

## Article

# A Rapid Analytical Approach for Monitoring Pharmaceuticals in Hospital Wastewater—A DPX-Based Procedure with Environmentally-Friendly Extraction Phase Coupled to High Performance Liquid Chromatography–Diode Array/Fluorescence Detectors

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**Abstract:** In this study, a novel analytical methodology based on disposable pipette extraction (DPX) was developed using an alternative extraction phase for the extraction/determination of six pharmaceutical compounds, including carbamazepine, diclofenac, naproxen, fluoxetine, losartan and 17 $\alpha$ -ethinylestradiol, in samples of hospital wastewater by high-performance liquid chromatography coupled to diode array and fluorescence detectors. The performance of three extraction phases was examined, including 3-n-propyl (3-methylpyridinium) silsesquioxane chloride (Si3Py<sup>+</sup>Cl<sup>−</sup>), the conductive polymer polypyrrole (PPy), and polypyrrole modified with cetyltrimethylammonium bromide (PPy.CTAB). The optimization of the experimental parameters was performed through univariate and multivariate approaches. The optimized condition was obtained with the use of 20 mg of Si3Py<sup>+</sup>Cl<sup>−</sup> as extraction phase; six extraction cycles with 700  $\mu$ L of sample in each cycle and 15 s of extraction time; three desorption cycles with 100  $\mu$ L of ACN (same aliquot) and 15 s of desorption time; and sample pH adjusted at 3.5 and addition of 15% (w/v) of NaCl in the sample. The methodology proposed exhibited environmentally-friendly aspects with a significantly reduced volume of organic solvent (only 100  $\mu$ L) and a small amount of extraction phase (20 mg). In addition, the extraction phase employed exhibits a simple synthetic procedure, low cost, and high stability in organic solvent. Moreover, the method developed exhibits high throughput (extraction time of 6.5 min per sample), and robustness. The analytical figures of merit were obtained using hospital wastewater, and the values were very satisfactory. The correlation coefficients were higher than 0.9710. LODs and LOQs ranged from 0.030  $\mu$ g L<sup>−1</sup> to 1.510  $\mu$ g L<sup>−1</sup> and 0.10  $\mu$ g L<sup>−1</sup> to 5.00  $\mu$ g L<sup>−1</sup>, respectively. Relative recoveries varied from 80 to 127%, and intra-day ( $n = 3$ ) and inter-day ( $n = 9$ ) precision was lower than 19%.

**Keywords:** hospital wastewater; pharmaceutical compounds; disposable pipette extraction; alternative extraction phases; sample preparation

## 1. Introduction

The growing population and higher life expectancy have led to an increased demand for pharmaceuticals [1]. Generally, after use, the final destination of these compounds or their metabolites, if not properly treated, is the environment. The main sources of environmental contamination consist of urine and feces from humans and animals in domestic sewage and also the incorrect disposal of drugs and the discharge of hospital

wastewater [2,3]. Hospital wastewater presents a particular concern because it can represent the main source of pollution with pharmaceutical compounds [4,5]. Even with wastewater treatment, some pharmaceuticals may not be completely removed and, therefore, they can be discarded in the environment [6]. Considering that this process can be continuous, the effect of these compounds in the environment can be similar to that caused by persistent pollutants and thus could cause damage in aquatic systems and to human beings [7]. Non-steroidal anti-inflammatory drugs (NSAID), such as naproxen (NAP) and diclofenac (DCL), exhibit analgesic and antipyretic properties and can be used for headache, muscle pain, and inflammation [8]. Estrogen-based hormones such as 17 $\alpha$ -ethinylestradiol (EE2) are widely used in the formulation of contraceptive pills. Another class of drugs that deserves attention consists of neuroactive drugs, which include antidepressants and anti-epileptic drugs. Among the various types of antidepressants, the most popular are selective serotonin reuptake inhibitors, including fluoxetine (FLU), which is indicated for the treatment of depression, anxiety, and some disorders. Among the anti-epileptic drugs, carbamazepine (CBZ) is widely used for seizures in the treatment of epilepsy and bipolar disorder [9]. Another widely used class consists of antihypertensive drugs, with losartan (LOS) as one of the most common compounds applied for the treatment of hypertension, the protection of the kidneys, and heart failure [10].

A number of methodologies used for the determination of pharmaceutical compounds described in the literature focus on a specific class of analytes [2,10–12]. However, the proposal of analytical methodologies with an emphasis on the determination of compounds of different chemical classes, simultaneously, is an analytical challenge since the compounds generally exhibit different physicochemical properties. Usually, before the instrumental analysis, a suitable sample preparation procedure is necessary in order to evaluate complex matrices since direct analysis is generally restricted. Sample preparation involves removing possible interfering compounds, transferring the analytes to a solution compatible with analytical instrumentation, and concentrating the analytes [13]. The choice of a suitable sample preparation procedure is typically based on the physicochemical properties of the analytes and the extraction phase, as well as the compatibility of the extraction technique with the analytical instrumentation.

Disposable pipette extraction (DPX) is a technique based on the miniaturization of solid phase extraction (SPE), and it consists of a device with capacity of 1 and 5 mL, with a sorbent material dispersed between two filters. The extraction steps involve conditioning the extraction phase to activate the sorbent sites; aspiration of the sample followed by aspiration of air, which promotes a dynamic mixture between the sample and the sorbent phase that can enhance the extraction kinetics; sample cleanup (optional); and desorption of the analytes using an appropriate solvent [14,15]. This technique exhibits some advantages, such as rapid extractions, use of small mass of extraction phase, and the use of low sample and organic solvent volumes.

