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ARTICLE

Anticancer Drug Docetaxel in Hospital Effluent
Development of Chromatographic Method, Occurrence, and Degradation via Ozonation

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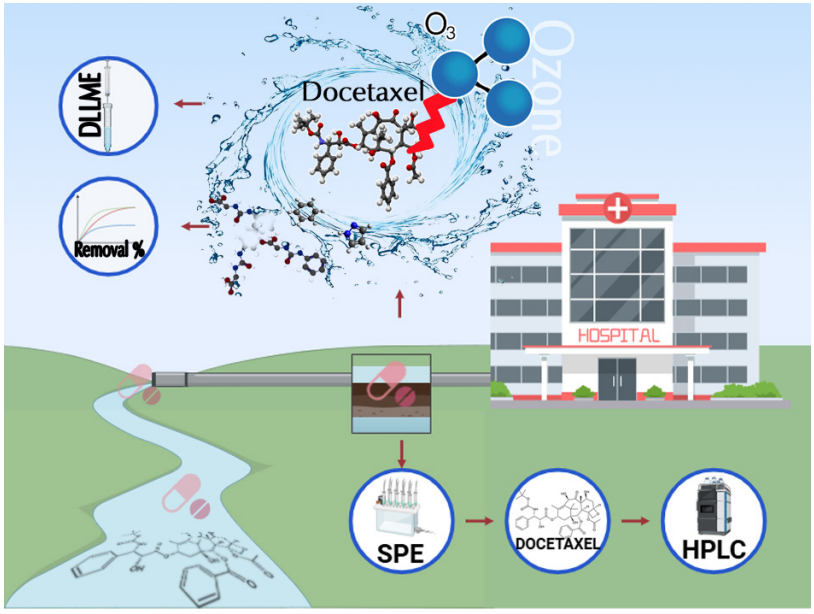

Anticancer agents, specifically designed to induce cell death in neoplastic cells, have become increasingly prevalent contaminants in various environmental matrices. Their extensive use has resulted in their detection across multiple settings, including hospital and pharmaceutical factory effluents, domestic wastewater, and surface waters. This study aimed to develop an analytical method for determining docetaxel residues in hospital effluent using Solid-Phase Extraction (SPE) and HPLC-DAD. The SPE method demonstrated R^2 greater than 0.99, with recovery rates reaching 95% and an RSD of less than 2%. The LOQ was established at 10.0 μg L^{-1}, with no significant matrix effects observed for docetaxel. The validated SPE method was deemed suitable for its intended application, as all evaluated parameters met the specifications outlined in current regulatory guidelines. Docetaxel was quantified at 29.9 μg L^{-1} (±1.3%) in one of the 14 effluent samples collected over a week from the Federal University of Santa Maria Hospital (HUSM). Given that the microbiological treatment system at HUSM does not effectively remove this drug, an advanced degradation process using ozonation was investigated. A Dispersive Liquid-Liquid Microextraction (DLLME) method was developed for bench-scale degradation studies and subsequently applied to assess the degradation of docetaxel via ozonation in real samples. The optimized extraction conditions for docetaxel involved a 10 mL hospital effluent sample at pH 9, with ionic strength adjusted using Na_2SO_4. Methanol served as the disperser solvent, while chloroform was the extracting solvent. The ozonation process achieved

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a removal efficiency exceeding 97% after 60 minutes of reaction, under optimal conditions of pH 7 and an average ozone production rate of 1.5 g O_3 h^{-1}. These findings indicate that ozonation is an effective treatment strategy, significantly reducing docetaxel concentrations in hospital effluent within a short reaction timeframe.

Keywords: anticancer docetaxel, DLLME, SPE, HPLC-DAD, advanced oxidation process

INTRODUCTION

Cancer poses a major public health challenge, especially in developing countries.[1] It ranks among the top four leading causes of death before the age of 70. The global incidence and mortality rates of cancer have been rising, driven in part by increased exposure to risk factors, socioeconomic development, population aging, and growth.[2] The most diagnosed types include breast, colorectal, and lung cancers.[3] According to the World Health Organization, cancer remains the second most common cause of death worldwide, accounting for 9.6 million fatalities in 2018.[4] Projections indicate that the number of new cancer cases could surpass 25 million by 2030. In Brazil, it is estimated that approximately 704,000 new cancer cases will be diagnosed annually from 2023 to 2025, excluding non-melanoma skin cancer, which is expected to contribute an additional 483,000 cases each year.[5]

The increasing prevalence of cancer among the population has led to a surge in the consumption of anticancer drugs.[6,7] This rise in usage directly influences the amount of these substances introduced into the environment, suggesting a likely increase in their discharge in the coming years.[8] Cytotoxic drugs, widely used in cancer therapy, exert their effects by damaging DNA, inhibiting synthesis, and disrupting cell replication. Due to their non-selective action, these medications impact all proliferating cells, not just cancerous ones.[9] As a result, they pose significant health risks, exhibiting potential genotoxic, cytotoxic, carcinogenic, mutagenic, teratogenic, and endocrine-disrupting effects on non-target organisms.[7,8]

Anticancer drugs are among the pharmaceutical classes with the greatest potential to cause harmful environmental impacts. However, despite growing scientific interest in their presence in environmental samples, the number of studies on these compounds remains limited.[8,10-12] Developing analytical methods for detecting anticancer compounds in both aqueous and solid environmental matrices is critically important, yet only a few such methods are currently available. Several studies have reported the presence of specific anticancer drugs in hospital effluents at concentrations ranging from ng L^{-1} to µg L^{-1}.[8,14,16,18,19] The detection of antineoplastic agents in aquatic environments highlights significant risks to both human and ecological health, emphasizing the need for robust analytical techniques capable of identifying and quantifying these compounds at trace levels.[8,20,21]

Many of these studies have reported the inefficacy of conventional microbiological treatment processes in entirely removing or mineralizing anticancer drugs from wastewater widely employed due to their capacity to produce effluents that meet quality standards with relatively low operating and maintenance costs;[6,11,22,23] they often fail to eliminate pharmaceutical contaminants effectively. Implementing a complementary pre-treatment or post-treatment step specifically designed to target pharmaceutical residues could serve as a viable strategy to mitigate the continuous release of these contaminants into the environment.[24] Among the treatment technologies, Advanced Oxidation Processes (AOPs) have shown particular promise for degrading emerging contaminants, including pharmaceuticals and other micropollutants, offering a robust alternative for enhancing effluent quality.[11,25]

Among AOPs, ozonation is an effective and promising technique for removing various microcontaminants, including anticancer drugs, from aqueous environmental matrices.[26] Recent studies have demonstrated the efficacy of ozonation in degrading anticancer compounds across different water samples, including ultrapure water,[27] drinking water,[28] hospital effluent,[10,11] and urban wastewater.[29-31] Docetaxel, in particular, is recognized as one of the most potent chemotherapeutic agents used in the treatment of solid tumors, such as breast cancer. This study aimed first to establish a robust analytical method for quantifying docetaxel in hospital effluent samples using solid-phase extraction (SPE). Subsequently, this validated method was applied to assess the occurrence of docetaxel in real effluent

samples. Finally, the research focused on optimizing a dispersive liquid-liquid microextraction (DLLME) procedure to facilitate degradation studies, investigating the potential of ozonation as an effective strategy for removing and degrading docetaxel in hospital effluent.

