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Environmental Pollution Monitoring via Capillary Zone Electrophoresis and UHPLC Simultaneous Quantification of Some Antipsychotic Drug Residues in Industrial Wastewater Effluents

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Citation: Alabbas, A.B.; Slimani, R.; El Ouahabi, I.; Zarrouk, A.; Lazar, S.; Azzallou, R.; Shalaby, N.F.; Abdel-Gawad, S.A. Environmental Pollution Monitoring via Capillary Zone Electrophoresis and UHPLC Simultaneous Quantification of Some Antipsychotic Drug Residues in Industrial Wastewater Effluents. *Chemosensors* **2024**, *12*, 123. https://doi.org/10.3390/chemosensors12070123

Received: 23 April 2024
Revised: 20 June 2024
Accepted: 26 June 2024
Published: 2 July 2024

Abstract: Monitoring and measuring pharmaceutical pollutants in environmental samples is a vital and complex task due to their potential detrimental effects on human health, even at low levels. Using capillary zone electrophoresis (CZE) and ultra-high-performance liquid chromatography (UHPLC), it was possible to separate and measure three commonly used antipsychotic drugs, chlorpromazine (CPZ), haloperidol (HAL), and risperidone (RIS), in wastewater of the pharmaceutical industry. The technique of solid-phase extraction (SPE) was developed and implemented as a very effective method for preparing samples prior to analysis. The settings of the capillary electrophoretic and chromatographic techniques were adjusted to obtain the most efficient separation profile for the medications being studied. The concentration of all the medicines being investigated ranged from 0.5 to 50 µg/mL. SPE was used to treat real wastewater samples after a thorough validation process that followed the rules set by ICH-Q2B. The developed assays were then effectively employed to identify the tested antipsychotic substances in the real wastewater samples. The provided methodologies may be efficiently utilized to monitor the extent of environmental contamination caused by the investigated pharmaceuticals.

Keywords: environmental sustainability; water pollution; environmental analysis; wastewater; resource conservation

1. Introduction

Antipsychotic drugs, sometimes referred to as neuroleptics, are used to treat and control symptoms associated with a variety of mental illnesses. Two categories of antipsychotics can be distinguished: first-generation antipsychotics, also called "typical" antipsychotics, and second-generation antipsychotics, also called "atypical" antipsychotics. Antipsychotics of the first and second generations are used to treat a range of neuropsychiatric conditions. Addiction to food, behavioral disorders, obsessive-compulsive disorder

(OCD), attention-deficit hyperactivity disorder (ADHD), geriatric agitation, eating, and behavioral abnormalities in dementia are among these conditions [1].

Chlorpromazine (CPZ) and haloperidol (HAL) are classified as typical antipsychotics, whereas risperidone (RIS) is categorized as an atypical antipsychotic [2]. Figure 1 presents the chemical structures of CPZ, HAL, and RIS.