There are few solid phases commercially available for DPX, with different extraction mechanisms for specific classes of compounds. Therefore, the possibility of using alternative extraction phases is a positive trend for this technique [16–19]. Among these alternative extraction phases are those based on polyaniline composites and cork [16,18]. Herein, the hybrid material named Si3Py<sup>+</sup>Cl<sup>−</sup> ([3-*n*-propyl (3-methylpyridinium) silsesquioxane chloride]) and the conductive polymers PPy (polypyrrole) and PPy.CTAB (polypyrrole modified with cetyltrimethylammonium bromide) are promising due to the hydrogen,  $\pi$ - $\pi$ , and hydrophobic interactions that can take place with the pharmaceutical compounds under study. In addition, the hybrid material Si3Py<sup>+</sup>Cl<sup>−</sup> has already been successfully applied for the extraction of phenolic endocrine-disrupting compounds using the DPX technique [17], and the conductive polymers PPy and PPy.CTAB have recently been used to develop an efficient methodology based on DPX procedure to determine hormones and polycyclic aromatic hydrocarbons [20]. These materials are easy to obtain, present a low cost, and have shown very satisfactory stability in organic solvents. The characterization of these materials has already been performed in previous studies [17,20–22].

Pharmaceutical compounds can be classified as emerging pollutants, which means that the concentrations of these compounds in the environment have not yet been regulated by any legislation [23]. Analytical methods capable of quantifying and monitoring the concentrations of these compounds in environmental matrices are urgently needed, mainly due to the risks that can be related to human health and safety regarding the incorrect disposal of pharmaceutical compounds [24].

This study aims to propose a high-throughput and environmentally-friendly DPX-based procedure using alternative extraction phases ( $\text{Si3Py}^+\text{Cl}^-$ , Ppy, and PPy.CTAB) for the determination of six pharmaceutical compounds (CBZ, DCL, NAP, FLU, LOS, and EE2) in hospital wastewater. The analytical methodology was fully optimized, and the analytical parameters of merit were determined.

## 2. Materials and Methods

### 2.1. Reagents and Materials

Analytical standards of fluoxetine (FLU), carbamazepine (CBZ), losartan (LOS), naproxen (NAP),  $17\alpha$ -ethinylestradiol (EE2), and diclofenac (DCL) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). The chemical structure and some physicochemical properties of the analytes are shown in Table 1. Stock solutions at  $1000 \text{ mg L}^{-1}$  were prepared in methanol (MeOH), and a working solution containing a mix of the analytes at concentration of  $30 \text{ mg L}^{-1}$  was also prepared in MeOH. Acetonitrile (ACN), MeOH, and acetone (ACE) HPLC-grade were supplied by JT Baker (Mallinckrodt, NJ, USA). Hydrochloric acid used for pH adjustment, sodium chloride used to evaluate the salting-out effect, and formic acid used for pH adjustment of the mobile phase were obtained from Vetec (Rio de Janeiro, Brazil). Water was purified in an ultrapure water system (Mega Purity, Billerica, MA, USA). DPX pipette tips of 1 mL without sorbent material (DPX-Blank) were acquired from DPX Labs (Columbia, SC, USA). Tetraethylorthosilicate (TEOS), ethanol, chloropropyltrimethoxysilane (CPTS), toluene, and 3-methylpyridine used in  $\text{Si3Py}^+\text{Cl}^-$  synthesis as well as a pyrrole monomer (Py) used in PPy and PPy.CTAB synthesis were obtained from Sigma-Aldrich (Milwaukee, WI, USA) and stored at  $4^\circ\text{C}$  before use. Iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) was obtained from Dinâmica Química Contemporânea (Indaiatuba, SP, Brazil), and the surfactant cetyltrimethylammonium bromide (CTAB) was supplied by Vetec (Rio de Janeiro, Brazil). The hospital effluent samples were collected at disposal stations of sewage treatment plants of hospitals in Florianópolis, SC, Brazil and kept under refrigeration at  $4^\circ\text{C}$  in amber vials. The samples were left to decant, and the supernatant was used for extraction procedure prior to each analysis.

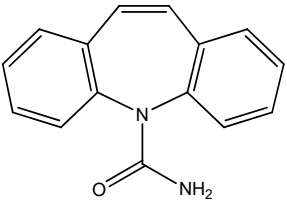
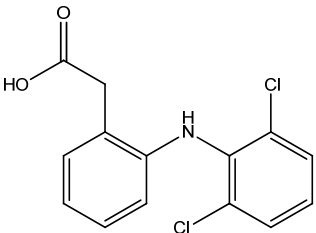
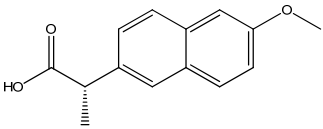
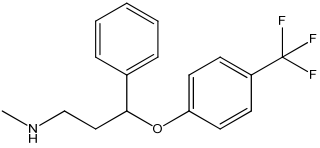
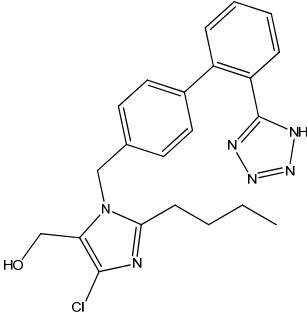
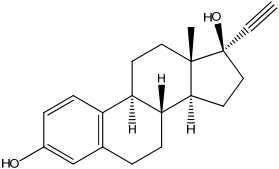
### 2.2. Synthesis and Characterization of the Extraction Phases

The  $\text{Si3Py}^+\text{Cl}^-$  was synthesized by the sol-gel processing method, in accordance with previous work [17]. Briefly, tetraethylorthosilicate (TEOS), ethanol, and an aqueous HCl solution were mixed, and the resulting solution was stirred in a round-bottomed flask for 2.5 h at room temperature. Afterwards, a solution of 3-*n*-chloropropyltrimethoxysilane (CPTS) was added in the flask and the mixture was stirred for 2 h at room temperature. The temperature of the solution was raised to 328 K, and the mixture was allowed to stand open for 60 h under atmospheric conditions until the gelation process occurred. The resulting solid was washed with ethanol and then dried under vacuum at room temperature. The dry solid was mixed in a round-bottomed flask with a solution prepared by pure 3-methylpyridine and dry toluene. So, the mixture was heated at the reflux temperature of the solvent for approximately 3 h. The solid was filtered, washed with ethanol, and dried for 2 h under vacuum at room temperature.