MATERIALS AND METHODS

Chemicals

Docetaxel ≥97% was used (Sigma Aldrich, United States of America). The following solvents and reagents were used: carbon tetrachloride, chloroform, and acetone from Merck (Germany). Methanol, formic acid, acetonitrile, and sodium chloride were obtained from J.T. Baker (United States of America). Chlorobenzene and ammonium formate were purchased from Sigma Aldrich (United States of America), sodium hydroxide, and sulfate sodium (Mallinckrodt, Mexico). Ultrapure water (18 MΩ cm, MerckMillipore, United States of America).

HPLC-DAD conditions

The analysis to quantify the docetaxel was performed using a Prominence liquid chromatograph system connected to a diode array detector (DAD) (Shimadzu, Kyoto, Japan). The HPLC was equipped with a C18 Inertsil ODS column (150 x 4,6 mm, 5 µm) (GL Sciences, Torrance, USA), maintained at a temperature of 35 °C. The mobile phase consisted of (A) acetonitrile and (B) ultrapure water, pH 4.5, adjusted with formic acid, with a flow rate of 0.7 mL min^{-1}. Isocratic elution mode comprised the following ratios: Phase A (55%) and Phase B (45%). The injection volume was set to 15 µL.

Extraction using SPE

Three types of SPE cartridges (3 mL/500 mg) were tested: Chromabond® C18 EC (end-capped octadecyl modified sílica, carbon content 14%) and C18 (octadecyl modified silica phase for SPE, not end-capped, carbon content 14%), both purchased from Macherey-Nagel (Düren, Germany); and Supelcosil® C8 (monomerically bonded, octyl, end-capped, carbon content 7%), from Supelco (Milford, EUA). The influence of sample pH (pH 5.0, 7.0, and 9.0) was investigated due to its significant impact on extraction efficiency.

The SPE procedure was optimized based on a standard protocol[33] recommended by the manufacturer and is described as follows. A Phenomenex vacuum manifold was used for this purpose. Each SPE cartridge was initially conditioned and pre-equilibrated with 6 mL of methanol, followed by 6 mL of water. A 100 mL sample was then passed through the cartridge, followed by a washing step using 3 mL of a methanol-water mixture (5:95, v/v) to remove impurities. The analytes were eluted with 2 × 2 mL of methanol and evaporated to near dryness under a gentle stream of nitrogen in a temperature-controlled bath set at 40 °C. Finally, the sample was reconstituted in 1 mL of methanol.

After the optimization phase, the SPE method was validated and applied to determine the occurrence of docetaxel anticancer in effluent from the University Hospital of the Federal University of Santa Maria (HUSM).

Analytical method validation procedures

The analytical method was validated by assessing various parameters, including linearity, precision, accuracy, LOQ, and matrix effect. The validation process adhered to the guidelines specified in ISO/IEC 17025 and the SANTE/11312/2021.[34,35]

For the linearity study, docetaxel analytical solutions were prepared in methanol and a matrix extract (“blank”) in seven concentrations between 0.8 – 75 mg L^{-1} (equivalent to 8.0 – 750 µg L^{-1}) in the hospital effluent samples (considering the 100x concentration factor).

Using the area and concentration values, an analytical calibration curve was obtained, linear function ($y = ax + b$), as well as the values of the coefficient of determination (R^2), angular coefficient (a), and linear coefficient (b). The linearity was further confirmed by residual analysis, which helps assess the goodness of fit of regression models and identify potential issues like outliers or nonlinearity.

The matrix effects were quantitatively evaluated by comparing the slope of the matrix-matched calibration curve with that of the docetaxel standard calibration curve prepared in solvent solutions (methanol), as described in Equation 1. The matrix effects within the range of ± 20% are not significant.[35]

$$Matrix\ Effect\ (\%) = \left[\left(\frac{slope\ curve, std.\ in\ effluent\ matrix\ extract}{slope\ curve, std.\ in\ neat\ methanol}\right) - 1\right] x\ 100 \qquad \text{(Equation 1)}$$

The accuracy was evaluated through recovery experiments using docetaxel standards at three concentration levels (10, 50, and 200 μg L^{-1}), and recovery values between 70 and 120% were considered acceptable.[35] Precision refers to the degree of agreement among multiple measurements expressed as the relative standard deviation (RSD%), with RSD ≤ 20% considered as the acceptability level.[35]

The limit of quantitation (LOQ) was defined as the lowest concentration of the docetaxel that can be measured with acceptable accuracy and precision.[35]

This validation process was applied to the optimized SPE and DLLME methods.

Occurrence of docetaxel in real samples

The HUSM, located in Santa Maria city, Rio Grande do Sul state, Brazil, serves a population of nearly 1 million people. As a reference center for oncology, it provides care to approximately 25,000 patients monthly.

Effluent treatment at HUSM employs a basic biological treatment system, after which the treated wastewater is released into a nearby water body. The hospital has two separate treated effluent streams labeled A and B, which are processed separately. For this study, samples from these two discharge points were collected over 7 days using a daily composite sampling method to ensure a representative effluent analysis.

Each subsample was homogenized, placed in an amber bottle, and transported to the laboratory in a cooler. Upon arrival, the samples were filtered using 47 mm hydrophilic polyvinylidene fluoride (PVDF) membrane filters with a 0.45 μm pore size (Millipore). The filtered samples were then stored at temperatures below 8 °C and analyzed preferably on collection day. Strict safety protocols were followed throughout the process due to the toxic nature of the samples.[36-38]

For the ozonation study, samples were taken from the discharge point of the oncology ward effluent (Point A). These samples were filtered using a cellulose qualitative filter, stored in amber glass containers, and kept below 8 °C until analysis.

