**).

Table 32 The results of the content of oxygen, nitrogen, hydrogen and carbon in MWCNTs before and after regeneration

Type of MWCNTs	Oxygen content [%]	Carbon content [%]	Hydrogen content [%]	Nitrogen content [%]
Pristine	5.44 ± 0.42	87.72 ± 0.10	0.840 ± 0.019	0.159 ± 0.014
After 5 cycles of contamination and thermal regeneration	6.59 ± 0.35	87.24 ± 0.21	0.804 ± 0.015	0.272 ± 0.010
After 5 cycles of contamination, thermal and chemical regeneration	7.71 ± 0.22	85.28 ± 0.12	1.339 ± 0.028	0.495 ± 0.044

The increasing amount of oxygen indicates that the oxidation of the MWCNTs occurred to some extent. Moreover, both thermal and chemical regeneration have that effect, but the influence of the acid flushing is much more pronounced. It is well known that chemical treatment of CNTs with acid, such as nitric acid, introduces additional functional groups and modifications onto their surface. These groups and formations are carbonyl, ester (lactone), aldehyde, phenol, quinone, carboxylic anhydride or carboxylic acid [262]. Some of them contain hydrogen, which explains the increase of its content after chemical regeneration. The increased content of nitrogen could be a consequence of remains of the organic compounds adsorbed onto the MWCNTs and not fully mineralized as well as the introduction of nitro groups after the treatment with HNO_3 [265]. Moreover, the Raman spectroscopy was used to further confirm changes in the adsorbent (**Figure 28**).

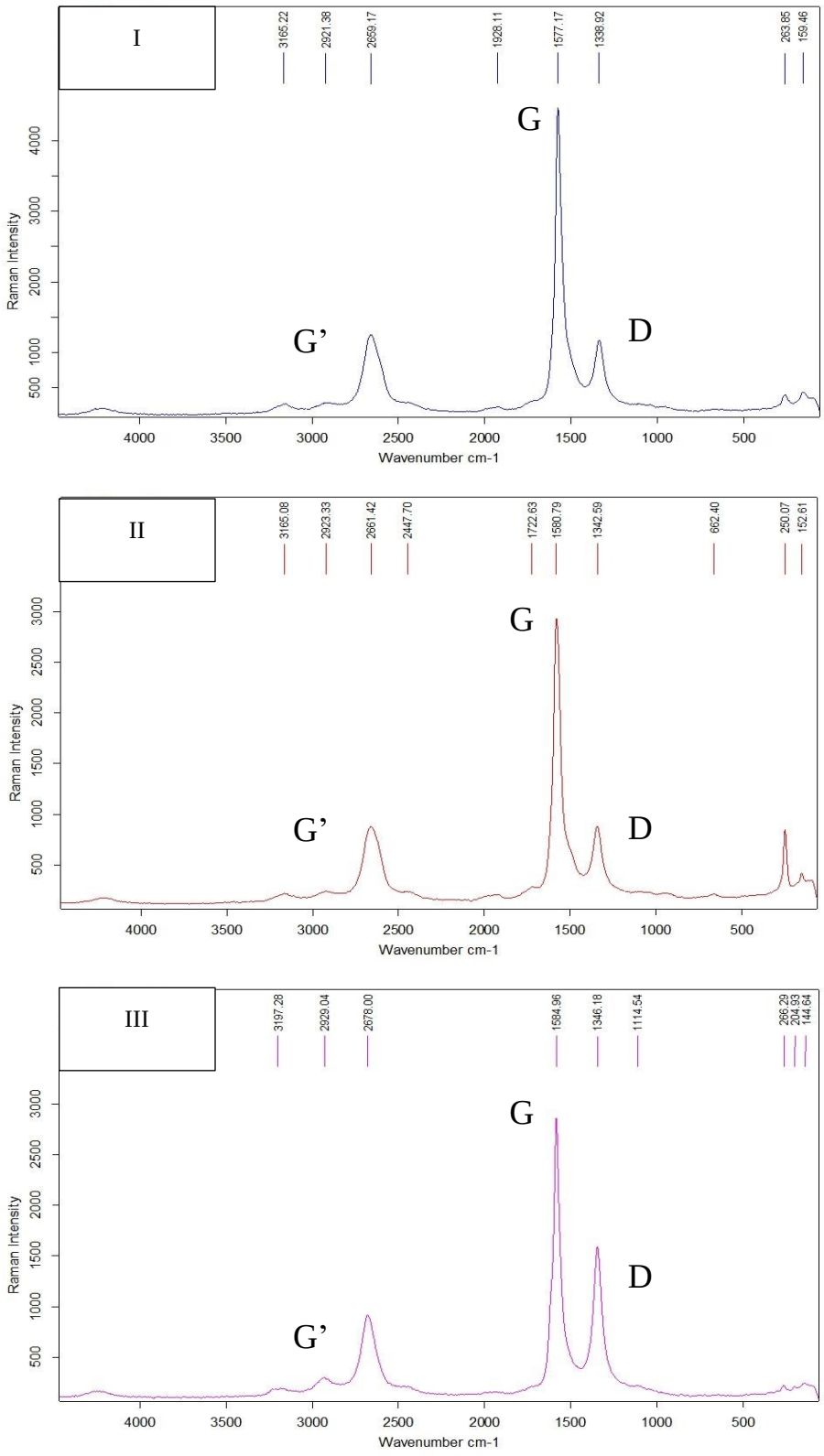


Figure 28 The Raman spectra for (I) pristine, (II) thermally regenerated and (III) thermally and chemically regenerated MWCNTs

Two peaks are the most important in the Raman spectra regarding CNTs, the D-band and G-band. The D-band reflects the disordered sp^2 – hybridized carbon atoms and the G-band the structural

integrity of the sp^2 – hybridized carbon atoms [266]. The ratio between signal intensity of D-band and G-band describes the level of the disorder density of the CNTs walls [267]. The ratio I_D/I_G for pristine MWCNTs was 0.26, for thermally regenerated 0.29 and for thermally and chemically regenerated 0.55. Therefore, it can be concluded that after contact with the acid more structural defects are present within MWCNTs structure. Nevertheless, these defects can be also the spots where the oxidation and modification of the MWCNTs occurs, so the increase of the I_D/I_G ratio may as well suggest introduction of the functional groups. The literature provides different I_D/I_G ratio values for CNTs; in the study of Li et al. the results were in agreement with this study, where for the pristine MWCNTs the ratio was 0.34 and was increasing with the more oxidized material up to 0.88 [268]. On the other hand, in the study of Lu and Chiu the ratio was smaller for the MWCNTs treated with different oxidants than for the pristine material [266]. This would suggest that the amorphous carbon was removed with the treatment. It seems that the result of chemical treatment vary between the experiments and fully controlled process should be developed to obtain the wanted result.

Additionally, SEM (**Figure 29**) and TEM (**Figure 30**) images were obtained of MWCNTs before and after thermal and chemical regeneration. The SEM images indicate, that the MWCNTs after the acid treatment seem to have less defined structure. There are much better visible spaces between bundles of pristine and thermally regenerated MWCNTs, whereas those treated with acid show less porous structure, like they were creating more compressed space. Moreover, higher magnitude revealed, that after thermal treatment there is probably bigger number of pores visible; it seems that these structures could be unblocked through the removal of amorphous carbon and other entities among the MWCNTs. From the more accurate TEM images it can be seen that there is a visible cover on the surface of pristine MWCNTs, which is significantly reduced after thermal treatment, which may indicate that it was amorphous carbon removed with the high temperature. Moreover, in the picture D there are visible metals (Co, Mo) left after manufacturing of the material with disrupted structure of MWCNTs around them. What is also interesting, picture F clearly indicates that there are sidewall damages after the acid treatment visible. The general structure of MWCNTs after acid treatment looks like it was altered, almost smoothed.