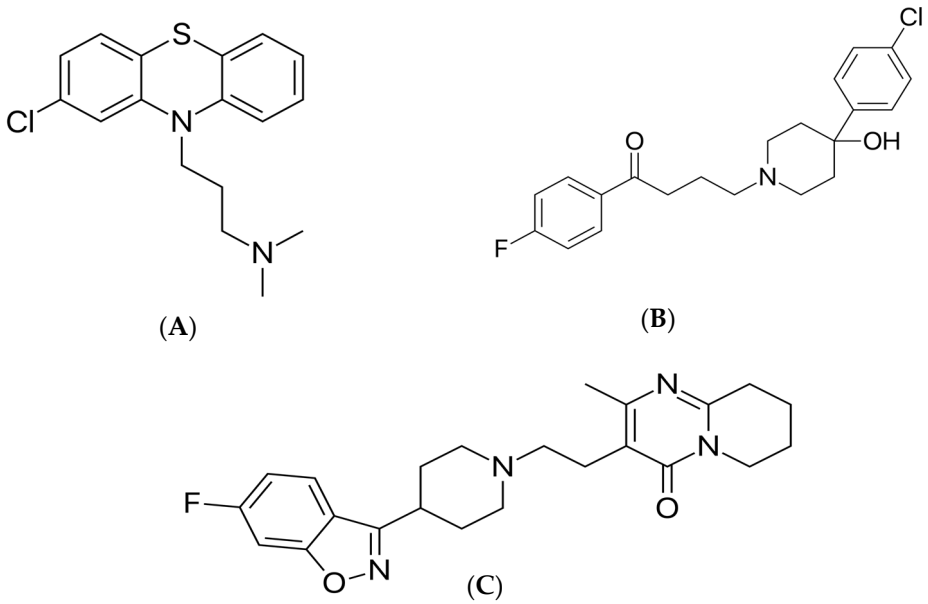

Figure 1. Chemical structure of chlorpromazine (CPZ) (**A**), haloperidol (HAL) (**B**), and risperidone (RIS) (**C**).

The widespread use of antipsychotic drugs in recent years has significantly endangered human life due to the heightened likelihood of their escape into the environment. Regular use of antipsychotic medications can result in severe health complications such as cognitive impairment, Alzheimer's disease, high blood sugar levels, abnormal breast enlargement in males, and symptoms resembling Parkinson's disease. Neuroleptics have detrimental effects on humans when they are found in the surrounding environment. Therefore, there is no acceptable level for their presence in soil or water [3,4]. From this vantage point, it is important to keep an eye on the amounts of the tested drugs that are being released into industrial wastewater effluents. The subsequent measure is a crucial aspect that must be examined in order to regulate environmental pollution caused by pharmaceutical pollutants, which may be regarded as a significant form of environmental contamination due to its detrimental impact on human health.

Spectrophotometry [5–7], high-performance liquid chromatography (HPLC) [8–11], gas chromatography coupled to mass spectroscopy (GC-MS) [12], liquid chromatography coupled to a mass spectrometric detector (LC-MS/MS) [13,14], and electrochemistry [15–17] are among the analytical techniques used to quantify the studied drugs in the various sample forms.

The application of HPLC allowed for the simultaneous determination of the investigated medicines in biological fluids [18,19]. Alternatively, LC-MS/MS was employed to measure the studied drugs at the same time, either in water environments [20] or biological materials [21,22]. In addition, the medications under investigation were measured in dosage form using capillary zone electrophoresis (CZE) [23].

Capillary electrophoresis (CE) has emerged as a significant analytical technique in recent years, mostly due to its superior separation efficiency, minimal usage of materials and reagents, short analysis time, and ability to handle a wide range of samples [24,25]. Ultra-high-performance liquid chromatography (UHPLC) is a chromatographic technique that employs a mixture of reversed-phase packing materials with a particle size of 1.7 µm and operates at pressures between 41.40×10^6 Pa and 103.40×10^6 Pa. This approach has

several benefits over conventional HPLC, one of which is enhanced sensitivity as indicated by a better signal-to-noise (S/N) ratio. The decrease in zone broadening is the cause of this. It is also noteworthy for its accelerated analytical speed and improved chromatographic peak resolution [26,27].

In one study, the studied drugs were determined in wastewater samples using GC-MS/MS [28]. In this method, Logarinho et al. used solid-phase extraction (SPE) as a sample preparation technique. The volatility of the studied drugs was increased by microwave-assisted derivatization before their analysis. Also, the studied drugs were quantified in hospital wastewater using LC-MS/MS [20]. In this method, dispersive liquid–liquid microextraction and SPE were applied for sample preparation. Massano et al. used ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS/MS) to determine different antipsychotic drugs in wastewater after their extraction by SPE [29].

The proposed work in this article overcomes the drawbacks encountered during the application of the GC-MS/MS technique, which include the low repeatability of the method indicated by the high values of the relative standard deviation (RSD) percent that reach 15%. Also, the presented work does not need the complex and tedious derivatization procedure needed for the GC analysis. In the same way, the proposed work overcomes the problems encountered with the application of the LC-MS/MS analysis, which are the limited throughput and high operational and maintenance costs [25,27].