Degradation studies on the lab scale

For ozonation studies involving real samples, it is essential to employ an extraction method that can effectively pre-concentrate the analyte, eliminate matrix interferences, and operate with minimal sample volumes, particularly given the limited capacity of bench-scale reactors. In this regard, exploring DLLME as a sample preparation technique for aliquots collected during degradation studies becomes highly relevant. Although SPE offers several advantages, it is unsuitable for this specific application. SPE typically requires a larger sample volume than feasible in bench-scale experiments. Additionally, reaction kinetics studies demand multiple sampling points, resulting in a substantial need for SPE cartridges, escalating the cost, duration of the procedure, and the sample volume required. Given these limitations, the initial step before conducting degradation studies was to develop and optimize the DLLME methodology. DLLME was selected due to its ability to work with smaller sample volumes, its lower solvent volume requirements, and the elimination of SPE cartridges, thereby reducing overall costs and providing a more efficient alternative.

Ecotoxicological risk assessment

The Risk Quotient (RQ) method is successfully applied to evaluate ecotoxicological risk by evaluating exposure and hazardous concentration. RQ was calculated as the measured environmental concentrations (MEC) ratio to the Predicted No-Effect Concentration (PNEC). For docetaxel, the RQ was estimated using the effluent concentration and a PNEC value sourced from the literature (5.50 μg L^{-1}).[39] The PNEC value for docetaxel was obtained using the Ecological Structure-Activity Relationships (QSAR) predictive model when experimental data were unavailable.

Optimizing the extraction process using DLLME

For the DLLME optimization study, the optimal extractor and disperser solvent combination was determined to maximize analyte extraction efficiency. Dichloromethane, chloroform, chlorobenzene, and carbon tetrachloride were evaluated as extraction solvents due to their density and immiscibility in water. Additionally, acetonitrile (ACN), acetone (ACE), ethanol (EtOH), and methanol (MeOH) were tested as disperser solvents.

The tests were carried out by matching each extractant with each dispersant in pairs, generating sixteen extraction solvent/disperser solvent pairs: carbon tetrachloride/ACE, carbon tetrachloride/MeOH, carbon tetrachloride/EtOH, carbon tetrachloride/ACN, dichloromethane/ACE, dichloromethane/MeOH, dichloromethane/EtOH, dichloromethane/ACN, chloroform/ACE, chloroform/MeOH, chloroform/EtOH, chloroform/ACN, chlorobenzene/ACE, chlorobenzene/MeOH, chlorobenzene/EtOH, chlorobenzene/ACN. The initial volumes of the disperser and extractor solvents tested were 750 μL and 250 μL, respectively.

The initial DLLME tests were performed according to the protocol described by Souza et al:[8] A 10 mL hospital effluent sample, adjusted to pH 7 and spiked with docetaxel, was used for the extraction process. A mixture of 1 mL of extraction and disperser solvents was added, creating a turbid solution. After allowing the solution to stand for 1 minute, the mixture was centrifuged at 4000 rpm for 5 minutes, resulting in a sedimented phase at the bottom of the tube. The solvent was evaporated in a temperature-controlled bath at 40 °C under a gentle nitrogen stream. The resulting extract was reconstituted in 100 μL of methanol and analyzed using HPLC-DAD. To assess the influence of ionic strength, 0.5 mol L^{-1} of salts (Na_2SO_4 and NaCl) was introduced, and the outcomes were compared to those from the procedure without salt addition.

A multivariate experimental designer was employed using a Plackett-Burman Design (PBD) to evaluate the influence of several factors, including sample pH, initial salt concentration in the sample (ionic strength), volumes of extraction and disperser solvents, interaction time, centrifugation duration, and centrifugation speed. Following identifying significant variables from the PBD, further optimization was performed using a Central Composite Design (CCD) to fine-tune these key parameters.

Study of degradation via ozonation

The degradation of docetaxel in treated effluent from HUSM was studied using a batch microdispersion bubble reactor connected to an ozone generator (Ozonebras, Brazil). The system operated with dry air, achieving an average ozone production rate of 1.5 g O_3 h^{-1}, was adapted from the system described by Souza et al.[11] For the degradation experiments, real hospital effluent was employed as the matrix, using 2 L of sample per batch.

The first stage of the degradation experiments involved spiking hospital effluent samples with a standard docetaxel solution at a concentration of 10 mg L^{-1}, higher than environmental concentrations to enable degradation monitoring and kinetic studies. Degradation experiments were conducted under three different pH conditions (5.0, 7.0, and 9.0), with ozonation performed for 60 minutes. Aliquots were collected at specific time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, and 60 minutes), extracted using the optimized DLLME method from the previous step, and analyzed by HPLC-DAD.

The optimized method was applied to a hospital effluent sample containing 29.9 μg L^{-1} of docetaxel, a concentration similar to that observed in the hospital effluent analyzed in this study.

RESULTS AND DISCUSSION

Extraction using SPE

The selection of sorbent is a crucial factor that must be optimized when developing a new SPE procedure. The properties of both the sample matrix and the physicochemical characteristics of the target analytes influence this decision. In addition to a C8 cartridge, two types of C18 cartridges were tested (*endcapped and non-endcapped*). *Endcapped* cartridges have methylated silica surfaces to reduce polar interactions, enhancing selectivity for nonpolar compounds. In contrast, *non-endcapped* cartridges retain free silanol groups, increasing interactions with polar analytes and making them suitable for compounds that benefit from additional hydrogen bonding or dipolar interactions. The choice depends on the analytes' polarity and desired interactions. The Supercosil® C8 cartridge was selected in this study due to its higher recovery efficiency for docetaxel under the experimental conditions (Table I).

Table I. Docetaxel recoveries (%) for different tested SPE cartridges

Sorbent	Recovery (%)		
	pH 5	pH 7	pH 9
Chromabond® C18 ec	79.9	81.3	73.2
Chromabond® C18	87.3	95.1	72.5
Supercosil® C8	99.6	88.8	75.9

As shown in Table I, the Supercosil® C8 cartridge exhibited the highest recovery rate, particularly notable at a sample pH of 5. While the Chromabond® C18 cartridge also demonstrated promising results, the selection of the Supercosil® C8 cartridge was motivated by its better cost-effectiveness ratio.

The optimized SPE extraction procedure involved the following steps: initially, the Supercosil® C8 cartridge (3 mL/500 mg) was conditioned with 5 mL of methanol, followed by 5 mL of ultrapure water adjusted to pH 7. Subsequently, a 100 mL aliquot of hospital effluent was passed through the cartridge. After loading the sample, the cartridge was rinsed with 2 mL of ultrapure water and dried for 2 minutes. Docetaxel was eluted using 2 mL of methanol. The eluates were evaporated using a temperature-controlled bath at 40 °C under nitrogen flow. The detailed steps of the optimized SPE procedure are depicted in the flowchart in Figure 1.