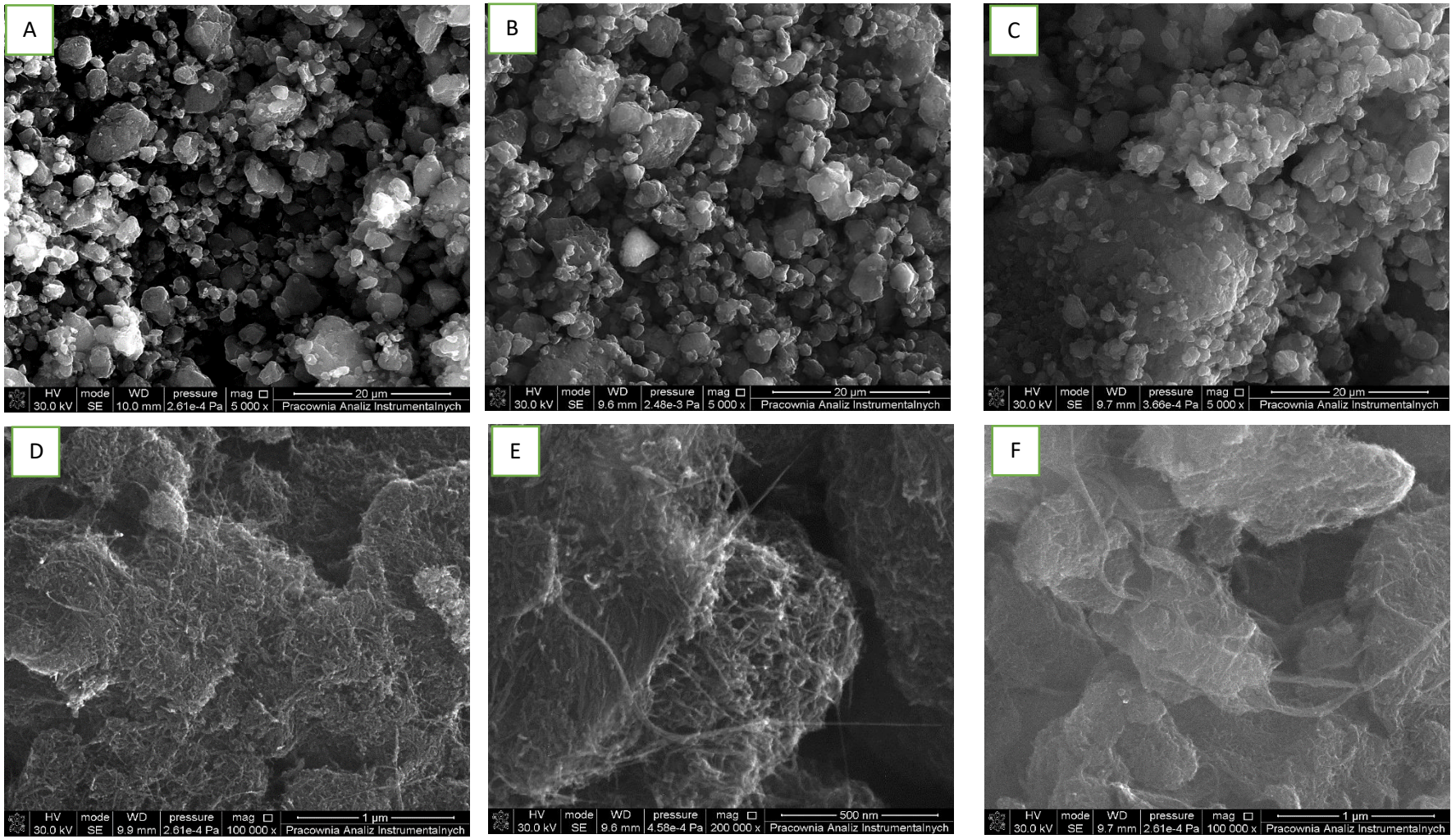


Figure 29 SEM images of (A, D) pristine MWCNTs, (B, E) MWCNTs after thermal regeneration, (C, F) MWCNTs after thermal and chemical regeneration

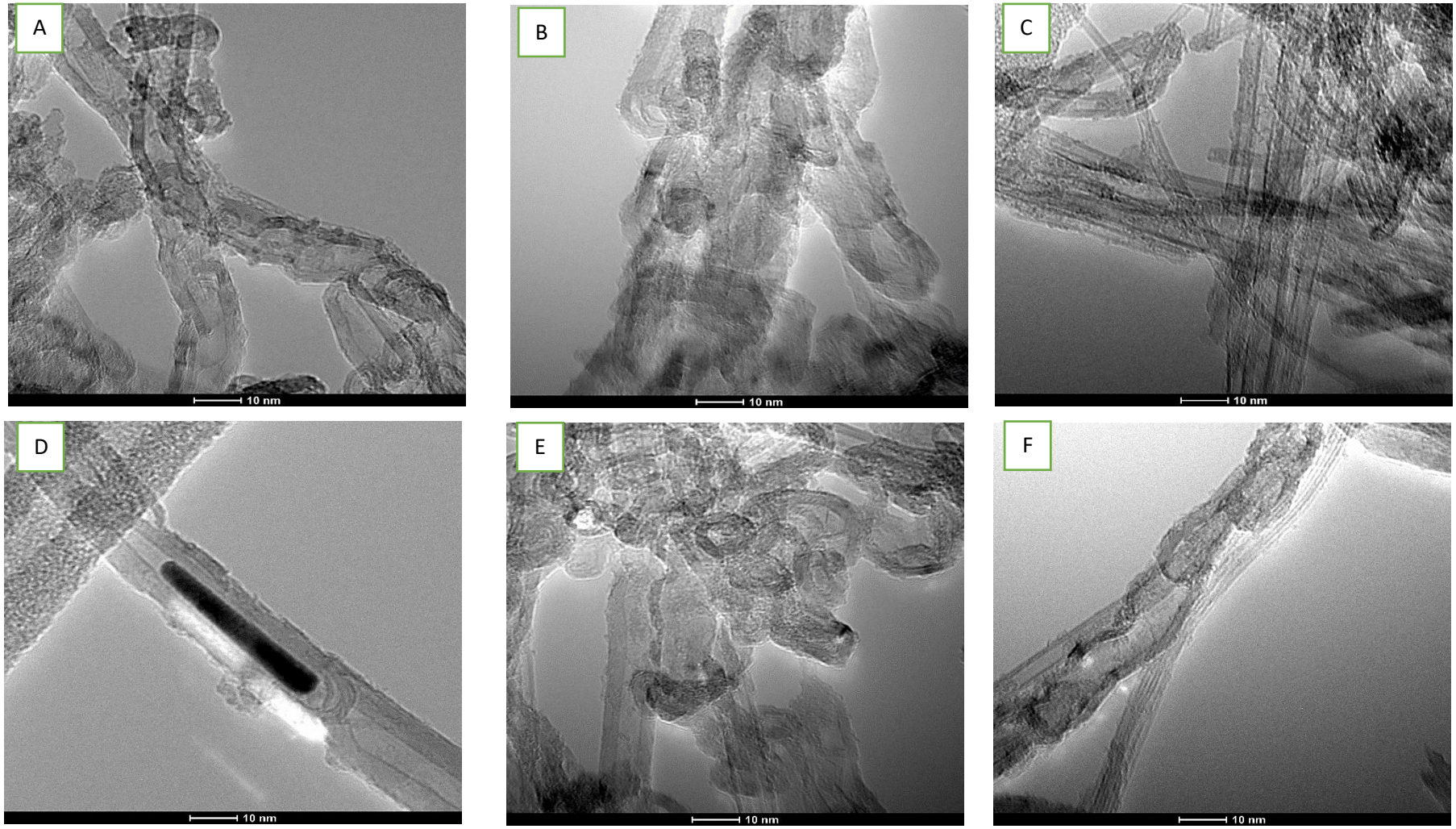


Figure 30 TEM images of (A, D) pristine MWCNTs, (B, E) MWCNTs after thermal regeneration, (C, F) MWCNTs after thermal and chemical regeneration

After such comprehensive study of the regeneration and description of MWCNTs, it was decided that thermal and chemical regeneration should be repeated for new batch of the material to verify the results. For this purpose, new batch of MWCNTs, purchased separately from the first one, was used in the same cycle of contamination and thermal regeneration as the first one using the same WWTPs influents. This time, the adsorption of CP as a model compound was verified only before and after 5 cycles of contamination/regeneration. Interesting observation was made for pristine MWCNTs; the level of adsorption of CP was only around 25 %, while it was around 50 % for the previous batch of pristine and thermally regenerated material. It suggests that even though the same product was purchased from the same supplier, it is not identical. Nevertheless, after thermal regeneration the adsorption was again on the level of around 50 %. Therefore, it suggests that thermal treatment caused purification and restoration of the material properties regarding the adsorption capacity towards CP. It is important observation, which indicates that MWCNTs before any application could be treated with high temperature to maximize their performance. Nevertheless, this aspect should be further investigated in order to confirm this hypothesis.

Moreover, since the previous chemical treatment with 3 M HNO_3 caused a certain decrease of adsorption level of CP from 50 to 30 %, several other variants were tested this time, namely 0.5 M HCl, 3 M HCl, 0.5 HNO_3 and 3 M HNO_3. It has to be underlined, that for each type of the acid 30 mg of MWCNTs was used and 3 fractions of 30 mL of the acid for the flushing, while in the first experiment it was 10 mg and 3 fractions of 10 mL. The adsorption levels for CP are presented in the table below.

Table 33 The adsorption level of CP onto MWCNTs after different types of chemical regeneration

Type of the acid used for flushing	Level of the adsorption of CP [%]
0.5 M HCl	35.8 ±3.2
3 M HCl	0
0.5 M HNO_3	31.3 ± 2.1
3 M HNO_3	2.11 ± 0.82

The adsorption of CP was reduced from around 50 % for thermally regenerated MWCNTs to around 36 % after treating the adsorbent with 0.5 M HCl and around 31 % after 0.5 M HNO_3. When it comes to more concentrated acids, the adsorption almost did not occur. At first glance the results for 3 M HNO_3 can be surprising, because in a previous experiments with this acid the adsorption dropped from 50 to 30 %. However, there was a difference in mass of MWCNTs and volume of the acid. In the first attempt, there was 10 mg of MWCNTs and 30 mL of acid, while in the second 30 mg and 90 mL. Even though the proportions were maintained, bigger amounts of the products generated longer contact time (the flushing was performed by gravity in both cases). Therefore, it seems that contact time has a crucial meaning to the functionalization of MWCNTs

and should be controlled. Nevertheless, when comparing the results of less concentrated to more concentrated acids it is clear, that milder conditions of chemical regeneration has less negative influence on the adsorption of CP. Moreover, it is possible that optimisation of contact time would maintain the adsorption level much closer to the initial. Furthermore, the ability to remove selected metals from MWCNTs was verified for each acid (**Table 34**). This time only concentration of metals that were the most abundant in previous test are presented.

Table 34 Concentration of selected metals in fractions of different acids used for the chemical regeneration of MWCNTs

Metal	0.5 M HNO_3			3 M HNO_3			0.5 M HCl			3 M HCl		
	fr. 1	fr. 2	fr. 3	fr. 1	fr. 2	fr. 3	fr. 1	fr. 2	fr. 3	fr. 1	fr. 2	fr. 3
Mn	4.1	n.d.	n.d.	4.0	1.1	0.1	4.1	0.2	n.d.	5.7	1.4	1.5
Co	4 378.4	461.4	83.3	4 314.5	264.3	12.0	4 559.1	271.3	18.3	4 342.4	67.3	88.4
Mo	212.5	30.0	28.3	221.2	27.9	4.4	203.7	62.4	10.5	215.6	27.5	32.0

fr. – fraction, n.d. – not determined, <LOQ

These results indicate that the 0.5 M acids are as well sufficient as 3 M acids in terms of metal removal. Therefore, the application of less concentrated acids is recommended for such treatment, since they cause less changes to the adsorbent with the same extraction efficiency of the metals.