Undoubtedly, the presence of pharmaceutical contaminants in industrial effluents poses a significant concern for the environment. This assertion confirms the importance of monitoring and quantifying the antipsychotic drugs being discharged from pharmaceutical factories.

After an extensive review of the existing literature, it came to light that there are currently no published studies that have specifically utilized CZE and UHPLC to simultaneously measure the quantities of research drugs in industrial wastewater effluents. The main goal of this study is to improve, verify, and use the CZE and UHPLC methods for measuring desired medicines in real wastewater effluents.

2. Materials and Methods

2.1. Chemicals, Reagents, and Standard Solutions

The solvents used were of high-performance liquid chromatography (HPLC) quality, whereas the compounds employed were of analytical grade. The following items were supplied by Merck (Darmstadt, Germany): triethyl amine, acetonitrile, methanol, potassium dihydrogen phosphate, o-phosphoric acid, and NaOH. Millipore, located in Bedford, Massachusetts, USA, makes the Milli-Q Plus system, which was utilized to obtain the distilled water needed for this study.

The background electrolyte (BGE) was a 30 mM phosphate buffer that had *o*-phosphoric acid added to bring its pH down to 3.0. The cation present in the BGE was the potassium ion (K^+) with a concentration of 25 mM.

Cymit Quimica S.L. (Barcelona, Spain) provided the pure materials CPZ (CAS no. 50-53-3), HAL (CAS no. 52-86-8), and RIS (CAS no. 106266-06-2). Clozapine (CLO), used as internal standard (IS) in the CZE experiments, was kindly supplied by Novartis (Ryaiadh, KSA). According to the certificate, CPZ, HAL, RIS, and CLO had purities of 100.92%, 99.45%, 101.14%, and 100.33%, in that order.

Cartridges of IST (Hengoed, UK) Isolute® cyanopropyl (CN) (50 mg, 1 mL) were used to process the samples. For filtering the BGE and final extract, Acrodisc nylon membrane syringe filters (0.2 µm, 13 mm; Pall Corp., Washington, DC, USA) were utilized. A Millipore solvent filter system and nylon membranes (0.2 µm, 47 mm; Supelco, Bellefonte, PA, USA) were used to filter the wastewater samples.

2.2. Instrumentation

The Agilent 7100 CE device, made in Waldbronn, Germany, was used for the CZE analysis. This apparatus is equipped with a UV-Vis detector and an automated injector. A

fused-silica capillary from Polymicro Technologies in Phoenix, AZ, USA, was employed in the separation procedure. The total capillary length is 64.5 cm, with an effective length of 56 cm. The inner diameter (I.D.) of the capillary used is 75 µm, and the outer diameter (O.D.) is 375 µm. The Agilent Chem-Station software (D version) was used to measure peak areas and migration times, in addition to other relevant data.

Ultra-performance liquid chromatography was conducted with a Waters™ Acquity system (Milford, CT, USA) with column dimensions of 100 × 2.1 mm, 1.7 µm. The pore size was 130 Å. The detector was a UV-visible wavelength detector (Waters, 2489, Milford, CT, USA).

The solutions' pH was measured using a pH meter manufactured by Mettler Toledo company, which is headquartered in Greifensee, Switzerland.

2.3. Wastewater Sample Collection

Three samples of wastewater free from the studied drugs were taken from a pharmaceutical plant in Cairo, Egypt. The samples were stored in opaque glass vials under refrigeration to avoid any deterioration.

2.4. Method Development

2.4.1. CZE Method

Agilent Technologies (Waldbronn, Germany) proposed a new capillary preconditioning procedure. A 1 M NaOH solution was used for 20 min to flush new capillaries, followed by a 0.1 M NaOH solution for 20 min, water for two minutes, and a BGE solution for thirty minutes. The pressure during washing was 20 kilopascals (kPa). The capillaries were thereafter submerged in water for the whole night.

The optimal separation of the materials under investigation was achieved at a temperature of 25 °C with a voltage of 16 kV and currents typically less than 50 µA. The sample solution was injected hydrodynamically for a duration of 10 s, using a pressure of 3.5 kPa. The analytes under investigation were detected at a wavelength of 238 nm.