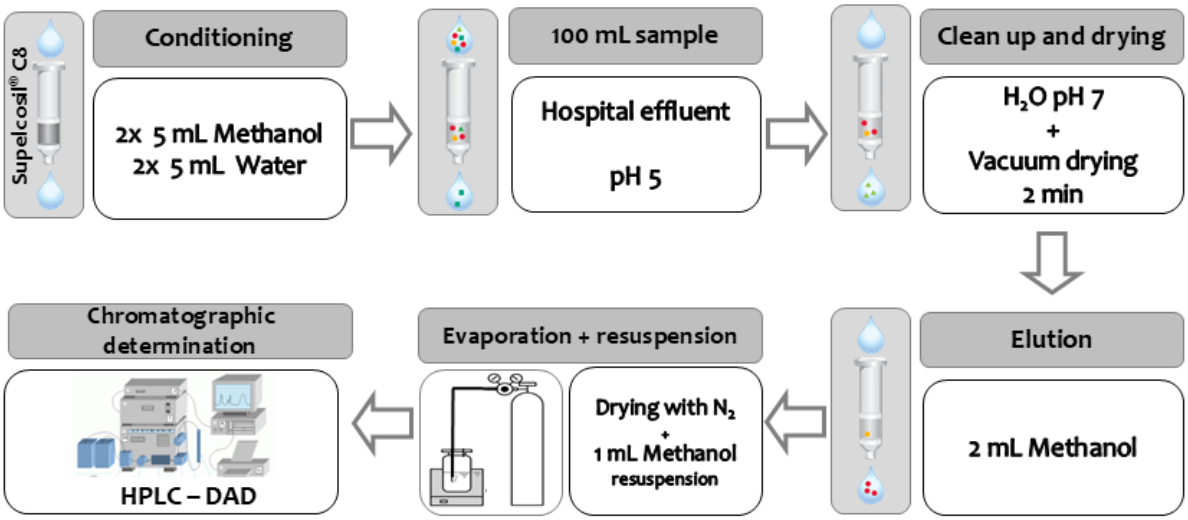


Figure 1. Diagram illustrating the optimized SPE method for extracting docetaxel from hospital effluent samples.

Samia et al.[40] developed an analytical method for determining pharmaceutical compounds in natural water sources, achieving optimal analyte recoveries using Waters Oasis HLB SPE cartridges. Similar to our study, the pre-treatment step of acidifying samples before SPE was optimized to improve compound recovery. SPE has been effectively employed in various studies to detect anticancer drugs in aqueous matrices, including hospital effluent, surface water, and urban wastewater.[8,19,21,31,39]

Analytical method validation procedures

Method validation is a crucial aspect of any analytical process. It involves defining the analytical requirements and confirming that the method in question possesses the necessary performance characteristics to meet the specific application needs. In essence, method validation ensures that the results are demonstrably "fit for purpose".

Accordingly, the method developed and optimized in this study was validated following the performance criteria outlined in the SANTE/11312/2021 guideline[35] and by the ISO/IEC 17025:2017[34] quality standards. The validation results are summarized in Table II.

Table II. Analytical method validation parameters evaluated for the SPE method: coefficient of determination (R^2), precision (expressed in terms of RSD%), accuracy (expressed as recovery %), and matrix effect (%).

Linearity (R^2)		Docetaxel	Extraction by SPE		
Analytical curve prepared in		µg L^{-1}	Recovery (%)	RSD (%)	Matrix Effect (%)
Methanol	Matrix extract				
0.9997	0.9996	10	94.9	1.3	0.7%
		50	95.0	1.1	
		200	95.5	0.4	

For standard solutions prepared in methanol, an organic solvent, the coefficient of determination (R^2) was ≥ 0.999, while for standard solutions prepared in matrix extracts of hospital effluent, R^2 values were **≥** 0.998 (Figure 2). However, the correlation coefficient alone is not sufficient to ensure the adequacy of the linear fit to the calibration curve; it is essential to evaluate the potential presence of residuals or poorly distributed points across the linear range. The residuals (differences between the observed values and their fitted values) were also examined for outliers and patterns that might invalidate the calibration curve. The assumptions that the regression residuals follow a normal distribution (Anderson-Darling test), are homoscedastic (Cochran test), and are independent (Durbin-Watson test) were confirmed, ensuring the reliable application of the analytical curve for docetaxel quantification. The calibration curve equations were estimated using the ordinary least squares (OLS) method and employed to calculate the concentrations of the analytes.

The method exhibited excellent linearity within the range of 0.8 to 75 mg L^{-1}, equivalent to 8.0 to 750.0 µg L^{-1} in the effluent sample, considering a 100-fold concentration factor. As for the matrix effect, a value of 0.7% was observed for the SPE method, as shown in Table II. Docetaxel recoveries exceeded 94%, confirming the high accuracy of the SPE method.

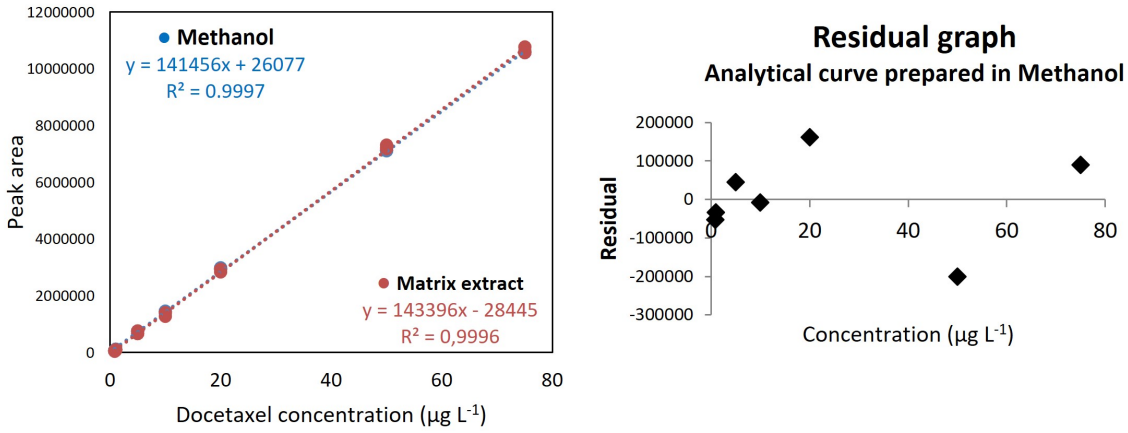


Figure 2. Representation of the analytical calibration curve for docetaxel prepared in solvent (methanol) and in matrix extract, along with the residuals of the regression analysis of the calibration curve.