The extraction phases PPy and PPy.CTAB were also synthesized, based on previous work, through chemical in situ polymerization of pyrrole (Py) with the use of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  as oxidant in aqueous solution containing a cation surfactant CTAB in the following proportions: oxidant/Py molar ratio = 2.3/1 and surfactant/Py molar ratio = 1/5 [20]. In this case,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  was dissolved in distilled water and the aqueous solution containing

CTAB was slowly added under magnetic stirring at room temperature. After 15 min, pyrrole dispersed in distilled water was added dropwise. The polymerization was carried out for 1 h under magnetic stirring at room temperature. After 24 h, the conductive polymer PPy.CTAB was filtered, washed with distilled water, and dried at 60 °C. The synthesis of neat PPy was also carried out according to the same previously mentioned procedure but without the use of CTAB. The characterization of the extraction phases was reported in previous studies [17,20].

**Table 1.** Chemical structures and some physicochemical properties of the analytes (ChemAxon).

Analyte	Chemical Structure	pKa	log K <sub>o/w</sub>
CBZ		15.96	2.77
DCL		4.00	4.26
NAP		4.19	2.99
FLU		9.80	4.17
LOS		5.85	5.00
EE2		10.33	3.90

### 2.3. Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed on a Shimadzu Prominence LC 20AT series HPLC system (Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (RF 20A

series) and a diode array detector (RF 20A series). The injection volume was 20  $\mu\text{L}$  using a manual injector Rheodyne 7725i (Rohnert Park, CA, USA) at a flow rate of 1  $\text{mL min}^{-1}$  of mobile phase in gradient mode. The gradient method initially applied 40% of mobile phase A (ACN acidified with 0.1%  $v/v$  of formic acid) and 60% of mobile phase B (water acidified with 0.1%  $v/v$  of formic acid), and this condition was maintained up to 17 min. Next, mobile phase A was increased from 40% to 70% from 17 to 25 min. Finally, the mobile phase was returned to the initial condition and maintained up to 30 min. Chromatographic separations were performed in reversed phase mode using a C18 column (ZORBAX Eclipse XDB<sup>®</sup>, 250 mm  $\times$  4.6 mm i.d., 5 mm film thickness, Agilent, CA, USA). The analytes CBZ and DCL were detected using a diode array detector at a wavelength of 280 nm. The other analytes were detected in a fluorescence detector using excitation and emission wavelength as follows: FLU (230/290), LOS (248/410), NAP (284/352), and EE2 (280/310). The chromatographic data were evaluated with LCsolution software (Shimadzu, Kyoto, Japan).

#### 2.4. Optimization of DPX Procedure

The DPX procedure was fully optimized in order to obtain the best extraction conditions for the analytes using both univariate and multivariate approaches, with data processing assessed through Statistica 8.0 software (STATSOFT, Palo Alto, CA, USA).

#### 2.5. Evaluation of Extraction Phase

Initially, the best extraction material was chosen using a univariate strategy. For this, 20 mg of each material ( $\text{Si3Py}^+\text{Cl}^-$ , PPy and PPy.CTAB) was inserted into the DPX-Blank tips. The extractions were performed in water spiked with 100  $\mu\text{g L}^{-1}$  of each analyte; the pH was adjusted at 4 based on the analyte pKa values; the extraction parameters were fixed in 5 cycles of extraction using 800  $\mu\text{L}$  of sample (using a new aliquot in each cycle) and 15 s of extraction time per cycle. The desorption parameters were fixed in 5 cycles of 100  $\mu\text{L}$  of ACN: MeOH (1:1  $v/v$ ) (using the same aliquot in all cycles) and 15 s of desorption time per cycle.

#### 2.6. Optimization of Desorption Solvent

A simplex-lattice design with 9 experiments was used to examine the desorption solvent. In this particular case, ACN, MeOH, ACE, and mixtures of these solvents were adopted. The triangular response surface was generated using the geometric means of the chromatographic peak areas of the analytes. In this step, the best extraction phase was optimized according to the previous section, and the other parameters of the DPX procedure were fixed as previously described.

#### 2.7. Optimization of Extraction and Desorption Steps

The extraction step was optimized according to a central composite design with 17 experiments. The variables mass of extraction phase (5 to 30 mg), volume of sample (200 to 810  $\mu\text{L}$ ), and the number of extraction cycles (1 to 9) were evaluated. The extraction time was fixed at 15 s in all experiments.

Regarding the desorption step, number of cycles (1 to 9) and volume of desorption solvent (100 to 300  $\mu\text{L}$ ) were optimized through a Doehlert design with 9 experiments. The desorption time was set at 15 s in all experiments.

#### 2.8. Evaluation of pH and Salting-Out Effect

Other important parameters to be evaluated were the sample pH and salting-out effect. In this step, a Doehlert design with 9 experiments was adopted. The pH varied from 2 to 7 based on the analyte pKa values, and the salting-out effect was evaluated through addition of NaCl in the sample solution at concentrations ranging from 0 to 25%  $w/v$  of NaCl.

## 2.9. Analytical Figures of Merit and Analysis of Hospital Wastewater Samples

Calibration curves were obtained using hospital wastewater samples spiked with the analytes at different concentration ranges, as follows: for CBZ and DCL 5 to 100  $\mu\text{g L}^{-1}$ , for NAP 0.1 to 10  $\mu\text{g L}^{-1}$ , for FLU 1 to 50  $\mu\text{g L}^{-1}$ , for LOS 0.5 to 100  $\mu\text{g L}^{-1}$ , and for EE2 0.5 to 25  $\mu\text{g L}^{-1}$ . Coefficients of correlation ( $r$ ) were calculated based on calibration curves. Limits of quantification (LOQs) were determined as the first concentration of linear range, and the limits of detection (LODs) were obtained by dividing the LOQs by 3.3. Accuracy of the method was evaluated at three concentration levels through relative recovery. Precision of the method was evaluated through intra-day assays at three concentration levels, and inter-day assays at one concentration level. In addition, robustness of the method was evaluated through Youden test. The main factors that can influence the response of the method were identified, and small variations were performed, simultaneously. Results were expressed through Lenth's graphic.

Two hospital wastewater samples were collected at the outflow of sewage treatment plants of hospitals in Florianópolis, Santa Catarina state, Brazil. The samples were stored at 4 °C, before analysis they were allowed to settle, and the supernatant was submitted to the optimized DPX procedure.