2.4.2. UHPLC Method

Chromatographic settings were altered to obtain an optimal separation pattern for the analytes that were being investigated. To achieve the best possible separation, several attempts were undertaken to match the stationary phase and mobile phase perfectly.

2.5. Method Validation

The proposed methodologies were confirmed by adhering to the ICH-Q2B criteria [30].

2.5.1. Linearity

Various amounts of the medications under investigation, ranging from 5 to 500 µg, were separately transferred into separate 10 mL volumetric flasks. Then, 100 µg of CLO (IS) was added to the flasks in the case of the CZE analysis. Subsequently, water (using the CZE technique) or mobile phase (using the UHPLC method) was introduced into each flask until the desired studied drugs' concentration of 0.5–50 µg/mL was achieved. The IS concentration in all CZE experiments was 10 µg/mL. The BGE was utilized for the elution procedure in order to conduct the electrophoretic analysis of the samples. Alternatively, UHPLC separation was conducted utilizing a C8 reversed-phase Waters™ column. The column dimensions were 100 × 2.1 mm, 1.7 µm. The pore size was 130 Å. The developing system consisted of a blend of potassium dihydrogen phosphate buffer containing 0.5% *v*/*v* trimethylamine (with pH adjusted to 3.0 using *o*-phosphoric acid) and acetonitrile in a ratio of 7:3, *v*/*v*. The flow rate was set at 1.0 mL/min. The UV detection was conducted at a wavelength of 238 nm.

2.5.2. Accuracy

Accuracy is measured as the proportion of an analyte that is successfully recovered from a given quantity [30]. Applying the conditions under linearity, data from nine samples with 5, 10, and 30 µg/mL of each drug under study were analyzed.

2.5.3. Precision

Both intra- and inter-day precision are examples of precision, and they may be measured as the percentage relative standard deviation across a range of statistically significant samples. Three repeats of each drug's three concentrations (5, 10, and 30 µg/mL) were conducted, either during the same day (intra-day) or over the course of three days (inter-day).

2.5.4. LOD and LOQ

The lowest quantity of a medication that can be accurately and consistently measured is known as the limit of quantification (LOQ), whereas the lowest amount that can be detected above background noise is known as the limit of detection (LOD). The procurement of these items was conducted in compliance with the regulations specified by the United States Pharmacopeia (USP) [31]. The limit of quantification (LOQ) and limit of detection (LOD) can be determined by identifying concentrations that produce peaks with heights that are ten and three times higher than the baseline noise, respectively.

2.5.5. Robustness

The robustness of the offered strategies may be assessed by measuring the impact of minor modifications. This was achieved by making small adjustments to the BGE pH in the CZE technique or modifying the quantity of acetonitrile in the mobile phase in the UHPLC method.

2.6. Method Applications

Cartridges of the IST (Hengoed, UK) Isolute® cyanopropyl (CN) (50 mg, 1 mL) were used for the SPE process. One milliliter of methanol was added to the cartridge three times to activate it, and one milliliter of water was added to the cartridge three times to condition it.

A volume of 10 mL of the wastewater sample (free from the studied drugs and IS) was agitated for a duration of 1 min. Subsequently, it was allowed to stand in the absence of light for a minimum of 30 min. Following this, the sample was filtered to eliminate any suspended particles. The sample was spiked with 10 µg of each investigated drug. Moreover, it was loaded with the same concentration of the IS in the case of the CZE analysis. The sample was then applied onto the preconditioned cartridge. The cartridge was rinsed twice with 1.0 mL of water and once with 1.0 mL of a mixture containing equal parts water and methanol (1:1, v/v). It was then eluted with 1.0 mL of methanol. The eluate was evaporated under vacuum using a rotary evaporator. It was then dissolved again with 1.0 mL of BGE for the CZE technique or mobile phase for the UHPLC method. Finally, the optimized CZE and UHPLC procedures were utilized.

3. Results

Safeguarding human health necessitates meticulous consideration of environmental contamination. Because drug residues enter the body gradually and might have disastrous effects, their presence in household water or crops may pose a serious risk to human health.