Occurrence of docetaxel in real samples

The docetaxel was investigated in 14 samples, seven at each sampling point. Docetaxel was determined in only one of the 14 samples at a concentration of 29.9 µg L^{-1} (±1.3%). Considering that docetaxel is not used continuously, it is acceptable that it was only quantified on a specific day. It is possible that on the other days, the drug could be present, however, in concentration <LOQ method. The occurrence of this drug in the effluent (the HUSM releases that into the environment) is worrying from an environmental point of view since docetaxel, according to Environmentally Classified Pharmaceuticals, has maximum risk in terms of toxicity, bioaccumulation, and persistence in the environment. Furthermore, predicted values of log K_{ow} for docetaxel (2,83) suggest bioaccumulation in aquatic biota.[39]

The calculated RQ indicates a moderate risk to aquatic organisms (RQ = 5.44). The risk is classified into four levels based on RQ values: RQ < 0.1 indicates insignificant risk (no adverse effects expected); 0.1 < RQ < 1 denotes low risk; 1 < RQ < 10 signifies moderate risk (probable adverse effects); and RQ > 10 indicates high risk (adverse effects).[19,41,42]

Souza et al.[8] previously reported the presence of four anticancer drugs in the treated effluent of HUSM, highlighting the inefficiency of the microbiological treatment methods employed for the degradation of these compounds. The authors detected irinotecan, doxorubicin, epirubicin, and daunorubicin in the effluent samples from HUSM at concentrations that suggest a high-risk environment. Also, in Brazil, the Klein research group[39] reported the detection of three anticancer drugs – gemcitabine, 5-fluorouracil, and cyclophosphamide – along with two metabolites in the effluents from a cancer hospital, as well as in the influent and effluent of the municipal wastewater treatment plant in Barretos, São Paulo.

Despite its toxicity, docetaxel has been little studied, and there are few works in the literature about its occurrence in environmental matrices.[43] Ferrando-Climent and coworkers[14,22] in Spain reported the occurrence of docetaxel in hospital and urban effluent samples. Values of up to 79 ng L^{-1} in hospital effluent and up to 219 ng L^{-1} in urban effluent have been reported. The authors highlighted that docetaxel was the only anticancer drug found at higher levels in urban sewage than in hospital effluent among the 10 anticancer drugs investigated. According to the authors, this fact can be explained by its slow metabolism in the human organism: docetaxel can be excreted in up to seven days both in the urine and in the feces (6% and 75%, respectively). Consequently, the patient is no longer hospitalized.

Degradation studies in Lab Scale

The first step involved optimizing a DLLME extraction method to facilitate the kinetic study of degradation. This method was selected based on its applicability to small aliquots of effluents, its rapid execution, efficiency in removing interferences, and compatibility with the chromatographic system. Since hospital

effluent is a complex matrix, injecting it without prior sample treatment could compromise the system. After optimizing the DLLME method, it was applied to the study of docetaxel ozonation in effluent samples.

Extraction using DLLME

The selection of appropriate extractor and disperser solvents is a critical factor influencing the efficiency of the DLLME procedure. Chloroform and methanol were identified as the optimal extractor and disperser solvents, respectively, as shown in Figure 3. Chloroform possesses several characteristics that render it an effective solvent, including a higher density than water (1.49 g mL^{-1}), good solubility for the target analytes, immiscibility with water, and a relatively low boiling point, facilitating rapid evaporation. Conversely, methanol, with a density of 0.79 g mL^{-1}, was selected as the disperser solvent due to its primary attribute of being soluble in both the donor and acceptor phases,[40] which is essential for the DLLME process.

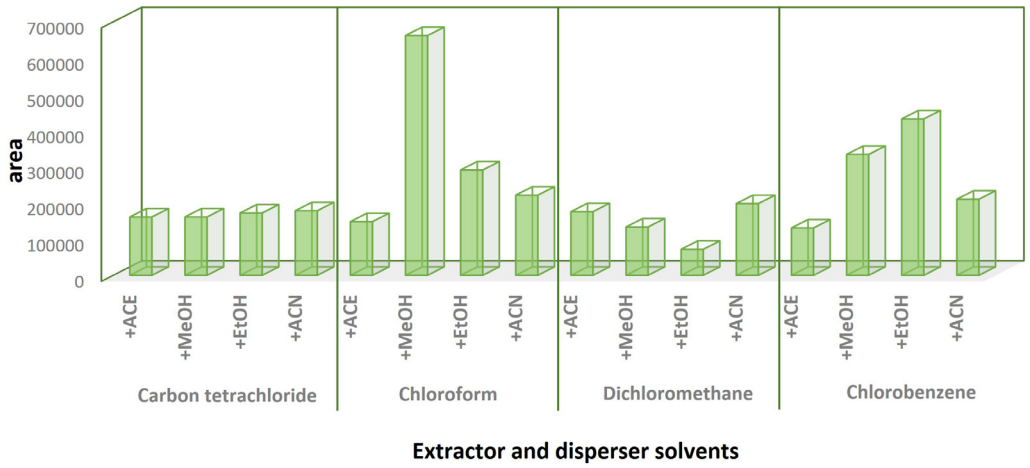


Figure 3. Influence of different combinations of extractor and disperser solvents on the efficiency of docetaxel extraction by DLLME. Abbreviations: ACE, acetone; MeOH, methanol; EtOH, ethanol; ACN, acetonitrile.

Another study conducted within our research group[8] explored the application of DLLME to determine additional anticancer drugs in hospital effluent. Consistent with these results, the combination of chloroform and methanol exhibited superior efficiency in extracting all analytes when compared to the alternative mixtures investigated.

Reichert et. al.[44] investigated the DLLME for the determination of antipsychotics in hospital effluent. According to the findings reported by the authors, the optimal combination of extractor and disperser solvents for extracting this class of drugs consisted of chlorobenzene and methanol, respectively.