## 3. Results and Discussion

### 3.1. Optimization of DPX Procedure

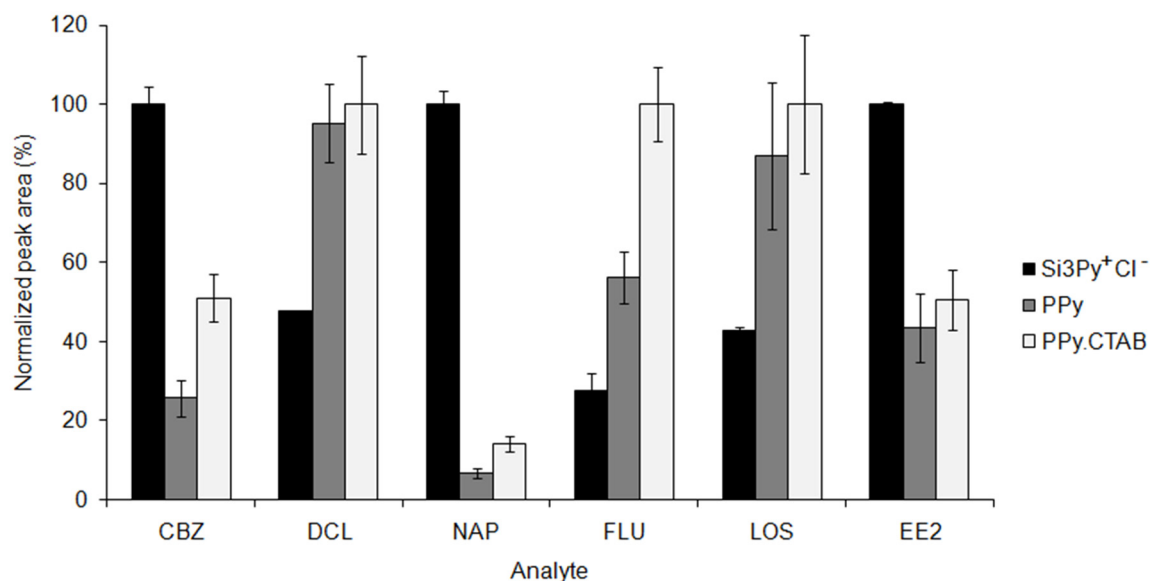
#### 3.1.1. Evaluation of the Extraction Phase

The selection of the most effective extraction phase for extracting the analytes is a crucial step. It is necessary to employ an extraction phase that has certain characteristics, such as functional groups that can interact with the analytes, a large surface area, and porosity that can favor the extraction of the analytes from aqueous samples. In this study, the hybrid material  $\text{Si3Py}^+\text{Cl}^-$ , which exhibits functional groups such as pyridinium and silanol that can interact with the analytes through  $\pi$ - $\pi$  and hydrogen interactions, was evaluated. Moreover, two conductive polymers, PPy and PPy.CTAB, were also examined. These materials also present pyridinium groups that can interact with the analytes through  $\pi$ - $\pi$  and hydrogen interactions. In addition, all the selected materials exhibit a large surface area, which can increase the contact surface of the analyte with the extraction phase [17,20].

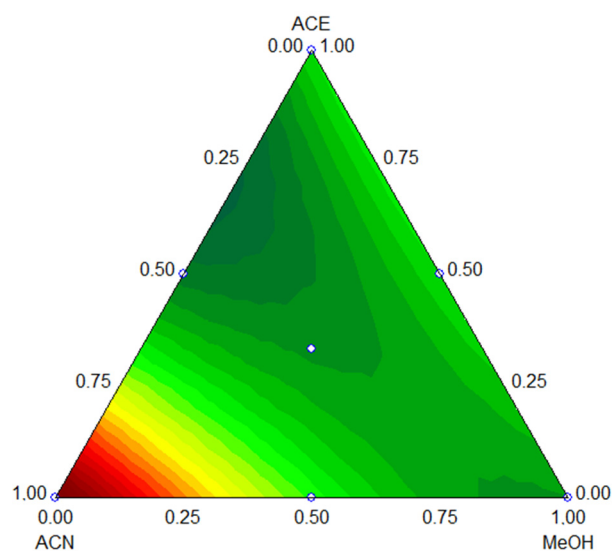
The results of the extraction efficiency comparison of each material are shown in Figure 1. According to the bar graph of Figure 1, it was observed that for CBZ, NAP, and EE2 the hybrid material  $\text{Si3Py}^+\text{Cl}^-$  presented the best extraction efficiency, and the conductive polymers presented the best extraction efficiency for DCL, FLU, and LOS. However, for NAP the extraction efficiency was overwhelming when using  $\text{Si3Py}^+\text{Cl}^-$ ; moreover, standard deviations were smaller when the extraction phase comprised of  $\text{Si3Py}^+\text{Cl}^-$  was used. Thus,  $\text{Si3Py}^+\text{Cl}^-$  was selected as the extraction phase for this study.

#### 3.1.2. Optimization of the Desorption Solvent

The choice of the most suitable solvent is an important step to ensure appropriate desorption of the analytes from extraction phase and to avoid any carryover effect. A triangular surface obtained from a simplex-lattice design is shown in Figure 2. It can be observed that ACN presented the highest performance as desorption solvent when 100% of this organic solvent was used. Therefore, ACN was used in further experiments.