The present study demonstrated the feasibility of quantifying different residues of antipsychotic drugs in wastewater effluents. This was achieved through the application of sensitive, fast, and accurate CZE and UHPLC techniques. The primary objective was to monitor the environmental levels of the investigated drugs and mitigate their potential negative impacts on human health.

3.1. Method Development

3.1.1. CZE Method

Since the electrolyte may alter electro-osmotic flow (EOF), Joule heating, ionic strength, and the current generated in the capillary, its concentration has a major impact on the quality of separation. Thus, the peak area and migration time will be influenced by the electrolyte concentration. An investigation was conducted to examine the impact of different phosphate concentrations (ranging from 10 mM to 50 mM) in the BGE, as shown in Figure 2. The pH was adjusted to 3.0 and the drug concentration under investigation was set at 15 µg/mL. The corrected peak area (peak areas normalized by the migration times) of the analytes rose gradually as the phosphate concentration rose from 10 mM to 30 mM. The migration time was prolonged, the peak area shrank, and the analysis time was increased at phosphate concentrations greater than 30 mM. Thirty mM of phosphate was found to be the ideal concentration.

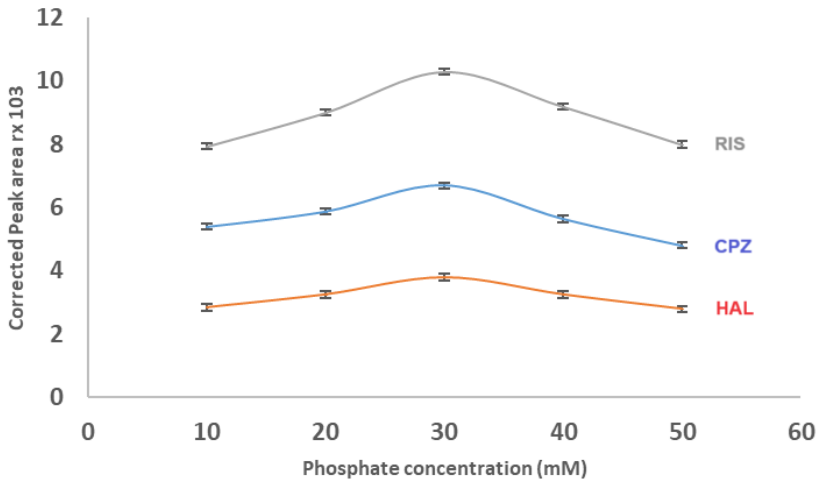

Figure 2. Effect of phosphate concentration on the CZE performance. Conditions: fused-silica capillary (64.5 cm total length, 56 cm effective length, 75 µm I.D., 375 O.D.), phosphate buffer (pH 3.0). Voltage 16 kV. UV detection at 238 nm. Sample concentration 15 µg/mL each, $n = 3$.

Since the pH of the buffer directly affects the electro-osmotic flow (EOF), it has a substantial effect on the CE analysis of the drugs being studied. As seen in Figure 3, an experiment was conducted to find out how the buffer pH affected the analytes' ability to separate. The analytes were fixed at a concentration of 15 µg/mL, and the phosphate concentration was 30 mM. The findings showed a positive relationship between the corrected peak area and the pH of the BGE, with an increase seen when the pH rose from 1.0 to 3.0. As the pH rose over 3.0, the corrected peak area showed a decrease. Consequently, a BGE pH of 3.0 was selected.

To achieve the optimal separation of the tested drugs, the applied voltage is essential. A voltage of 16 kV was used in the experiment to obtain an optimum migration time, peak area, and resolution. Upon increasing the applied voltage over 16 kV, the resolution was decreased. This may be attributed to the increased Joel heating effect. The electropherogram is given in the supplementary data under the name Supplementary Figure S1. Also, the BGE's temperature was adjusted to 25 °C. The CZE separation of the investigated drugs was tried at a higher temperature of 35 °C, and peak broadening was observed. The electropherogram at 35 °C is given in the supplementary data (Supplementary Figure S2).