The influence of different salts on DLLME efficiency was assessed by incorporating 0.5 mol L^{-1} (w/v) of NaCl and Na_2SO_4, compared to tests conducted without salt addition. The lowest recoveries for docetaxel were observed in the absence of salt, while the highest recoveries were achieved by adding Na_2SO_4. Adding salt can enhance extraction yields by reducing the solubility of the target compounds in the aqueous phase and facilitating their transfer to the organic phase, a phenomenon known as salting out.[44]

Following the selection of solvents and determination of the optimal salt type for extraction, quantitative factors potentially affecting docetaxel recovery were simultaneously investigated using a Plackett-Burman Design (PBD) with 16 experiments. This approach facilitated a screening process to identify variables significantly impacting the recovery of the target analytes, namely, ionic strength, volumes of extractor and disperser solvents, and sample pH (see Figure 4). Interaction time, speed, and duration of centrifugation were found to lack statistical significance at a confidence level of 95%.

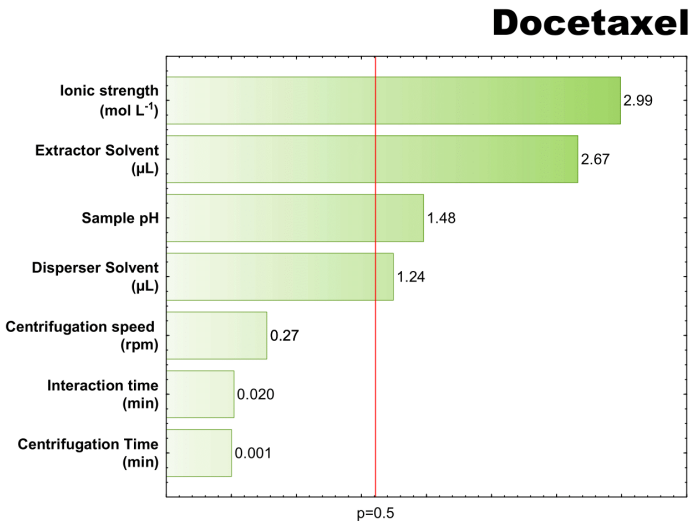


Figure 4. Pareto diagram of the docetaxel extraction parameters using DLLME. The values exceeding the red line represent the factors significantly affecting the extraction, with 95% confidence.

From identifying the parameters that significantly influence docetaxel extraction, a CCD involving 26 experiments was conducted to optimize the proposed methodology, according to data in Table III. The results obtained for the Predicted Values of the optimized DLLME variables for each parameter are shown in Figure 5.

Table III. Experimental design for the optimization of DLLME variables using a central composite design

	Levels				
Variables	**Low (-)**	**Center (0)**	**High (+)**	**-α**	**+α**
Sample pH	5	7	9	3	11
Disperser solvente – methanol (µL)	550	600	650	500	700
Extractor solvent – chloroform (µL)	210	240	270	180	300
Ionic strength – Na_2SO_4 (mol L^{-1})	0.3	0.5	0.7	0.1	0.9

Sample pH is an important parameter in optimizing the extraction, considering that it has an influence not only on increasing the efficiency of the process but also on the selectivity. Docetaxel is a compound with basic characteristics, with pK_a 10.96. Therefore, at acid pH values, it is positively charged, which is unfavorable to the extraction of analytes. Experimentally, it was possible to demonstrate this since there was an increase in the extraction efficiency and an increase in the sample pH. Based on the results obtained through the CCD, the pH condition of the sample was selected at 9. From the prediction graph, one could expect a reduction in the extraction of analytes at pH values >9. However, the interaction between pH and ionic strength must be considered.

When evaluating the graph of pH vs. ionic strength (Figure 5), it is noted that there would be a tendency to reduce the extraction of analytes at pH above 9 when in low or high concentrations of Na_2SO_4; however, in the intermediate conditions of study (center point), the increase in pH does not influence in the same way, it does not show this decrease, demonstrating the interaction between these two variables. This way, the concentration of 0.5 mol L^{-1} of Na_2SO_4 (ionic strength) was selected as the appropriate condition (Figures 5 and 6).

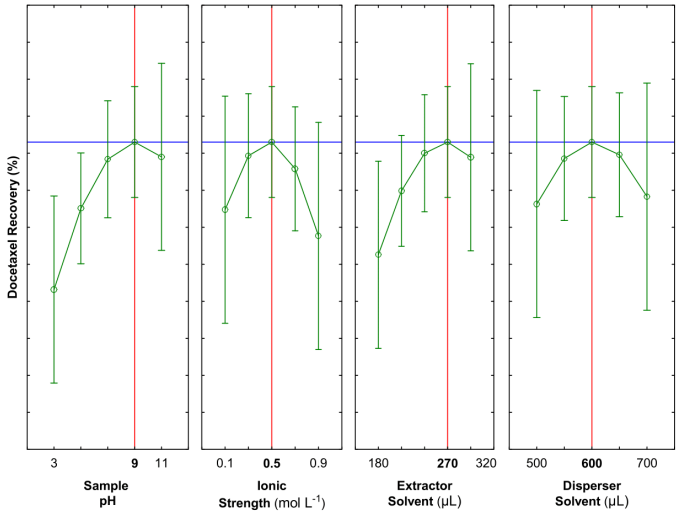
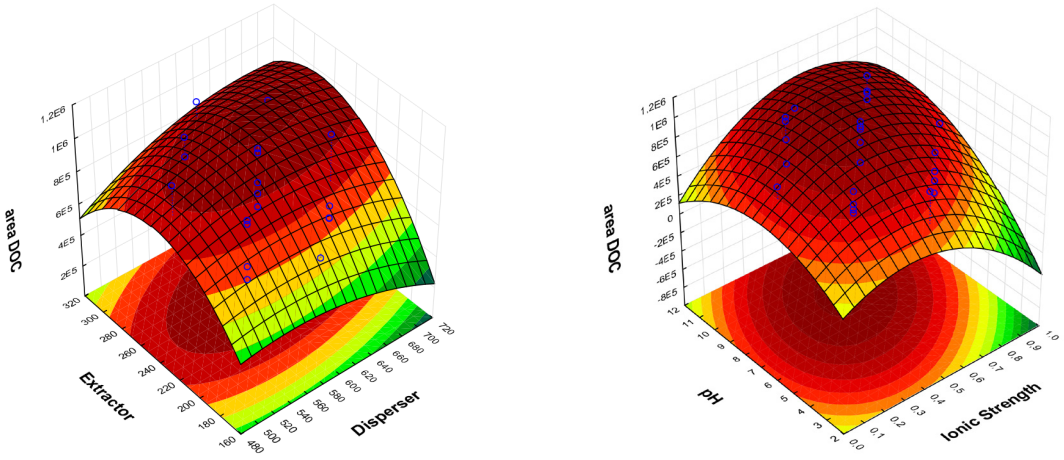


Figure 5. Predicted values of the optimized DLLME variables.