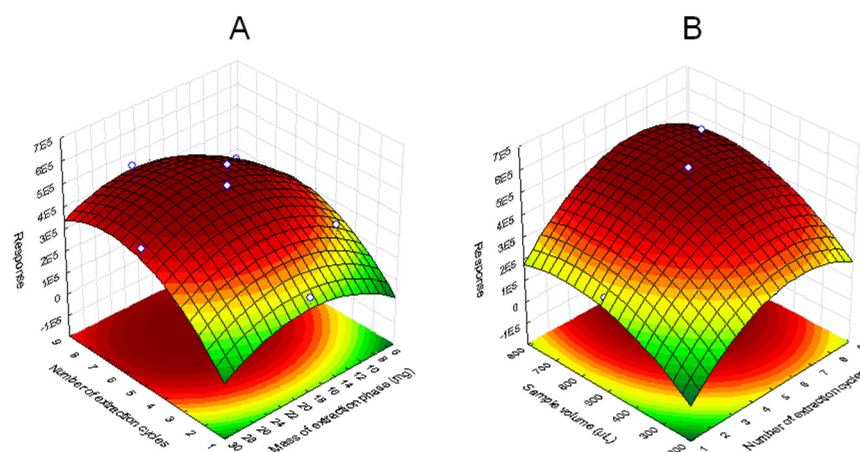
**Figure 1.** Univariate evaluation of extraction phases Si3Py<sup>+</sup>Cl<sup>-</sup>, PPy, and PPy.CTAB using the DPX procedure in the extraction of CBZ, DCL, NAP, FLU, LOS, and EE2. Extraction was performed with 20 mg of each material; 100 µg L<sup>-1</sup> of each analyte; five extraction cycles with 800 µL of sample in each cycle, and 15 s of extraction time; and five desorption cycles with 100 µL of ACN: MeOH, 1:1 v/v (same aliquot), and 15 s of desorption time.



**Figure 2.** Triangular surface obtained for the evaluation of the desorption solvent. Extraction was performed with 20 mg of Si3Py<sup>+</sup>Cl<sup>-</sup>; 100 µg L<sup>-1</sup> of each analyte, five extraction cycles with 800 µL of sample in each cycle and 15 s of extraction time; and five desorption cycles with 100 µL of each solvent and/or mixture of solvents and 15 s of desorption time.

### 3.1.3. Optimization of Extraction Step

In this step, a central composite design with 17 experiments was employed to evaluate the number of extraction cycles (1 to 9), sample volume (200 to 810 µL), and mass of extraction phase (5 to 30 mg). The response surfaces obtained are shown in Figure 3A,B.



**Figure 3.** Response surfaces obtained from a central composite design. (A) evaluation of the number of extraction cycles versus the mass of extraction phase (mg) and (B) evaluation of the sample volume (μL) versus the number of extraction cycles. Extraction was performed with 100 μg L<sup>-1</sup> of each analyte, 15 s of extraction and desorption time, and 5 desorption cycles with 100 μL of ACN.

According to Figure 3A, the chromatographic response using low mass of extraction phase was not satisfactory; nor did a large mass exhibit good responses. This is because a large mass of extraction phase can hinder the dynamic mixture between the sample and extraction phase. Therefore, the best response was obtained with 20 mg of Si3Py<sup>+</sup>Cl<sup>-</sup>. According to Figure 3B, it can be observed that enhanced chromatographic responses were obtained when the sample volumes were increased. However, the capacity of the DPX (1 mL) limits the sample volume. In this case, 700 μL of sample was the ideal volume for the DPX procedure. Regarding the number of extraction cycles, it can be noted that six cycles were enough to obtain satisfactory analytical responses. Therefore, six cycles were selected for further experiments. The mathematical function for this figure is presented in Equations (1) and (2).

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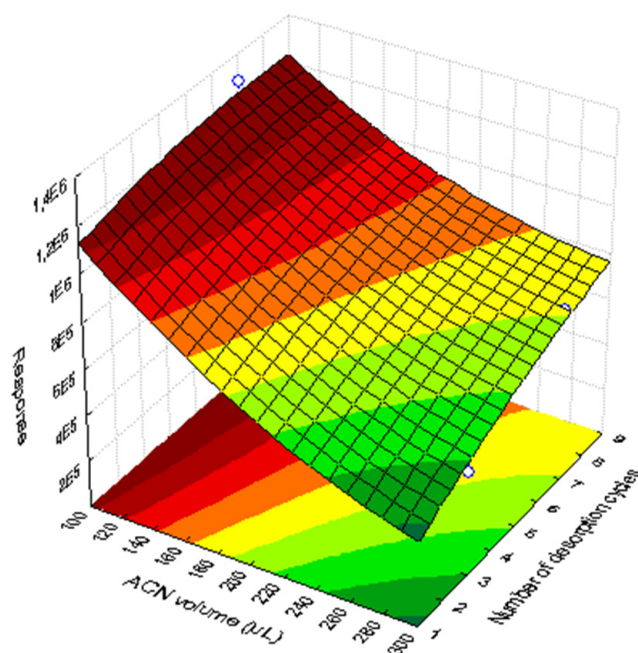
$$Z = -597122,13926468 + 127215,4901441 \times x - 10962,634303935 \times x^2 + 25803,003356547 \times y - 929,44970952146 \times y^2 + 431,83464609519 \times x \times y + 9,690852560031 \times 502,647059 \times x + 15,504551823089 \times 502,647059 \times y + 346746,501 \quad (1)$$

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### 3.1.4. Optimization of the Desorption Step

A Doehlert design was used to evaluate the number of desorption cycles and volume of desorption solvent. It can be observed in Figure 4 that the best response was obtained with 100 μL of ACN. The number of desorption cycles did not present a significant influence

on the overall response; thus, 3 cycles with 100  $\mu\text{L}$  of ACN (same aliquot) were selected for desorbing the analytes.