In general, thermal regeneration is a promising method for simple and efficient regeneration and possibility to reuse the adsorbent. In some cases, the thermal treatment can be treated as a pre-treatment for obtaining the best performance of the material. Moreover, chemical regeneration with acids is efficiently removing metals present in among the MWCNTs. However, the adsorption of model compound CP decreased after such treatment, which indicates that it can have negative influence on their further performance. Nevertheless, the oxidation of MWCNTs surface may also have a positive influence on the adsorption of pollutant.

The obtained results provide a comprehensive data on the possibility of the reuse of MWCNTs as adsorbents, as well as the influence of thermal and chemical regeneration on their performance. It is a significant contribution to the knowledge on their possible regeneration and reuse. There are only several papers available, which contain information on thermal regeneration and studies of the adsorption of pharmaceuticals. For example, the granular MWCNTs were successfully regenerated 5 times in a row at 400 °C [217]. The temperature was higher than used in this study, but it is not clear why the MWCNTs used in our experiments could not be regenerated at higher temperature. It could be due to the differences in size; the MWCNTs used in the cited paper had higher outer diameter, and length. Therefore, it is possible that MWCNTs with smaller diameter, like used by us (<8 nm) are not that resistant to temperature. It could be also due to the granular form of the MWCNTs. Nevertheless, they also have not noticed any decrease in the adsorption level of CBZ and DIC, which is in accordance with our results for CP, IF and 5-FU. However, the

additional step of polluting MWCNTs before each regeneration introduced in our study only emphasises the extraordinary performance of MWCNTs as adsorbents and the usefulness of the thermal treatment. Additionally, the study of Wei et al. presented thermal regeneration of the hybrid MWCNTs/Al_2O_3 adsorbent, which was also thermally regenerated at 400 °C [198]. During 10 reuse cycles, only slight changes of the adsorption of CBZ and DIC were noticed. Therefore, presented results supplement the available knowledge on this topic. Moreover, the obtained results show, that different types and concentrations of acids were able to remove metals from MWCNTs, however, they have an influence on the adsorbent, which may have an impact on the adsorption of organic compounds. It is significant contribution to the knowledge on this topic.

5.3.2 The preparation and application of the MWCNTs/chitosan membranes for the removal of pharmaceuticals from water

MWCNTs are very good adsorbent, which can be reused. However, it is important to search for various ways of their implementation for water purification as well as improvements of their performance. There are some CNTs hybrids that has been prepared and described, such as magnetic MWCNTs decorated with calcium, MWCNTs modification with polyaniline, MWCNTs grafted with carboxymethyl cellulose etc. [171,269,270]. Moreover, some composite materials with MWCNTs and chitosan were introduced [172,184,271]. The composite materials made of CNTs and chitosan have been already investigated as the adsorbents for removal of some potential water pollutants. This hybrid is created when the CNTs are coated with the chitosan and they are bonded either by hydrophobic interactions or hydrophilic ones with the amino and hydroxyl groups of chitosan and functional groups on the surface of CNTs. For example, it was shown that they efficiently remove Cu or methyl orange (dye) from water in batch adsorption experiments [272]. Other experiments used these composite in a form of a sponge or hydrogel core-shell beds to remove fluoride or congo red [183,185]. However, such studies have not included pharmaceuticals.

Mixing of the chitosan with MWCNTs can reduce cost of the adsorbent, create a stable structure in which MWCNTs are combined with another material and create other adsorption sites due to the chitosan's amine and hydroxyl functional groups. Therefore, in this study the attempt of preparing membranes with chitosan and MWCNTs was made. For this purpose, the 2nd batch of pristine and thermally regenerated MWCNTs described in previous chapter was used, as well as another, 3rd batch purchased separately from the previous two, for a comparison. First of all, interesting observations were made during preparation of the MWCNTs/chitosan mixture. Initially, MWCNTs from 2nd batch were added to the dissolved chitosan on the surface of the

solution. It resulted in creation of thick film on the surface and distribution of MWCNTs in entire solution was very difficult. Therefore, it was prepared once again, but first the MWCNTs were added to the beaker and then the solution of chitosan. This way it was distributed much better. However, the same batch, but thermally regenerated was fully dispersed in the solution without creating any sort of film. It shows how hydrophobic the unmodified MWCNTs can be and how thermal treatment can positively influence the material to be more dispersive in water. It is probably due to the introduction of polar groups onto their surface and making them less hydrophobic. There are literature reports, which support the fact that thermal treatment of CNTs results in better dispersion [217,267]. Additionally, 3rd batch of the same MWCNTs was distributed much better than the 2nd batch, but not as good as the regenerated material. Nevertheless, it is another example of how seemingly the same kind of MWCNTs has different properties.

Furthermore, the membranes of MWCNTs/chitosan prepared on nylon membranes were subjected to the experiment, in which the solution of the mixture of pharmaceuticals and their TPs was put through them under vacuum. The goal was to verify if the membranes are suitable for application for quick removal of potential pollutants from water. For this purpose, 100 mL of 10 µg/L solution of analytes was passed through membranes prepared from 2nd batch of pristine MWCNTs, thermally regenerated 2nd batch of MWCNTs and 3rd batch of pristine MWCNTs. For each type of MWCNTs the experiment was performed 5 times (**Figure 31**).

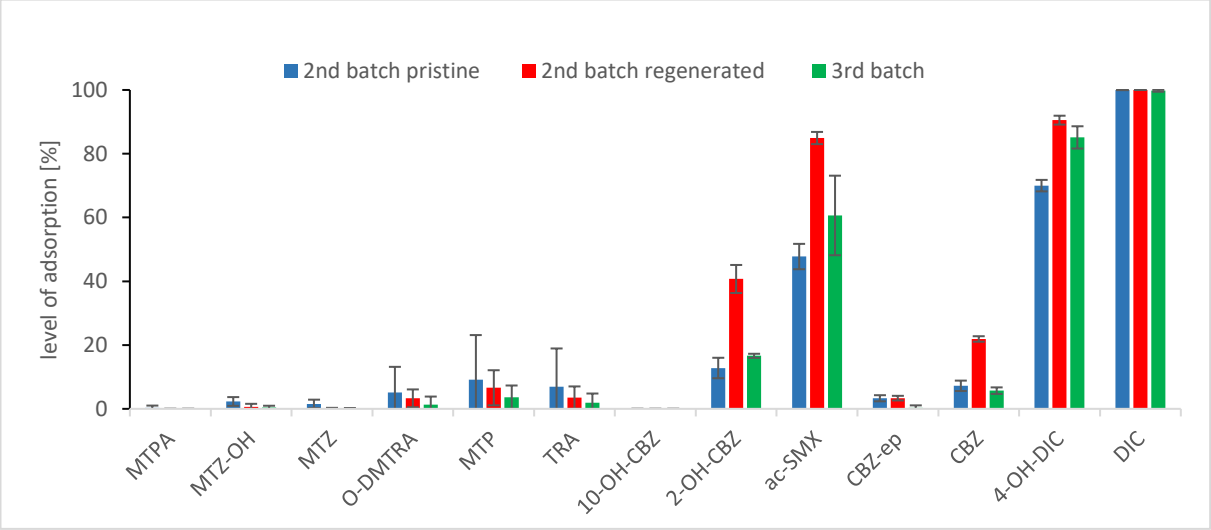


Figure 31 The average levels of adsorption on the MWCNTs/chitosan membranes (error bars represent SD)

Based on the obtained results it was observed that many of the analytes were adsorbed at a low level or not at all. It seemed that more polar compounds (log K_{OW} of MTZ, MTP, TRA and CBZ is in the range 0 – 2.5, while DIC 4.5) have lower affinity to the adsorbent. However, there are

two exceptions of this dependence, namely noticeable adsorption of 2-OH-CBZ and ac-SMX; it indicates that in this case additional factors besides their polarity are crucial for this process. Moreover, the results for MTP, TRA and O-DMTRA were quite different in each repetitions, which influenced high standard deviation. For those compounds that adsorption was noticeable, membranes with regenerated MWCNTs provided the best results. It is another indication that thermal treatment before application of the MWCNTs could be necessary to achieve the best adsorption results. However, due to the unsatisfactory results for many compounds, the amount of adsorbent was increased by combing two membranes together. They were put one on another and the same experiment was conducted. It has to be highlighted that similar time of contact (around 10 min) that was kept for single membranes was maintained only for membranes prepared from regenerated MWCNTs. In the case of pristine MWCNTs, either from 2nd or 3rd batch, the samples either were passing through the membrane so slowly that the experiment was terminated or it took at least one or two hours to do that. Therefore, these membranes were rejected as vastly inefficient when doubled. Nevertheless, the results for regenerated MWCNTs double-layered membranes are presented in the figure below.

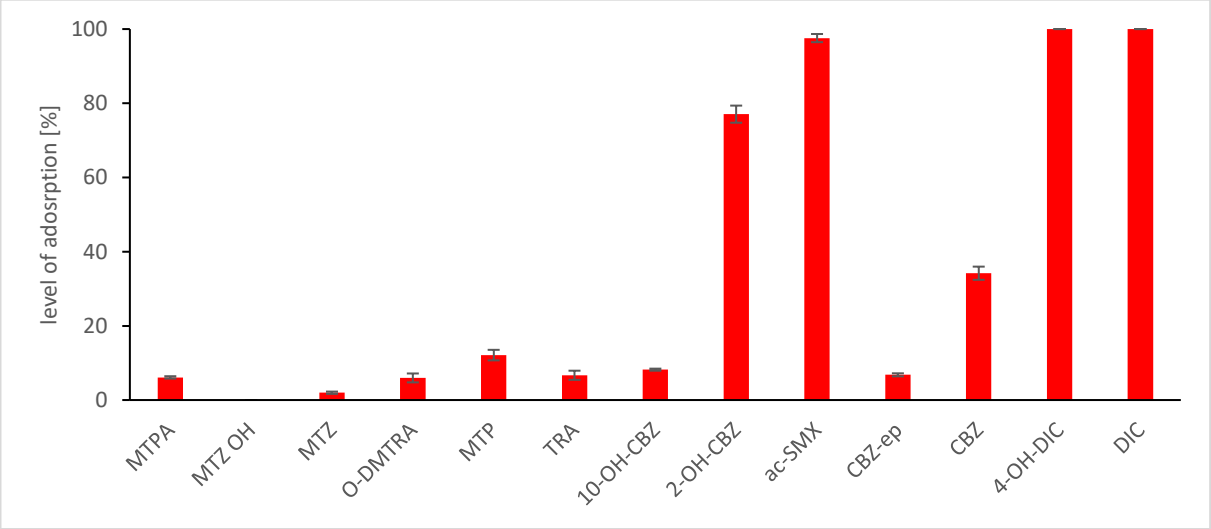


Figure 32 The level of adsorption for double membranes prepared from regenerated MWCNTs (error bars represent SD)

The results indicate that adsorption of those compounds, which were not efficiently removed from water by single membranes, increased only slightly, but still the process was not efficient for MTPA, MTZ-OH, MTZ, O-DMTRA, MTP, TRA, 10-OH-CBZ and CBZ-ep. On the other hand, the adsorption of DIC remained on 100 % and similar level was achieved for 4-OH-DIC and ac-SMX. Moreover, the removal of 2-OH-CBZ increased significantly (more than 35 %) in comparison to single membrane prepared from regenerated MWCNTs and adsorption of CBZ increased nearly 15 %. Therefore, to further explore the adsorption of these compounds and find

better conditions of their removal from water, two different pH of the water were tested, namely pH 3 and 9. However, it was performed only for the regenerated MWCNTs and 3rd batch of MWCNTs, due to their best performance in previous experiments (**Figure 33**).