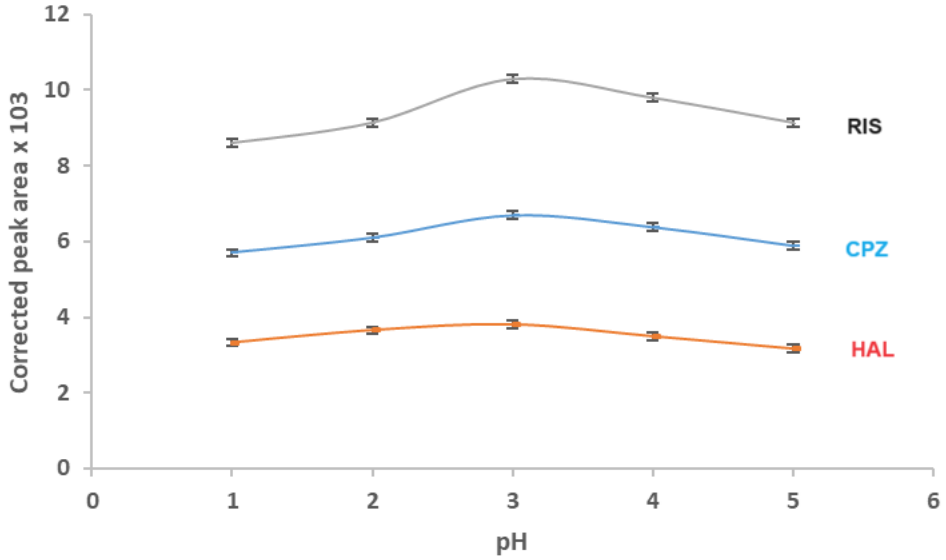

Figure 3. Effect of the BGE's pH on the CZE performance. Conditions: fused-silica capillary (64.5 cm total length, 56 cm effective length, 75 µm I.D., 375 O.D.), phosphate buffer (30 mM). Voltage 16 kV. UV detection at 238 nm. Sample concentration 15 µg/mL each, n = 3.

Following the adjustment of the experimental settings, an analysis was conducted on a combination of the investigated medications together with the CLO (IS), with each drug present at a concentration of 10 µg/mL. Figure 4 depicts the separation profile.

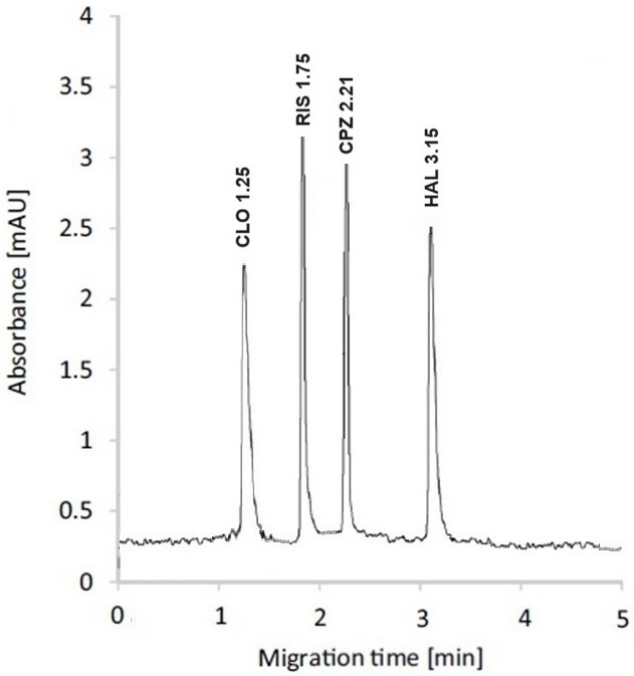

Figure 4. Capillary electrophoretic separation pattern of the studied antipsychotics and the IS. Conditions: fused-silica capillary (64.5 cm total length, 56 cm effective length, 75 µm I.D., 375 O.D.), phosphate buffer (pH 3.0, 30 mM). Voltage 16 kV. UV detection at 238 nm. Each drug concentration 10 µg/mL.