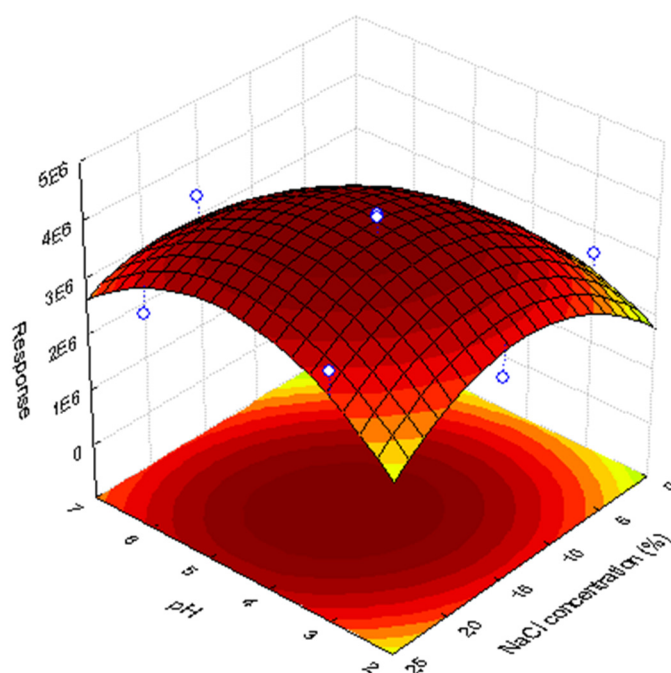


**Figure 4.** Response surface obtained from a Doehlert design employing volume of ACN and number of desorption cycles. Extraction was performed with 20 mg of  $\text{Si3Py}^+\text{Cl}^-$ ; 100  $\mu\text{g L}^{-1}$  of each analyte; six extraction cycles with 700  $\mu\text{L}$  of sample in each cycle and 15 s of extraction time; and five desorption cycles with 100  $\mu\text{L}$  of ACN and 15 s of desorption time.

### 3.2. Evaluation of PH and Salting-Out Effect

A Doehlert design with nine experiments was adopted to optimize the sample pH and salting-out effect. In most of the extraction procedures, it is preferable for the analytes to remain in the neutral form to enhance the chemical interactions with the extraction phase. For acidic compounds, the pH must be 1.5 units lower than the  $\text{pK}_a$  of the analytes [13]. The  $\text{pK}_a$  of the analytes under study are 15.96, 4.00, 4.19, 9.80, 5.85, and 10.33 for CBZ, DCL, NAP, FLU, LOS, and EE2, respectively. As DCL exhibits a lower  $\text{pK}_a$  value, the pH of the sample was evaluated from 2 to 7.

Salt addition may exhibit an influence on the mass transfer of the analyte due to the salting-out or salting-in effects. An increase in salt content can decrease the solubility of analytes in the aqueous sample, facilitating the migration to the extraction phase (salting-out effect). This effect is more pronounced in polar compounds [25]. In this particular case, it can be observed that the analytes exhibit non-polar to medium polarity aspects with  $\log K_{\text{O/W}}$  of 2.77, 4.26, 2.99, 4.17, 5.00, and 3.90 for CBZ, DCL, NAP, FLU, LOS, and EE2, respectively. So, this parameter was evaluated from 0 to 25%  $w/v$  of NaCl. The response surface obtained is shown in Figure 5.



**Figure 5.** Response surface obtained from Doehlert design for the evaluation of pH and NaCl content (%). Extraction was performed with 20 mg of  $\text{Si3Py}^+\text{Cl}^-$ ;  $100 \mu\text{g L}^{-1}$  of each analyte; six extraction cycles with 700  $\mu\text{L}$  of sample in each cycle and 15 s of extraction time; and three desorption cycles with 100  $\mu\text{L}$  of ACN and 15 s of desorption time.

As can be seen in Figure 5, the best chromatographic responses were achieved over a wide range of pH and NaCl concentrations. At pH 3.5, most of the analytes are in the neutral form, which can favor the interaction with the extraction phase. Regarding NaCl concentration, 15% (*w/v*) of NaCl exhibited the best extraction condition.

Thus, after optimizing the experimental conditions, the following parameters were adopted: 20 mg of  $\text{Si3Py}^+\text{Cl}^-$  as extraction phase; six extraction cycles with 700  $\mu\text{L}$  of sample in each cycle and 15 s of extraction time; three desorption cycles with 100  $\mu\text{L}$  of ACN (same aliquot) and 15 s of desorption time; and sample pH 3.5 and addition of 15% (*w/v*) of NaCl in the sample.

### 3.3. Analytical Figures of Merit and Analysis of Hospital Wastewater Samples

Calibration curves were obtained using hospital wastewater analyte-free samples spiked with the analytes at six concentration levels. The extractions/desorptions were performed under optimized conditions, and the results are shown in Table 2. The correlation coefficients (*r*) obtained were satisfactory, varying from 0.9710 to 0.9996, indicating a good correlation of instrument response (*y*) and analyte concentration (*x*) [26]. The LOD values ranged from  $0.030 \mu\text{g L}^{-1}$  for NAP to  $1.510 \mu\text{g L}^{-1}$  for CBZ and DCL, and the LOQ values ranged from  $0.10 \mu\text{g L}^{-1}$  for NAP to  $5.00 \mu\text{g L}^{-1}$  for CBZ and DCL.

**Table 2.** Linear equation, linear range, correlation coefficients, limits of detection, and quantification obtained for the pharmaceutical compounds in hospital wastewater using the fully optimized  $\text{Si3Py}^+\text{Cl}^-$ -DPX-based procedure.