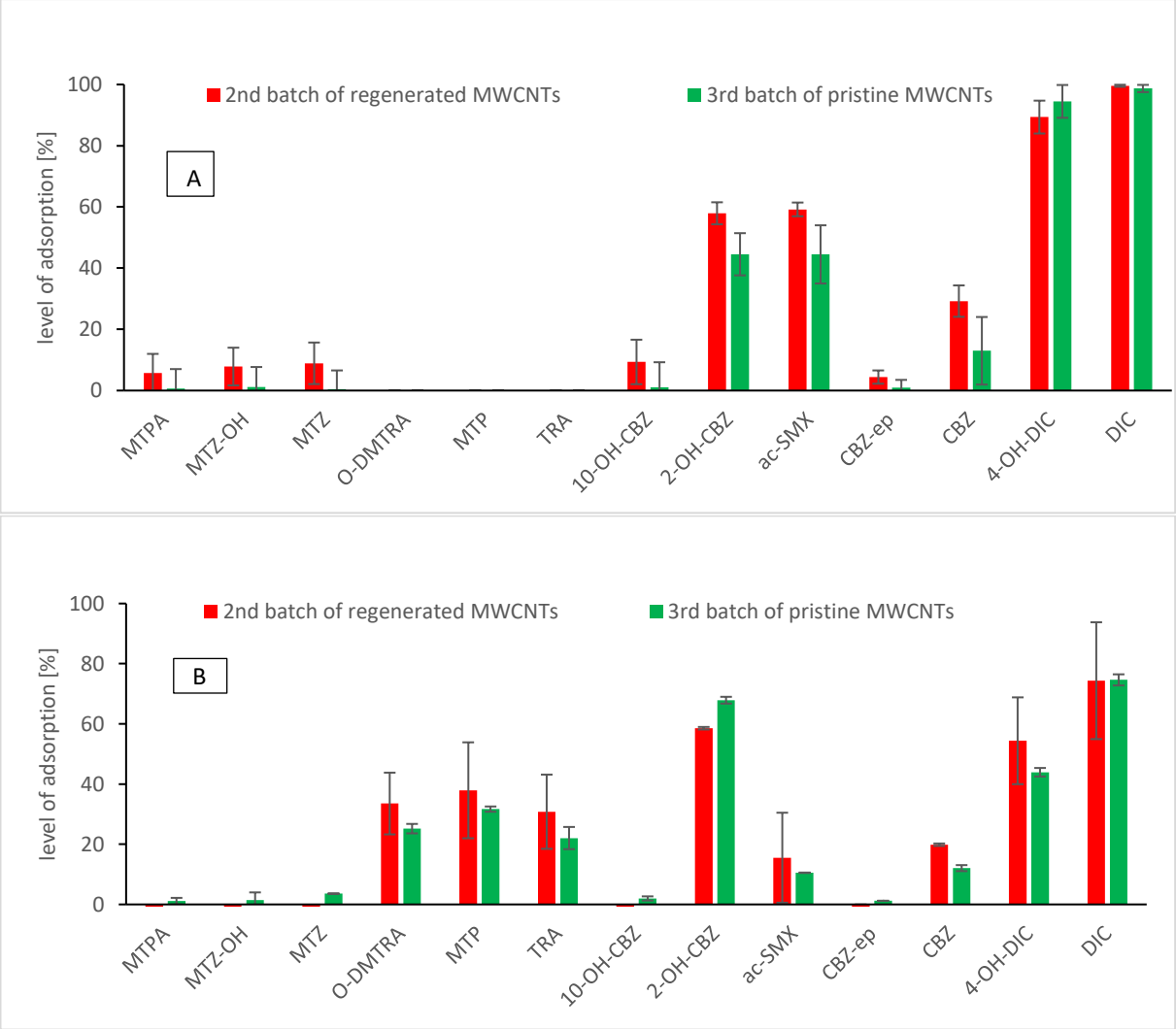


Figure 33 The level of adsorption of selected analytes at (A) pH 3 and (B) pH 9 on the membranes prepared from regenerated MWCNTs and 3rd batch of pristine MWCNTs (the error bars represent SD)

The obtained results are not unambiguous. First, the results for the best adsorbed compounds DIC and 4-OH-DIC remained at the same level at pH 3 as for the pure water, but decreased at pH 9. DIC with pKa around 4 is in ionic form above that value, whereas the amine groups at alkaline conditions become neutral, which reduces the interactions. The adsorption of ac-SMX dropped at pH 3 around 25 % for regenerated and 15 % for pristine MWCNTs and even more at pH 9. At pH 9 it can be similar reason as for DIC (the carboxyl group of ac-SMX is deprotonated, while amine groups of chitosan become neutral), whereas at pH 6 – 7 the carboxyl group is deprotonated, while amine groups of chitosan are positively charged, which creates electrostatic interaction. Moreover, there was increase in the removal rate for 2-OH-CBZ at both acidic and alkaline

conditions. Nevertheless, the results for the compounds that were poorly removed from water are still not satisfying. From these, O-DMTRA, TRA and MTP at pH 9 were adsorbed to some extent, so it seems that the alkaline conditions are more suitable for them, but still the adsorption level did not exceed 40 %. It may be caused by increased hydrophobic interactions due to the neutralization of amine groups, while MTP and TRA are still in their neutral form. Nevertheless, other forms of the interactions also must occur, especially for compounds with aromatic rings, which can interact with the MWCNTs surface or amine groups. Moreover, due to the presence of hydroxyl groups, hydrogen bonding is also possible to occur. To verify if the analytes are not attracted to the MWCNTs or is it the influence of combination with chitosan, similar experiments were performed but only for MWCNTs in dispersive mode. For this purpose 3, 4 and 5 mg of MWCNTs (pristine 2nd batch, regenerated 2nd batch and pristine 3rd batch) were prepared on the nylon membranes in the vacuum filtration system and 100 mL of the 10 μg/L solution was added for each. The time of filtration was the same as for the MWCNTs/chitosan membranes – around 10 min. The results are presented in the figures below.

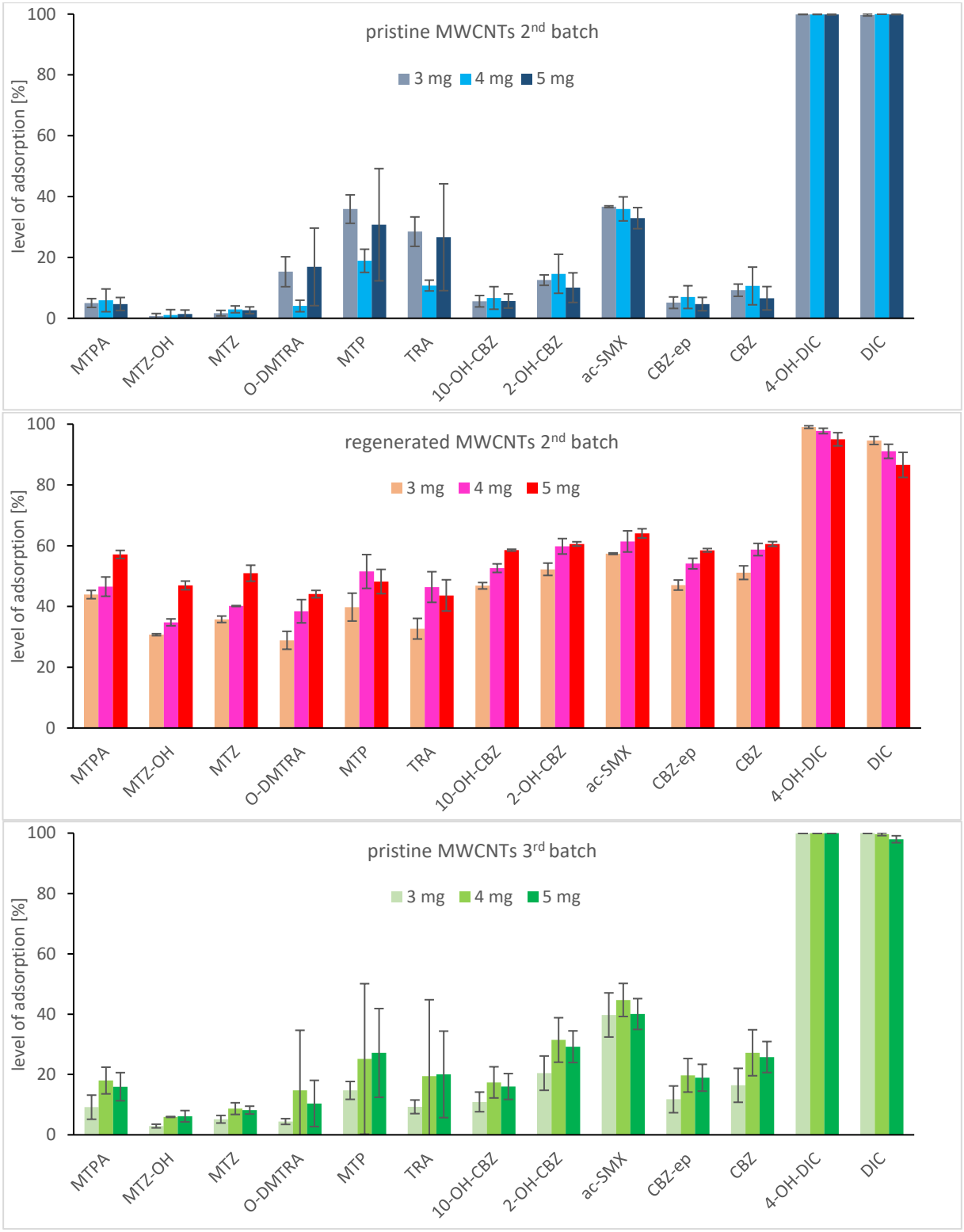


Figure 34 The results of dispersive adsorption of different masses of 3 types of MWCNTs performed in similar conditions to the membrane experiments

Moreover, to easily compare results between different types of MWCNTs, the results for 5 mg of the adsorbent are presented below.