(a) (b)

Figure 6. Response surface plots of (a) extractor solvent vs. dispersor solvent volume and (b) ionic strength vs. sample pH; on docetaxel recoveries after extraction by DLLME.

In a study conducted by Mostafa and collaborators,[45] concentrations of NaCl were evaluated in the range of 0 – 12% (w/v), for the extraction of sulfonamides in different water samples using DLLME. Similar to what was shown in this study, the authors reported that the increase in salt concentrations provided an increase in the extraction of analytes. However, they noted that concentrations greater than 10% did not promote improvements in extraction efficiency. On the other hand, Coutinho et al.[46] when optimizing the conditions for the determination of bisphenols and benzophenone in complex water matrices using DLLME, reported that the best extraction conditions were achieved when no type of salt was used. That is, the increase in ionic strength had a negative effect on the extraction of analytes.

The volume of the disperser solvent plays a crucial role in forming microdroplets consisting of water, disperser solvent, and extractor solvent. It also impacts the extent of solvent dispersion within the aqueous phase, affecting the overall extraction efficiency. Similarly, the volume of the extractor solvent can alter the volume of the sedimented phase. In this study, the volumes of methanol and chloroform were simultaneously optimized using CCD. The extraction efficiency was observed to increase with the addition of solvent up to 270 µL, after which a decline in efficiency occurred. A similar trend was noted with the disperser volume, where efficiency improved up to 600 µL, followed by a subsequent decrease.

The optimized extraction conditions for docetaxel involved using a 10 mL sample of hospital effluent at pH 9 with 0.5 mol L^{-1} Na_2SO_4. A volume of 600 µL of methanol was used as the disperser solvent, and 270 µL of chloroform served as the extractor solvent. After one minute, the mixture underwent centrifugation for 5 minutes at 2000 rpm, and the lower phase was collected with a microsyringe. The collected extract was then evaporated under a nitrogen stream in a temperature-controlled bath set at 40 °C, reconstituted with 100 µL of methanol, and subsequently analyzed using HPLC-DAD (Figure 7). The quantification limit for docetaxel, as determined by SPE, was set at 10.0 µg L^{-1}.

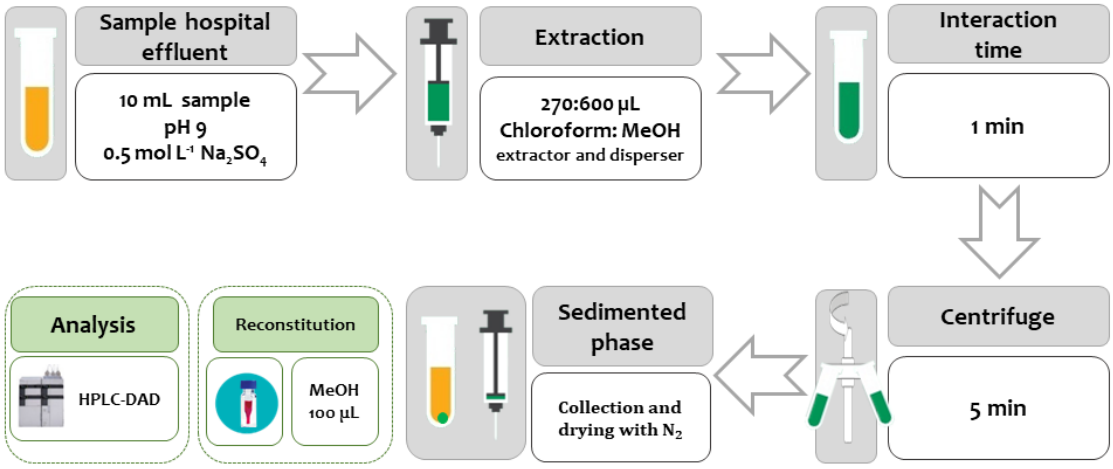


Figure 7. DLLME extraction diagrams.

DLLME was initially introduced as an alternative analytical technique, but it has gained attention due to its environmental friendliness and effectiveness in the pretreatment of aqueous samples. The method is characterized by its simplicity, low cost, and rapid processing time, requiring only minimal amounts of samples and organic solvents. These attributes make DLLME particularly suitable for bench-scale drug degradation studies, where sample quantities are often restricted. It is highly reproducible and provides satisfactory results for the intended application in degradation studies. The optimized procedure was evaluated and validated according to the figures of merit outlined in the guidelines, following the SANTE/11312/2021 standards.[35] The results are summarized in Table IV.

Table IV. Analytical method validation parameters evaluated for the DLLME method: coefficient of determination (R^2), precision (expressed in terms of RSD%), accuracy (expressed as recovery %), and matrix effect (%).

Linearity (R^2)		Docetaxel	Extraction by DLLME		
Analytical curve prepared in		µg L^{-1}	Recovery (%)	RSD (%)	Matrix Effect (%)
Methanol	Matrix extract				
0.9997	0.9984	50	67.1	2.6	5.8 %
		200	67.6	2.9	
		500	71.2	1.8	

According to the SANTE/11312/2021 document,[35] which provides guidelines for validating analytical methods for pesticide residues in the European Union, the recovery rates for analytical methods applied to agricultural and environmental samples should be adequate between 70% and 120%. However, suppose

the recovery falls outside this range. In this case, it may still be acceptable, provided it is between 60% and 140%, and the method demonstrates repeatability and precision, taking into account the matrix complexity and the low concentration levels of the analyte in the sample.

In a similar context, Becker et al.[47] optimized DLLME for use in degradation experiments. The researchers focused solely on the peak area of the analytes during the optimization process rather than recovery percentages. Their study selected chloroform as the extractor solvent, while acetonitrile was used as the disperser solvent. Within the tested range, the ionic strength of the solution did not significantly influence the analytical response. This method effectively extracted diazepam and its transformation products during the solar photo-Fenton process from different matrices, including ultrapure water, simulated wastewater, and hospital effluent.

Ozonation study in Lab Scale

The incomplete removal of pharmaceutical residues and their metabolites by conventional wastewater treatment systems has raised significant concerns due to the potential risks these compounds pose to human health and environmental integrity. To address the limitations of traditional microbiological treatment methods, an advanced oxidation process, specifically ozonation, was proposed for the degradation of docetaxel.