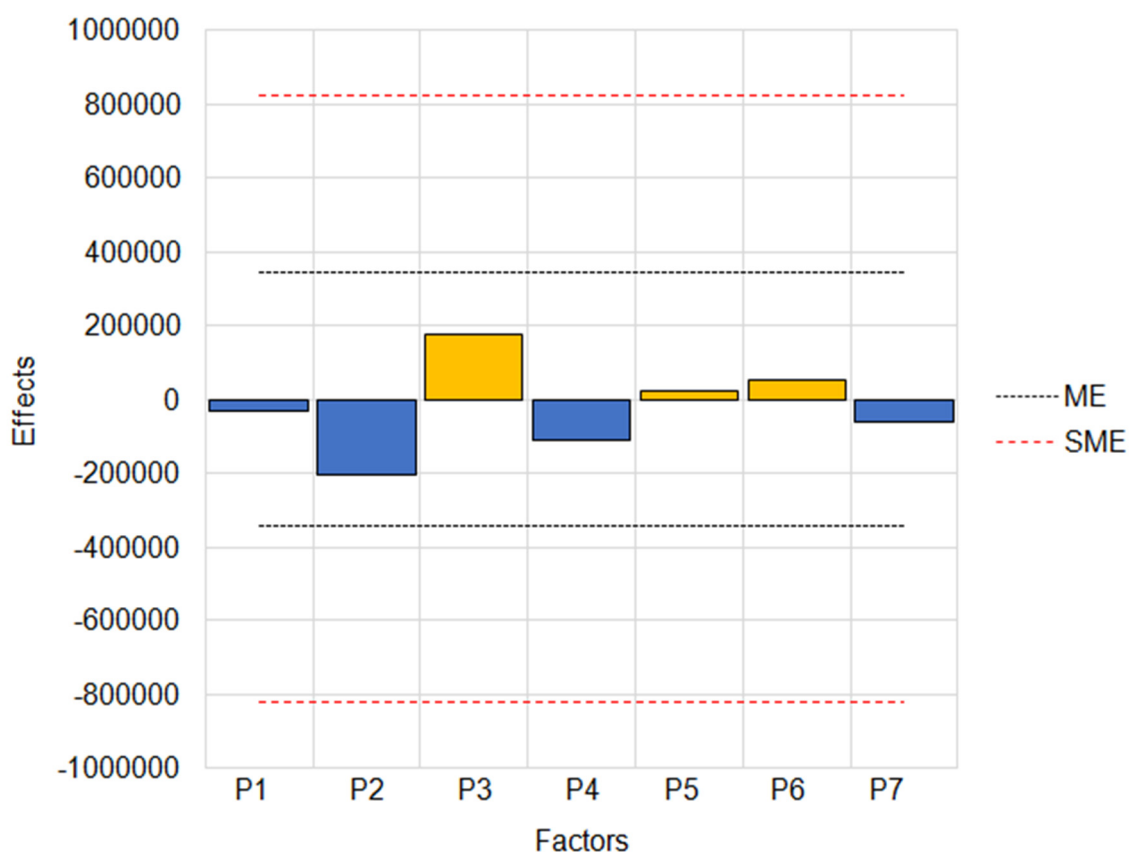
Analyte	Linear Equation	Linear Range ( $\mu\text{g L}^{-1}$ )	Correlation Coefficient ( <i>r</i> )	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )
CBZ	$y = 645.03x - 731.96$	5–100	0.9996	1.510	5.00
DCL	$y = 760.09x - 2089.9$	5–100	0.9990	1.510	5.00
NAP	$y = 262281x - 21877$	0.1–10	0.9996	0.030	0.10
FLU	$y = 9805.6x + 3435$	1–50	0.9710	0.303	1.00
LOS	$y = 11535x - 5496.7$	0.5–100	0.9991	0.151	0.50
EE2	$y = 101441x - 13706$	0.5–25	0.9994	0.151	0.50

The accuracy of the proposed method was evaluated using hospital wastewater samples spiked at three concentration levels. As can be seen in Table 3, the relative recovery obtained ranged from 80 to 127%. The precision was evaluated through intra and inter-day assays. The intra-day precision was evaluated in three concentration levels, and the inter-day precision was evaluated in one concentration level; both exhibited results lower than 19%. Most of the results are in accordance with validation guidelines [27]. Nevertheless, these results emphasized the applicability of the method developed for monitoring the pharmaceutical compounds of different classes in hospital wastewater.

**Table 3.** Relative recovery, and intra and inter-day precisions for the pharmaceutical compounds in hospital wastewater using the fully optimized  $\text{Si3Py}^+\text{Cl}^-$ -DPX-based procedure.

Analyte	Spiked Concentration ( $\mu\text{g L}^{-1}$ )	Relative Recovery (% , $n = 3$ )	Precision (RSD, %)	
			Intra-Day ( $n = 3$ )	Inter-Day ( $n = 9$ )
CBZ	5	118	6	15
	25	127	13	
	100	97	13	
DCL	5	86	8	19
	25	94	19	
	100	86	7	
NAP	0.10	110	19	18
	1	113	18	
	5	117	7	
FLU	1	83	5	10
	5	98	4	
	25	115	7	
LOS	0.50	120	1	19
	5	108	6	
	100	80	12	
EE2	0.5	97	13	17
	5	118	12	
	25	109	15	

The Youden test was performed to assess the robustness of the method. Small variations of the main parameters that can affect the extraction by DPX procedure were performed [28]. The parameters and variations adopted were used as follows: mass of  $\text{Si3Py}^+\text{Cl}^-$  (18 to 22 mg), concentration of NaCl (12 to 18%  $w/v$ ), sample pH (3.0 to 4.0), sample volume (680 to 720  $\mu\text{L}$ ), number of extraction cycles (5 to 7), volume of ACN for the desorption step (80 to 120  $\mu\text{L}$ ), and number of desorption cycles (2 to 4). The results were evaluated through the Lenth's graphic shown in Figure 6. Simultaneous margins of error (SME) are reference lines and should be considered, taking into account that multiple effects were assessed [29,30]. According to Figure 6, no parameter exceeded this limit, so the method can be considered robust.



**Figure 6.** Lenth's graphic to assess the robustness of the method developed based on previously optimized  $\text{Si3Py}^+\text{Cl}^-$ -DPX procedure.