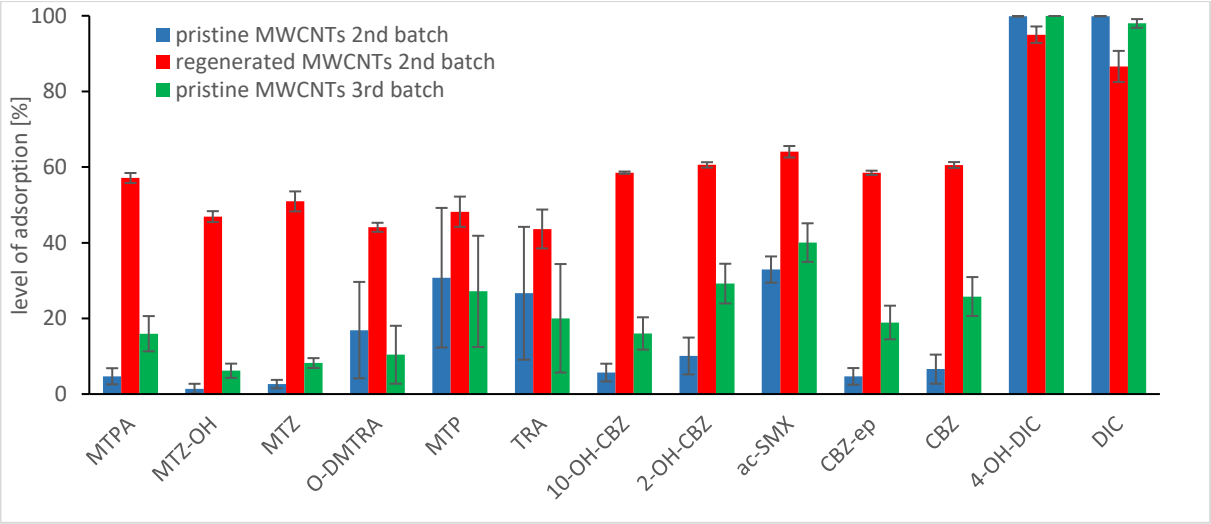


Figure 35 The results of dispersive adsorption of 5 mg of 3 types of MWCNTs performed in similar conditions to the membrane experiments

It can be concluded, that increasing mass of the adsorbent did not enhance the efficiency of the adsorption onto MWCNTs. Nevertheless, only in the case of regenerated MWCNTs there is clear pattern of increasing adsorption level with more adsorbent, for pristine MWCNTs it is more random. However, the adsorption of most of the compounds can be noticed, contrary to the performance of membranes with chitosan. Nevertheless, the adsorption of each analyte was clearly higher for the regenerated MWCNTs than for pristine. It can be concluded that the investigated analytes can be removed to some extent in such dynamic process, but it is recommended to prepare MWCNTs by thermal treatment for their best performance. Moreover, the membranes with chitosan and MWCNTs were not as efficient as MWCNTs alone. This could be due to the form in which they were used; dispersed material ensures good distribution and contact with the pollutants in water, whereas tightly and condensed mixture of MWCNTs and chitosan mounted on the membrane may not ensure such easy distribution. However, there were analytes like DIC and 4-OH-DIC which were adsorbed very efficiently on the membranes. Therefore, worse performance of the membranes can also be influenced by inaccurate proportions between chitosan and MWCNTs. Too much of chitosan could limit the physical access to MWCNTs, which are the main adsorbing material in this arrangement. The literature provides various weight ratios of CNTs and chitosan mixtures, like 1 : 100, 2 : 3.5 or 1 : 0.45 [185,272,273], while in this study the ratio was 1 : 5. Therefore, it cannot be concluded if this hypothesis is true, it requires further exploration. It is worth mentioning that often there are some additional components of the hybrid material, such as poly-2-hydroxyethyl methacrylate (pHEMA), iron oxide and various additives during preparation of such material, which may have an influence on their final performance as the adsorbents [187,214,274], and should be taken into account in further studies.

6. CONCLUSIONS

The pollution of the environment with pharmaceuticals is nowadays well recognized. However, it seems that similar problem exists for their transformation products. Therefore, it is important to explore the knowledge on their environmental fate, persistency, sources of pollution as well as on the methods for the reduction of their amounts in the inefficiently purified wastewaters and eventually environmental waters.

Therefore, taking all of these aspects into account, during the studies performed in this doctoral thesis different analytical methods for the identification of various pharmaceuticals and their transformation products were developed, which were applied as tools for further investigations. Firstly, the HPLC-UV-Vis equipment was used for developing determination methods for single analytes, which were successfully validated and applied in the model studies on hydrolytic stability of 7 pharmaceuticals and 10 PTPs as well as during the adsorption studies onto MWCNTs. Furthermore, the analytical method based on the application of LC-MS/MS technique with IT analyser was developed and used for the evaluation of the SPE procedure employed for the extraction of the selected pharmaceuticals and their TPs from water samples. Different conditions of the SPE method were tested, like pH of the sample, eluent and additional clean-up steps. Finally, new procedure for the analysis of these compounds, which was based on the application of the SPE Oasis® HLB (200 mg, 6 mL) sorbent and additional clean-up step with the use of MWCNTs was presented and fully evaluated in terms of its performance taking into account different kinds of surface waters and such parameters as: matrix effects (ME), absolute recovery (AR) and extraction efficiency (EE) for each analyte. The obtained results were satisfactory for most of the selected analytes. Afterwards, the method was transferred to more sensitive LC-MS/MS equipment with QqQ analyser, which was fully characterized and validated, and afterwards applied for the determination of selected analytes in different water samples collected from the selected regions in northern Poland. Almost all investigated pharmaceuticals and their TPs have been detected in the investigated wastewaters. Some of the native forms of pharmaceuticals, like TRA, CBZ or DIC reached a concentration of several μg/L in WWTP effluent and influent, whereas similar level was found for their transformation products like 10-OH-CBZ and 4-OH-DIC in WWTP influent. Nevertheless, concentrations of other analytes were often several hundred ng/L. It was also proved that some these pharmaceuticals and their TPs (CBZ, 10-OH-CBZ, TRA, O-DMTRA, MTPA and DIC) were present in the surface waters, especially those after the WWTP effluent discharge in the concentration from several to around a hundred ng/L. Based on the obtained results it was concluded that wastewaters can be are quite heavily loaded not only with

the parent pharmaceuticals but also their TPs, highlighting that its removal in WWTPs is not efficient and effluent waters might be recognized as a main source of these chemicals in the environmental waters. CBZ was found in almost all tested samples, which indicates that this pharmaceutical could be the marker of anthropogenic pollution in the environment. The presence of the selected TPs of pharmaceuticals has been rarely performed in terms of such broad number of TPs in samples collected in Poland, hence the presented results might be very useful in terms of their future risk assessment in Poland.

Taking into account the state of the knowledge and the obtained results on the presence of different pharmaceuticals and their TPs in different analysed waters as well as the limited knowledge on the stability of many pharmaceuticals, and especially their transformation products (as it was presented in the theoretical part of this thesis), it was decided to investigate their hydrolytic stability in the environmental waters according to the standardised OECD 111 procedure. It was proved that most of the investigated compounds at pH 4, 7 and 9 (CBZ, 10-OH-CBZ, 2-OH-CBZ, IBU, 2-OH-IBU, cx-IBU, TRA, O-DMTRA, 5-FU, MTX, 7-OH-MTX and ac-SMX) were stable. Only CP, IF (at all pH), 4-OH-DIC at pH 4 and 9, MTZ-OH at pH 9 and CBZ-ep at pH 4 were determined as hydrolytically unstable. However, at environmentally relevant conditions their degradation might be estimated as very slow (at 20 °C the degradation was either not observed for 30 days or the $t_{1/2}$ was from 80 to 8 days). The information on the stability of the investigated compounds, especially TPs of the pharmaceuticals, contribute to the knowledge on their persistency, which may be important in the context of the assessment of their environmental fate. Moreover, such standardized results can be utilised for the evaluation of the environmental risk posed by these compounds.