Hospital effluent with a natural neutral pH was used for the ozonation experiments. To evaluate the influence of pH on the degradation efficiency of docetaxel, additional tests were conducted at pH levels of 5.0 and 9.0. The impact of pH on the degradation of organic pollutants primarily manifests in two ways: (i) the transfer of ozone from the gas phase to the liquid phase (direct effect), and (ii) the decomposition of ozone into reactive radicals (indirect effect).[48] Generally, the ozone decomposition rate increases with higher pH, as hydroxide ions catalyze the formation of radicals and other reactive oxygen species.[49]

As shown in Figure 8, variations in pH did not significantly influence the reaction rates ($p > 0.05$), indicating that the oxidation of docetaxel was predominantly facilitated by molecular ozone rather than by radical-mediated mechanisms. Under the experimental conditions, the ozonation process achieved a removal efficiency exceeding 97%. Consequently, a neutral pH of 7 was selected, corresponding to the natural average pH of the effluent generated by the hospital's wastewater system. The degradation was rapid, with the concentration of docetaxel reduced to less than 3% within the first 15 minutes of treatment. Importantly, this residual concentration remained stable for up to 60 minutes,

Comparable findings were reported by Pérez Rey et al.,[50] who investigated various anticancer drugs, including azathioprine, cytarabine, methotrexate, and 5-fluorouracil, in aqueous solutions at pH levels of 3 and 7. Their results indicated complete degradation of the anticancer compounds to below detectable levels within 75 minutes, with no significant variation in degradation rates across the tested pH range.[50]

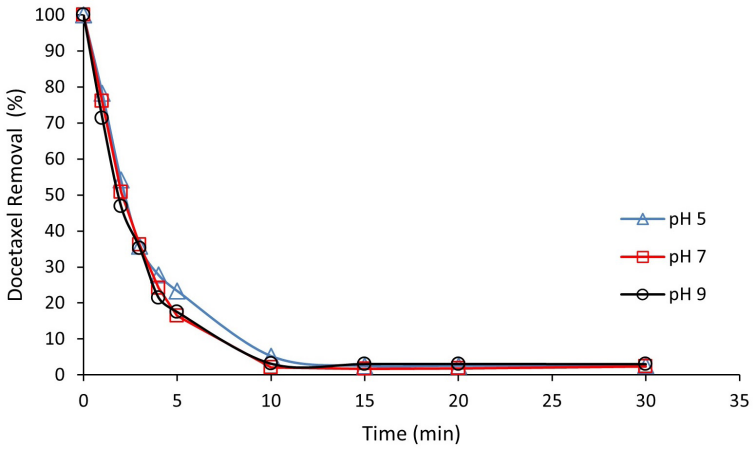


Figure 8. Effect of hospital effluent pH on the removal of docetaxel in O_3 system.

Ozonation is a promising technique for removing anticancer compounds from aqueous environments. However, data on the reaction kinetics between these pharmaceuticals and ozone are still scarce, which poses a challenge for conducting a comprehensive economic assessment of this treatment method. In the current study, the ozonation experiments revealed that the degradation of docetaxel followed pseudo-first-order reaction kinetics, as depicted in Figure 9.

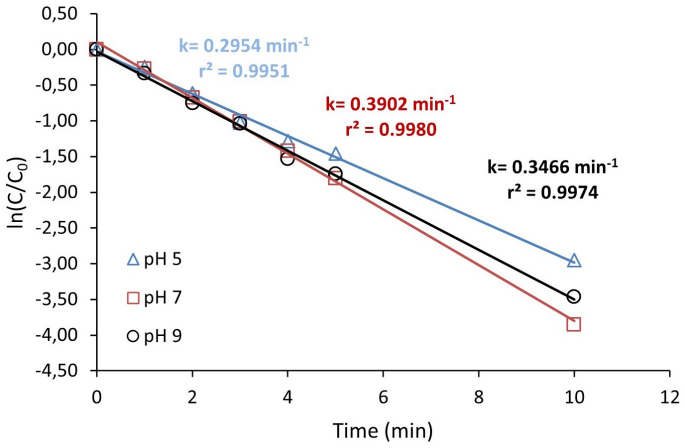


Figure 9. Degradation of docetaxel in different reaction systems and first-order kinetic constants for degradation reactions. Symbols represent experimental data, and a continuous line represents the first-order reaction kinetic model for Docetaxel degradation.

For degradation in pH = 5.0, a pseudo-zero-order rate constant of k = 0.2954 min^{-1} was observed. After 60 minutes of reaction under acidic conditions, 97.5% of the initial docetaxel concentration was degraded. Under natural conditions (pH = 7) a pseudo-first-order kinetic constant of k= 0.3902 min^{-1} was observed, resulting in 97.6% removal of docetaxel. Finally, at alkaline pH (pH = 9.0), the rate constant of k= 0.3466 min^{-1} achieved 96.8% degradation of docetaxel after 60 minutes of reaction.

In the study conducted by Zimmermann and colleagues, the degradation of three anticancer drugs by ozonation was best described by pseudo-first-order reaction kinetics. For the ozonation of capecitabine and irinotecan, reaction times for achieving over 95% removal were less than 20 minutes.[27]

An experiment using an initial docetaxel concentration of 30.0 μg L^{-1} at pH 7 was conducted to evaluate degradation at a concentration close to the observed in real wastewater samples. In these conditions, degradation to below the LOQ was achieved after 60 minutes.

CONCLUSIONS

The study successfully developed and optimized an SPE method for determining docetaxel in hospital effluent using HPLC-DAD analysis. The proposed method demonstrated excellent performance, meeting the acceptance criteria established by standardization agencies regarding linearity, precision, and accuracy while showing no significant matrix effects. Moreover, the SPE method provided an effective sample-cleaning step, ensuring accurate quantification. Docetaxel was quantified in one of the analyzed samples at a concentration that poses a potential moderate risk to aquatic organisms based on the calculated risk quotient.

DLLME was also explored due to its advantageous features, including simplicity, cost-effectiveness, rapid processing, and minimal sample volume requirements. These attributes make DLLME particularly well-suited for bench-scale studies of drug degradation, where limited sample volumes are a common constraint. The optimized DLLME method exhibited recovery rates exceeding 60%, proving to be reproducible and reliable for monitoring degradation processes.

Given the need for effective strategies to mitigate the presence of docetaxel in aquatic environments, ozonation was investigated as a potential treatment option. The results indicate that ozonation is a highly effective method, achieving substantial reductions in the concentration of docetaxel in hospital effluent within a short reaction time. These findings highlight the promise of advanced oxidation processes, such as ozonation, for efficiently removing anticancer drugs from wastewater, contributing to improved environmental safety.

Conflicts of interest

The authors have declared no conflict of interest.

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