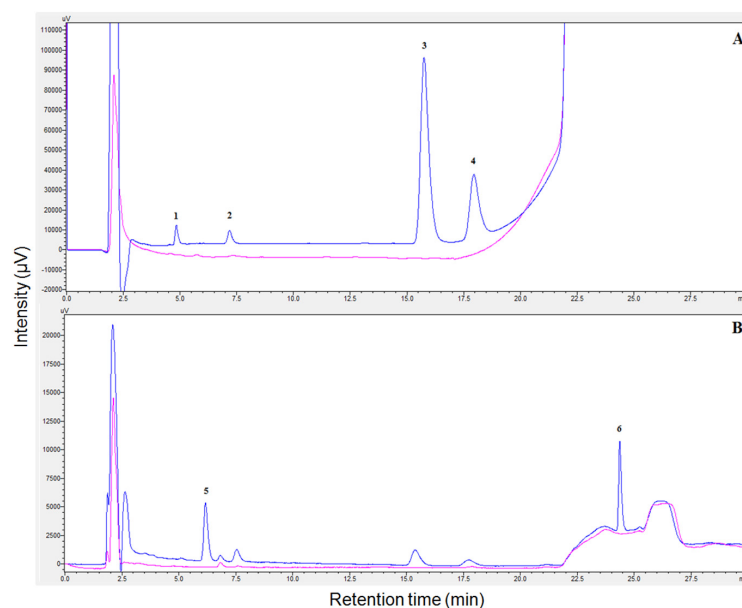
In addition, a comparison with other previously reported methods is shown in Table 4. As can be observed, in this work, the volume of organic solvent and mass of extraction phase are smaller in relation to the other methods, at only 100  $\mu\text{L}$  and 20 mg of  $\text{Si3Py}^+\text{Cl}^-$ , respectively. In previous studies, authors used 280  $\mu\text{L}$  to 41 mL of organic solvent and 40 to 200 mg of extraction phase. These values can evidence the environmentally-friendly aspects of the methodology developed. Another important factor is the sample preparation time; in this study, only 6.5 min were required to accomplish all sample preparation steps, including pH and ionic strength adjustments. According to literature, 21 min to almost 2 h were required as sample preparation time in previous studies listed in Table 4. This high-throughput aspect is particularly important when multiple samples need to be analyzed. In relation to LOQs, this method exhibits comparable or lower values using the FLD detector (for the analytes NAP and LOS) than those obtained using DAD or UV detectors for the same compounds [31–33]. For EE2, the LOQ obtained in this work was comparable to the results obtained using a methodology with GC-MS [34]. Therefore, the LOQs obtained with the methodology proposed can be considered very satisfactory since the instrumentation used is relatively simple compared to some studies when mass spectrometry detectors have been employed.

**Table 4.** Comparison of Si3Py<sup>+</sup>Cl<sup>−</sup>-DPX-based procedure with methods previously reported in literature.

Method	Analyte	Extraction Phase	Matrix	Linear Range (µg L <sup>−1</sup> )	LOQ (µg L <sup>−1</sup> )	Time of Sample Preparation	Solvent Volume
This work DPX and HPLC-DAD/FLD	CBZ	20 mg of Si3Py <sup>+</sup> Cl <sup>−</sup>	Hospital wastewater	5–100	5	6.5 min	100 µL
	DCL			5–100	5		
	NAP			0.1–10	0.1		
	FLU			1–50	1		
	LOS			0.5–100	0.5		
	EE2			0.5–25	0.5		
LLE and HPLC-UV (Ashfaq et al. 2017)	DIC NAP	40 mL of chloroform	Hospital wastewater	300–10000	12 8	-	42 mL
SBSE and HPLC-UV (Pebdani et al. 2016)	LOS	50 mg of Ni:ZnS-AC	Urine	0.4–50	0.38	Approximately 32 min	280 µL
RDSE and GC-MS (Arismendi, etal, 2019)	DIC	40 mg of Oasis <sup>®</sup> HLB	Drinking water and natural waters (well and river)	0.1–100	0.12	Approximately 115 min	10 mL
	EE2				0.21		
SPE and HPLC-DAD (Ngubane et al. 2019)	DCL	200 mg of Oasis <sup>®</sup> HLB	Estuary and seawater	100–5000	0.036	-	16 mL
	NAP				0.025		
DSPE and HPLC-DAD (Rashvand and Vosough 2016)	CBZ	43 mg of GO-PANI	Effluent from sewage Treatment Plants (STPs)	–	1.12	Approximately 21 min	3.5 mL
	DCL				0.36		
	NAP				0.15		
	NAP				0.15		
SPE and HPLC-MS (Matongo et al. 2015)	CBZ	60 mg of Oasis <sup>®</sup> HLB	Surface water and wastewater	0.297–100	0.891	Approximately 112 min	21 mL

HLB: hydrophobic-lipophilic balance; DSPE: dispersive solid phase extraction; GO-PANI: graphene oxide-polyaniline nanocomposite; LLE: liquid–liquid extraction; SBSE: stir bar sorptive extraction; Ni: ZnS-AC: nicel:zins sulphide nanoparticles loaded on activated carbon; and RDSE: rotating-disk sorptive extraction.

Two samples, namely, A and B, were used to evaluate the method proposed in monitoring pharmaceutical compounds in hospital wastewater. LOS and FLU were identified as lower than LOQ in effluent B. No analyte was identified in effluent A. A comparison between the extractions of hospital wastewater (sample B) without spiking (pink line) and spiking the analytes (blue line) is shown in Figure 7A comparison using an FLD detector with a sample spiked with the analytes at 10 µg L<sup>−1</sup> of 1-FLU, 2-LOS, 3-NAP, and 4-EE2 is shown in Figure 7A; and a comparison obtained with a DAD detector at 280 nm using a sample spiked with 100 µg L<sup>−1</sup> of 5-CBZ and 6-DCL is shown in Figure 7B.



**Figure 7.** Comparison between the extractions performed in hospital wastewater B without spiking (pink line) and with spiking (blue line) with the analytes. (A) comparison using FLD detector with sample spiked with 10 µg L<sup>−1</sup> of 1-FLU, 2-LOS, 3-NAP, and 4-EE2. (B) comparison using DAD detector at 280 nm with sample spiked with 100 µg L<sup>−1</sup> of 5-CBZ and 6-DCL.

#### 4. Conclusions

In this study, an environmentally-friendly method based on a DPX procedure using an alternative extraction phase was fully optimized and used for the determination of environmental pollutants in wastewater samples. The advantages of the method include the low volume of organic solvent employed (only 100 µL) and the high throughput since the extraction can be performed in 6.5 min. Three materials were evaluated, and the hybrid material  $\text{Si3Py}^+\text{Cl}^-$  exhibited the best analytical performance. This material is easy to synthesize, and it presents a low cost, which makes this method accessible for application in other laboratories. The proposed method was fully optimized through univariate and multivariate approaches, and the analytical parameters of merit were very satisfactory, with acceptable LOQs that are comparable to previously reported methodologies. Moreover, the applicability of the  $\text{Si3Py}^+\text{Cl}^-$ -DPX-based method was demonstrated in the analysis of hospital wastewater for monitoring different pharmaceutical classes using simple analytical instrumentation. This study has expanded the applicability of the hybrid material  $\text{Si3Py}^+\text{Cl}^-$  to wastewater complex matrices to determine compounds from different pharmaceutical classes.

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