Taking into account the obtained results, it was also decided to evaluate the application of multi-walled carbon nanotubes (MWCNTs) for their removal from water. Moreover, the possibility of their reuse was investigated by thermal and chemical regeneration of the material and the assessment of their adsorption capacity after the treatment. As a result it was shown that thermal regeneration at 300 °C for 2 h did not influence the adsorption of three model compounds: CP, IF and 5-FU. In fact, the isotherm models fitted to the results indicate, that the adsorption capacity increased after thermal treatment. However, it was also proved that flushing the MWCNTs with HNO_3 or HCl has an impact on the adsorbent as the adsorption of CP decreases significantly after such process. Nevertheless, it was shown that these acids are sufficient to remove metals from MWCNTs. These results provide information that carbon nanotubes can be regenerated and reused multiple times. However, the regeneration with acids needs further, comprehensive investigation. Furthermore, it was preliminarily assessed that the application of MWCNTs/chitosan membranes

were effectively removing only selected analytes (DIC, 4-OH-DIC, ac-SMX and to some extent CBZ and 2-OH-CBZ) of 13 in total and their efficiency increased after the thermal regeneration of MWCNTs. Short contact time and more tightly packed material in comparison to the dispersive mode could have had an influence on the overall performance. Nevertheless, knowing how good adsorbent the MWCNTs are and the successful development of hybrids with chitosan suggest, that selection of better conditions may have key role in the performance of the membrane. The presented studies on the removal of broad range of compounds, which included PTPs, by the MWCNTs and MWCNTs/chitosan membranes are a novelty in this field, which give a new insight of their application for such purpose.

7. ACKNOWLEDGEMENTS

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ABSTRACT

The main aim of this thesis was to develop novel and sensitive analytical methods for the determination of selected pharmaceuticals and their transformation products in aqueous environmental samples and apply them in the comprehensive analysis of these compounds in different matrices, during hydrolytic studies and in the evaluation of their removal with multi-walled carbon nanotubes - MWCNTs (which included investigation of the regeneration methods and the performance of the adsorbent after such treatment as well as application of MWCNTs/chitosan membranes) from water. The analytes investigated in this research included various representatives of different therapeutic groups, such as antiepileptic, non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers, antibiotics, opioid analgesics and anticancer drugs as well as their selected TPs.

The analytical methods based on the application of HPLC-UV/Vis technique have been successfully developed for 17 single compounds and applied in model experiments on hydrolytic stability and on the adsorption studies with MWCNTs. Furthermore, the analytical methods with the LC-MS/MS equipment were developed. First, the LC-MS/MS with ion trap analyser was used for the method development and selection of the best SPE conditions for the extraction of the analytes from water samples. The method was evaluated in terms of the matrix effects, extraction efficiency and absolute recovery. Afterwards, the analytical method has been transferred to another, more sensitive and faster LC-MS/MS with triple quadrupole analyser. It was evaluated, fully validated, and finally, applied for the determination of the investigated compounds in various environmental water samples, such as wastewaters and surface waters. Based on the obtained results it was proved that not only pharmaceuticals could be detected in the analysed samples but also their transformation products, including metabolites at the level from hundreds ng/L to even several μg/L in the case of wastewaters or even up to 90 ng/L in the surface water. However, it must be highlighted that in general, surface waters were much less polluted with these compounds and their concentration levels were one, two or even three orders of magnitude lower than in the wastewaters. Nevertheless, carbamazepine was found in almost all analysed samples. Presented data is crucial in terms of their future risk assessment in Poland, as the state of the knowledge on this problem (mainly for pharmaceuticals' transformation products) is still very limited.

Taking into account the obtained results on their inevitable presence in the aquatic environment and still limited data on their stability in the environment, investigations of the hydrolytic stability of 17 pharmaceuticals and their transformation products have been performed according to the standardized guideline OECD 111. It was proved that most of the selected analytes may be

classified as hydrolytically stable under environmental conditions (their $t_{1/2}$ > 1 year at 25 °C). Only cyclophosphamide, ifosfamide, carbamazepine-10,11-epoxide, hydroxymetronidazole and 4-hydroxydiclofenac have been evaluated as unstable; however, at the environmentally relevant temperatures (20 °C and less) almost all of them could be recognized as persistent. Only carbamazepine-10,11-epoxide at pH 4 was degraded quite quickly with the half-life of around 8 days at 20 °C. The presented data is crucial in their future risk assessment, as the state of the knowledge on their fate in the environment is still not sufficiently known.

Furthermore, special attention has been paid to the assessment of the potential application of selected type of the MWCNTs for the removal of such pollutants from water samples. This aspect included the evaluation of the possibility of their regeneration and reuse as well as possibility to use them in combination with chitosan as MWCNTs/chitosan based membranes. As a result, it was shown that thermal regeneration of MWCNTs can be successfully performed at 300 °C and no influence was observed on the adsorption of three anticancer drugs – cyclophosphamide, ifosfamide and 5-fluorouracil after treatment. The Freundlich and Langmuir isotherm parameters indicated that the adsorption capacity after such treatment even increased. However, after the chemical regeneration with HNO_3 or HCl there was a decrease in adsorption level of cyclophosphamide, but it was also proven that these acids efficiently remove metals from MWCNTs. Additionally, membranes with MWCNTs and chitosan were evaluated in different setups (type of MWCNTs, pH, number of membranes) for the elimination of pharmaceuticals and their TPs from water in quick process. It was observed that the removal was sufficient only for selected analytes (for example diclofenac, 4-hydroxydiclofenac, N^4-acetylsulfamethoxazole). Therefore, the composition with chitosan as a membrane should be further investigated to find a more efficient way of their performance. Nevertheless, such combination with the MWNCTs/chitosan based membranes was investigated for the first time for the removal of the mixture of different pharmaceuticals and their transformation products.

STRESZCZENIE

Głównym celem pracy było opracowanie nowych i czułych metod analitycznych do oznaczania wybranych farmaceutyków i ich produktów transformacji w wodnych próbkach środowiskowych oraz zastosowanie ich do kompleksowej analizy tych związków w różnych matrycach, a także podczas badań nad stabilnością hydrolityczną oraz do oceny ich usuwania z wody za pomocą wielościennych nanorurek węglowych - MWCNTs (co obejmowało badanie metod regeneracji i wydajności adsorbentu po takim procesie, jak również zastosowanie membran MWCNTs/chitozan). Anality wzięte pod uwagę w tych badaniach obejmowały przedstawicieli różnych grup terapeutycznych, takich jak leki przeciwpadaczkowe, niesteroidowe leki przeciwzapalne (NLPZ), beta-blokery, antybiotyki, opioidowe leki przeciwbólowe i przeciwnowotworowe oraz ich wybrane produkty transformacji.

Metody analityczne oparte na zastosowaniu techniki HPLC-UV/Vis zostały z powodzeniem opracowane dla 17 pojedynczych związków i zastosowane w modelowych eksperymentach dotyczących stabilności hydrolitycznej oraz w badaniach adsorpcji z MWCNTs. Ponadto, opracowano metody analityczne z wykorzystaniem techniki LC-MS/MS. W pierwszej kolejności, do opracowania metody i wyboru najlepszych warunków SPE do ekstrakcji analitów z próbek wodnych, wykorzystano technikę LC-MS/MS z pułapką jonową. Opracowana procedura analityczna została oceniona pod względem efektów matrycowych, wydajności ekstrakcji i odzysku bezwzględnego. Następnie dokonano transferu tejże procedury analitycznej do innego zestawu LC-MS/MS - bardziej czułego i szybszego, wyposażonego w analizator typu potrójny kwadrupol. Została ona oceniona i poddana pełnej walidacji, a następnie zastosowana do oznaczania badanych związków w różnych próbkach wód środowiskowych, takich jak ścieki i wody powierzchniowe. Na podstawie uzyskanych wyników udowodniono, że w analizowanych próbkach można wykryć nie tylko farmaceutyki, ale także ich produkty transformacji, w tym metabolity na poziomie od setek ng/L do nawet kilku µg/L w przypadku ścieków lub nawet do 90 ng/L w wodach powierzchniowych. Należy jednak podkreślić, że generalnie wody powierzchniowe były znacznie mniej zanieczyszczone tymi związkami, a ich stężenia były o jeden, dwa, a nawet trzy rzędy wielkości niższe niż w ściekach. Niemniej, karbamazepina występowała w prawie wszystkich analizowanych próbkach. Przedstawione dane są istotne z punktu widzenia przyszłej oceny ryzyka związanego z tymi substancjami w Polsce, gdyż stan wiedzy na ten temat (głównie w odniesieniu do produktów transformacji leków) jest nadal bardzo ograniczony.

Biorąc pod uwagę uzyskane wyniki dotyczące ich potwierdzonej i nieuniknionej obecności w środowisku wodnym oraz wciąż ograniczone dane na temat ich stabilności w środowisku, przeprowadzono badania stabilności hydrolitycznej 17 farmaceutyków i produktów ich przemian zgodnie ze standardowymi wytycznymi OECD 111. Wykazano, że większość z wybranych analitów można zaklasyfikować jako stabilne hydrolitycznie w warunkach środowiskowych (ich $t_{1/2}$ > 1 rok w temperaturze 25 °C). Jedynie cyklofosfamid, ifosfamid, epoksyd karbamazepiny, hydroksymetronidazol i 4-hydroksydiklofenak zostały ocenione jako niestabilne; jednakże w temperaturach występujących w środowisku (20 °C i mniej) prawie wszystkie z nich można uznać za trwałe. Jedynie epoksyd karbamazepiny w pH 4 ulegał dość szybkiej degradacji, a jego okres półtrwania w temperaturze 20 °C wynosił około 8 dni. Przedstawione dane mają kluczowe znaczenie dla przyszłej oceny ryzyka związanego z tymi substancjami, ponieważ stan wiedzy na temat ich losów w środowisku jest wciąż niewystarczający.

Ponadto, szczególną uwagę zwrócono na ocenę możliwości zastosowania wybranych typów MWCNTs do usuwania tego typu zanieczyszczeń z próbek wody. Aspekt ten obejmował ocenę możliwości ich regeneracji i ponownego wykorzystania, jak również możliwość zastosowania ich w połączeniu z chitozanem jako membran. W rezultacie wykazano, że regeneracja termiczna MWCNTs może być z powodzeniem przeprowadzona w temperaturze 300 °C i nie zaobserwowano negatywnego wpływu na adsorpcję trzech leków przeciwnowotworowych - cyklofosfamidu, ifosfamidu i 5-fluorouracylu po tym procesie. Parametry wyznaczonych izoterm Freundlicha i Langmuira wskazywały, że pojemność sorpcyjna po regeneracji termicznej nawet wzrosła. Natomiast po regeneracji chemicznej za pomocą HNO_3 lub HCl nastąpił spadek poziomu adsorpcji cyklofosfamidu, ale udowodniono również, że kwasy te skutecznie usuwają metale z MWCNTs. Dodatkowo, membrany z MWCNTs i chitozanem były oceniane w różnych konfiguracjach do eliminacji leków i ich produktów transformacji z wody w szybkim procesie. Zaobserwowano, że usuwanie było skuteczne tylko dla wybranych analitów (np. diklofenak, 4-hydroksydiklofenak, N^4-acetylosulfametoksazol). Dlatego też tego typu membrany opierające się o połączenie chitozanu i MWCNTs powinny być dalej badane w celu znalezienia bardziej efektywnego sposobu ich zastosowania. Jednakże, taki materiał został wykorzystany po raz pierwszy do zbadania usuwania mieszaniny leków i ich produktów transformacji z wody.

ACADEMIC ACHIEVEMENTS

Summarized impact factor (IF)	39.75
Summarized impact factor (IF) of the papers directly regarding the PhD thesis	22.50
Summarized points of MEiN (former MNiSW) category	630
Number of citations*	67
Number of citations (without self-citations)*	51

*according to Scopus database

PUBLICATIONS

1. Maculewicz Jakub, Kowalska Dorota, Świacka Klaudia, **Toński Michał**, Stepnowski Piotr, Białk-Bielińska Anna, Dołżonek Joanna: Transformation products of pharmaceuticals in the environment: their fate, (eco)toxicity and bioaccumulation potential, Science of the Total Environment, vol. 802, 2022, s. 1-31, DOI:10.1016/j.scitotenv.2021.149916, **IF 7.963**;

2. **Toński Michał**, Dołżonek Joanna, Stepnowski Piotr, Białk-Bielińska Anna: Hydrolytic stability of anticancer drugs and one metabolite in the aquatic environment, Environmental Science and Pollution Research, vol. 28, nr 41, 2021, s. 57939-57951, DOI:10.1007/s11356-021-14360-0, IF **4.223**;

3. **Toński Michał**, Paszkiewicz Monika, Dołżonek Joanna, Flejszar Mariusz, Bielicka-Giełdoń Aleksandra, Stepnowski Piotr, Białk-Bielińska Anna: Regeneration and reuse of the carbon nanotubes for the adsorption of selected anticancer drugs from water matrices, Colloids and Surfaces A – Physicochemical and Engineering Aspects, Elsevier BV, vol. 618, 2021, s. 1-9, DOI:10.1016/j.colsurfa.2021.126355, **IF 4.539**;

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5. **Toński Michał**, Dołżonek Joanna, Paszkiewicz Monika, Wojsławski Jerzy, Stepnowski Piotr, Białk-Bielińska Anna: Preliminary evaluation of the application of carbon nanotubes as potential adsorbents for the elimination of selected anticancer drugs from water matrices, Chemosphere, vol. 201, 2018, s. 32-40, DOI:10.1016/j.chemosphere.2018.02.072, **IF 5.108**;

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7. Mioduszewska Katarzyna, Dołżonek Joanna, Wyrzykowski Dariusz, Kubik Łukasz, Wiczling Paweł, Sikorska Celina, **Toński Michał**, Kaczyński Zbigniew, Stepnowski Piotr, Białk-Bielińska Anna: Overview of experimental and computational methods for the determination of the pKa values of 5-fluorouracil, cyclophosphamide, ifosfamide, imatinib and methotrexate, Trac-Trends in Analytical Chemistry, vol. 97, 2017, s. 283-296, DOI:10.1016/j.trac.2017.09.009, **IF 7.034**.

ORAL PRESENTATIONS AT THE CONFERENCES

1. Godlewska Klaudia, **Toński Michał**, Paszkiewicz Monika, Stepnowski Piotr: Badanie zależności między czynnikami środowiskowymi a szybkością poboru β-blokerów i sulfonamidów z wody przez CNTs-PSDs, 2020, VI Ogólnopolska Konferencja Interdyscyplinarna "EUREKA" 2020;

2. **Toński Michał**, Godlewska Klaudia, Białk-Bielińska Anna, Stepnowski Piotr: Wielościenne nanorurki węglowe jako materiał do usuwania zanieczyszczeń z wody: sorpcja oraz możliwość wielokrotnego stosowania wobec wybranych leków przeciwnowotworowych, 2020, VI Ogólnopolska Konferencja Interdyscyplinarna "EUREKA";

3. **Toński Michał**, Flejszar Mariusz, Paszkiewicz Monika, Białk-Bielińska Anna, Stepnowski Piotr: Ocena potencjału sorpcyjnego nanorurek węglowych poddanych regeneracji wobec wybranych leków przeciwnowotworowych, 2019, XVII Ogólnopolskie Seminarium Doktorantów "Na Pograniczu Chemii i Biologii";

4. Stepnowski Piotr, Mioduszewska Katarzyna, **Toński Michał**, Białk-Bielińska Anna: Wybrane leki przeciwnowotworowe w środowisku: analityka, trwałość, rozprzestrzenianie i efekty toksykologiczne, 2018, 61. Zjazd Naukowy Polskiego Towarzystwa Chemicznego;

5. Toński Michał, Białk-Bielińska Anna, Dołżonek Joanna, Borecka Marta, Wojsławski Jerzy, Paszkiewicz Monika, Stepnowski Piotr: Determination of pharmaceuticals and their transformation products in water samples using SPE-LC-MS/MS method, 2018, 3rd International Caparica Christmas Conference on Sample Treatment;

6. Wojsławski Jerzy, **Toński Michał**, Białk-Bielińska Anna, Stepnowski Piotr, Dołżonek Joanna: Approach for the assessment of chemicals mobility in soil using percolation column test, 2018, 3rd International Caparica Christmas Conference on Sample Treatment;

7. Białk-Bielińska Anna, Mioduszewska Katarzyna, Dołżonek Joanna, **Toński Michał**, Marcin Stokowski, Mulkiewicz Ewa, Stepnowski Piotr: Assessment of mobility, hydrolytic stability and ecotoxicity of anticancer drugs and their transformation products in the environment, 2017, VIII International Scientific Conference "Toxic substances in the environment";

8. **Toński Michał**, Maszkowska Joanna, Paszkiewicz Monika, Wojsławski Jerzy, Stepnowski Piotr, Białk-Bielińska Anna: Ocena zastosowania nanorurek węglowych jako efektywnych adsorbentów do usuwania pozostałości wybranych leków przeciwnowotworowych z wód ściekowych, 2016, V Ogólnopolska Konferencja Młodych Naukowców "Człowiek, Nauka, Środowisko";

9. Wojsławski Jerzy, Białk-Bielińska Anna, **Toński Michał**, Mioduszewska Katarzyna, Paszkiewicz Monika, Stepnowski Piotr, Maszkowska Joanna: Ocena możliwości zastosowania nanorurek węglowych jako efektywnych adsorbentów do usuwania wybranych cieczy jonowych z matryc wodnych, 2016, V Ogólnopolska Konferencja Młodych Naukowców "Człowiek, Nauka, Środowisko".

POSTER PRESENTATIONS

1. Godlewska Klaudia, **Tońsk**i **Michał**, Paszkiewicz Monika, Stepnowski Piotr: Wpływ rodzaju nanorurek węglowych użytych jako sorbent w próbnikach pasywnych na szybkość poboru wybranych farmaceutyków z wody, 2020, VI Ogólnopolska Konferencja Interdyscyplinarna "EUREKA";

2. **Tońsk**i **Michał**, Białk-Bielińska Anna, Godlewska Klaudia, Stepnowski Piotr: Trwałość farmaceutyków oraz ich metabolitów w roztworach wodnych, 2020, VI Ogólnopolska Konferencja Interdyscyplinarna "EUREKA";

3. Tońska Elżbieta, Klepacka Joanna, Michalak Joanna, **Tońsk**i **Michał**: Lead and cadmium in conventional and organic carrots, 2019, 13th European Nutrition Conference "Malnutrition in an obese world: European perspectives";

4. Tońska Elżbieta, Michalak Joanna, Klepacka Joanna, **Tońsk**i **Michał**: Variability of selected microelements in organic carrots, 2019, 13th European Nutrition Conference "Malnutrition in an obese world: European perspectives";

5. **Tońsk**i **Michał**, Flejszar Mariusz, Paszkiewicz Monika, Białk-Bielińska Anna, Stepnowski Piotr: Wpływ kwasu azotowego (V) na strukturę i pojemność sorpcyjną regenerowanych termicznie nanorurek węglowych, 2019, XIII Kopernikańskie Seminarium Doktoranckie;

6. **Tońsk**i **Michał**, Wojsławski Jerzy, Białk-Bielińska Anna, Stepnowski Piotr: Application of the HPLC technique for the assessment of the hydrolytic stability of carbamazepine and its transformation products, 2018, 41st Symposium "Chromatographic Methods of Investigating Organic Compounds";

7. **Tońsk**i **Michał**, Wojsławski Jerzy, Białk-Bielińska Anna, Stepnowski Piotr: Stabilność hydrolityczna produktów transformacji popularnych farmaceutyków, 2018, X Polska Konferencja Chemii Analitycznej "Od chemii wszystko się zaczyna";

8. Wojsławski Jerzy, **Tońsk**i **Michał**, Białk-Bielińska Anna, Dołżonek Joanna, Stepnowski Piotr: Ocena potencjału sorpcyjnego wybranych farmaceutyków oraz ich produktów przemiany w układzie gleba/roztwór, 2018, X Polska Konferencja Chemii Analitycznej "Od chemii wszystko się zaczyna";

9. Wojsławski Jerzy, **Tońsk**i **Michał**, Białk-Bielińska Anna, Stepnowski Piotr, Dołżonek Joanna: The assessment of carbamazepine and 10,11-dihydro-10-hydroxy carbamazepine sorption in soil, 2018, 41st Symposium "Chromatographic Methods of Investigating Organic Compounds";

10. Białk-Bielińska Anna, **Tońsk**i **Michał**, Waniek Ewelina, Karnialiuk Aliona, Dołżonek Joanna, Stepnowski Piotr: Badanie stabilności hydrolitycznej wybranych leków przeciwnowotworowych o charakterze jonowym i jednego metabolitu, 2017, V Konferencja Naukowa "Monitoring i analiza wody. Chromatograficzne metody oznaczania substancji o charakterze jonowym";

11. Białk-Bielińska Anna, Mioduszewska Katarzyna, Dołżonek Joanna, **Tońsk**i **Michał**, Marcin Stokowski, Mulkiewicz Ewa, Stepnowski Piotr: Liquid chromatography in the modeling studies on the mobility, hydrolytic stability and ecotoxicity of selected anticancer drugs and their transformation products in the environment, 2017, 24th International Symposium on Electro- and Liquid Phase-Separation Techniques (ITP2017) & XI Polska Konferencja Chromatograficzna "Chromatography in pharmacy and bioanalysis" (PKChrom 2017);

12. **Toński Michał**, Białk-Bielińska Anna, Dołżonek Joanna, Borecka Marta, Wojsławski Jerzy, Paszkiewicz Monika, Stepnowski Piotr: Development of the LC-MS/MS method for the determination of selected pharmaceuticals and their transformation products in water samples, 2017, VIII International Scientific Conference "Toxic substances in the environment";

13. **Toński Michał**, Karnialiuk Aliona, Waniek Ewelina, Grabarczyk Łukasz, Wojsławski Jerzy, Dołżonek Joanna, Białk-Bielińska Anna, Stepnowski Piotr: Ocena stabilności hydrolitycznej wybranych farmaceutyków i ich produktów transformacji z zastosowaniem techniki HPLC-UV/Vis w oznaczeniach końcowych, 2017, V Konferencja Naukowa "Monitoring i analiza wody. Chromatograficzne metody oznaczania substancji o charakterze jonowym";

14. **Toński Michał**, Waniek Ewelina, Karnialiuk Aliona, Wojsławski Jerzy, Dołżonek Joanna, Białk-Bielińska Anna, Stepnowski Piotr: Określenie stabilności hydrolitycznej jako element oceny ekspozycji na popularne farmaceutyki i ich produkty transformacji w środowisku, 2017, VI Ogólnopolska Konferencja Młodych Naukowców "Człowiek, Nauka, Środowisko";

15. Wojsławski Jerzy, **Toński Michał**, Białk-Bielińska Anna, Stepnowski Piotr, Dołżonek Joanna: Ocena mobilności tramadolu oraz o-desmetyl tramadolu w środowisku glebowym, 2017, VI Ogólnopolska Konferencja Młodych Naukowców "Człowiek, Nauka, Środowisko";

16. Wojsławski Jerzy, **Toński Michał**, Białk-Bielińska Anna, Stepnowski Piotr, Dołżonek Joanna: The assessment of imidazolium ionic liquids mobility in soil, 2017, VIII International Scientific Conference "Toxic substances in the environment";

17. Wojsławski Jerzy, **Toński Michał**, Białk-Bielińska Anna, Stepnowski Piotr, Dołżonek Joanna: Wykorzystanie metod chromatograficznych do oznaczeń końcowych w ocenie sorpcji tramadolu oraz O-desmetyltramadolu, 2017, V Konferencja Naukowa "Monitoring i analiza wody. Chromatograficzne metody oznaczania substancji o charakterze jonowym";

18. Tońska Elżbieta, Paszczyk Beata, **Toński Michał**: Mleko różnych gatunków ssaków jako źródło wybranych mikroelementów, 2016, XVI Międzynarodowa Konferencja Polskiego Towarzystwa Magnezologicznego im. prof. Juliana Aleksandrowicza "Jawny i Utajony Niedobór Magnezu" & XIII Sympozjum "Pierwiastki Śladowe w Środowisku - Problemy Ekologiczne i Analityczne";

19. Tońska Elżbieta, Klepacka Joanna, Łuczyńska Joanna, **Toński Michał**: Nasiona warzyw strączkowych, jako źródło wybranych składników mineralnych, 2016, IX Ogólnopolska Konferencja Naukowa Technologów Przetwórstwa Owoców i Warzyw "Owoce, Warzywa, Grzyby - Żywność i Technologia";

20. Tońska Elżbieta, Paszczyk Beata, **Toński Michał**: Porównanie zawartości magnezu i wapnia w mleku krowim, owczym i kozim, 2016, XVI Międzynarodowa Konferencja Polskiego Towarzystwa Magnezologicznego im. prof. Juliana Aleksandrowicza "Jawny i Utajony Niedobór Magnezu" & XIII Sympozjum "Pierwiastki Śladowe w Środowisku - Problemy Ekologiczne i Analityczne";

21. Siemianowska Ewa, Wesołowski Andrzej, Barszcz Agnieszka, Tońska Elżbieta, **Toński Michał**: Wzbogacanie kruchych ciastek w składniki mineralne poprzez dodatek wytłoków owocowych, 2016, XVI Międzynarodowa

Konferencja Polskiego Towarzystwa Magnezologicznego im. prof. Juliana Aleksandrowicza "Jawny i Utajony Niedobór Magnezu" & XIII Sympozjum "Pierwiastki Śladowe w Środowisku - Problemy Ekologiczne i Analityczne";

22. Tyburski Józef, Skibniewska Krystyna, Siemianowska Ewa, Warechowska Małgorzata, Sadowski Tadeusz, Tońska Elżbieta, Rychcik Bogumił, **Toński Michał**: Ziarno niewymłacanych pszenic (samopszy, płaskurki i orkiszu) jako źródło mikroelementów, 2016, XVI Międzynarodowa Konferencja Polskiego Towarzystwa Magnezologicznego im. prof. Juliana Aleksandrowicza "Jawny i Utajony Niedobór Magnezu" & XIII Sympozjum "Pierwiastki Śladowe w Środowisku - Problemy Ekologiczne i Analityczne";

23. Łukaszewicz Paulina, Antczak Dominika, Mioduszewska Katarzyna, Toński Michał, Wojsławski Jerzy, Stepnowski Piotr: Testowanie wybranych mieszanin ekstrakcyjnych do wydzielania leków przeciwbakteryjnych z gleb rolniczych przy wykorzystaniu ekstrakcji wspomaganej promieniowaniem mikrofalowym, 2016, III Ogólnopolska Konferencja Młodych Naukowców Nauk Przyrodniczych "Wkraczając w świat nauki";

24. Mioduszewska Katarzyna, Łukaszewicz Paulina, Toński Michał, Wojsławski Jerzy, Stepnowski Piotr: Sprawdzenie potencjału sorpcyjnego 5-fluorouracylu w układzie gleba/roztwór, 2016, III Ogólnopolska Konferencja Młodych Naukowców Nauk Przyrodniczych "Wkraczając w świat nauki";

25. Tońska Elżbieta, Klepacka Joanna, Łuczyńska Joanna, Siemianowska Ewa, Barszcz Agnieszka, Toński Michał: Toxic elements (Pb, Cd and Hg) in organic and conventional carrots, 2016, XVI Międzynarodowa Konferencja Polskiego Towarzystwa Magnezologicznego im. prof. Juliana Aleksandrowicza "Jawny i Utajony Niedobór Magnezu" & XIII Sympozjum "Pierwiastki Śladowe w Środowisku - Problemy Ekologiczne i Analityczne";

26. Tońska Elżbieta, Łuczyńska Joanna, Klepacka Joanna, Siemianowska Ewa, **Toński Michał**: Wpływ zawartości miazgi kakaowej na poziom magnezu w czekoladach, 2016, XVI Międzynarodowa Konferencja Polskiego Towarzystwa Magnezologicznego im. prof. Juliana Aleksandrowicza "Jawny i Utajony Niedobór Magnezu" & XIII Sympozjum "Pierwiastki Śladowe w Środowisku - Problemy Ekologiczne i Analityczne";

27. **Toński Michał**, Maszkowska Joanna, Paszkiewicz Monika, Wojsławski Jerzy, Mioduszewska Katarzyna, Stepnowski Piotr, Białk-Bielińska Anna: Ocena właściwości sorpcyjnych nanorurek węglowych jako potencjalnych sorbentów do usuwania pozostałości cyklofosfamidu z wód ściekowych, 2015, IV Ogólnopolska Konferencja Studentów i Doktorantów Nauk Ścisłych "Człowiek, Nauka, Środowisko";

28. Wojsławski Jerzy, Maszkowska Joanna, Białk-Bielińska Anna, **Toński Michał**, Mioduszewska Katarzyna, Paszkiewicz Monika, Stepnowski Piotr: Sorpcja cieczy jonowych do nanorurek węglowych jako potencjalnego adsorbentu w oczyszczaniu ścieków, 2015, IV Ogólnopolska Konferencja Studentów i Doktorantów Nauk Ścisłych "Człowiek, Nauka, Środowisko".

PARTICIPATION IN THE PROJECTS

1. Participant of the intercollegiate and international Ph.D. programme: „Chemistry for Health and the Environment (INTERCHEM)" including the InterPhD2 (PO WER) programme: "Rozwój interdyscyplinarnego programu studiów doktoranckich o wymiarze międzynarodowym", co-financed by European Union from the European Social Fund – Operational Programme Knowledge, Education, Development: POWR.03.02.00-IP.08-00-DOK/16;

2. Investigator in the grant OPUS „Pharmaceuticals and their transformation products in the environment: analytics, ecotoxicology and risk assessment", Project number: 2015/17/B/NZ8/02481; from April 2016 to January 2019;

3. Annual project leader in BMN programme (Badania Młodych Naukowców) – 2017, 2018 and 2019.

INTERNSHIPS

1. Grupo de Reacção e Análises Químicas (GRAQ), Instituto Superior de Engenharia do Porto, Polytechnic Institute of Porto. November 2021 – May 2022;

2. Institute of Water Chemistry, Department of Hydrosciences, Technical University of Dresden. April 2019 – August 2019.