3.1.2. UHPLC Method

Optimizing chromatographic parameters is a vital process in attaining an optimal separation pattern with suitable times of retention for the analytes. Many attempts were performed to reach an optimum match between the stationary phase and the mobile phase.

The effect of variation in the mobile phase composition on the separation of the studied drugs was checked (Supplementary Figure S3).

Optimum separation was attained by using a C8 reversed-phase Waters^{TM} column that was 100 × 2.1 mm and had particles that were 1.7 µm in size and a porosity of 130 Å, as seen in Figure 5.

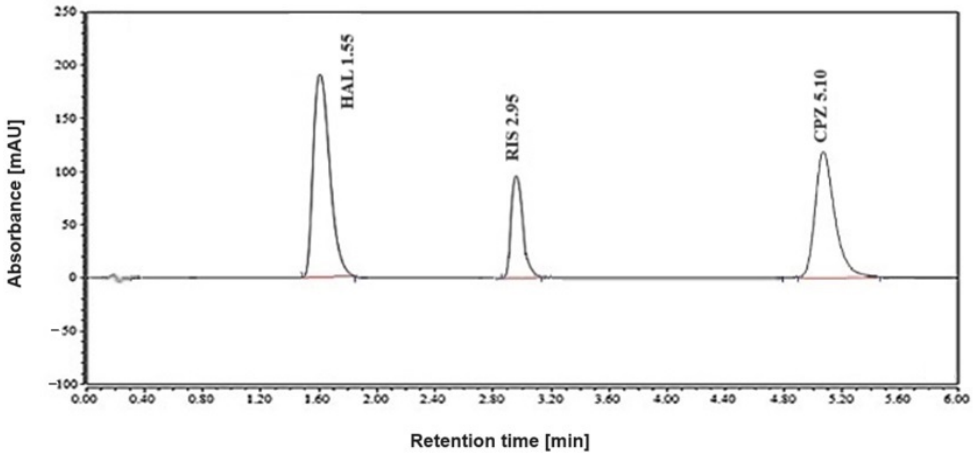

Figure 5. UHPLC chromatogram for the separation pattern of the studied antipsychotics. Conditions: stationary phase, C8 reversed-phase Waters^{TM} column (100 × 2.1 mm, 1.7 µm, 130 Å). Mobile phase is a blend of potassium dihydrogen phosphate buffer containing 0.5% v/v trimethylamine (with pH adjusted to 3.0 using o-phosphoric acid) and acetonitrile in a ratio of 7:3, v/v. Flow rate 1.0 mL/min. UV detection at 238 nm. Each drug concentration 10 µg/mL.

The optimal composition for the mobile phase consisted of potassium dihydrogen phosphate buffer with 0.5% trimethylamine (pH adjusted to 3.0 using o-phosphoric acid) and acetonitrile at a 7:3 v/v ratio. The flow rate was set at 1.0 mL/min. The UV detection was conducted at a wavelength of 238 nm.

3.2. Method Validation

This study was carried out in compliance with the ICH-Q2B standards [30]. The linearity of the proposed methods was confirmed by graphing the corrected peak area ratio (CPAR) or peak area (PA) against the concentrations of the pharmaceuticals under investigation within the concentration range of 0.5 to 50 µg/mL. The regression equations were calculated to depict the relationship between the corrected peak area ratios or peak areas and the concentrations of the tested pharmaceuticals using the CZE technique (Equations (1)–(3)) and the UHPLC method (Equations (4)–(6)).

$$\text{CPAR (CPZ)} = 0.1199\ \text{C—}0.0128\ \text{r} = 0.9997 \quad (1)$$

$$\text{CPAR (HAL)} = 0.0801\ \text{C—}0.0091\ \text{r} = 0.9998 \quad (2)$$

$$\text{CPAR (RIS)} = 0.1793\ \text{C—}0.0148\ \text{r} = 0.9998 \quad (3)$$

$$\text{PA (CPZ)} = 1590.10\ \text{C—}115.44\ \text{r} = 0.9999 \quad (4)$$

$$\text{PA (HAL)} = 1380.10\ \text{C} + 68.47\ \text{r} = 0.9999 \quad (5)$$

$$\text{PA (RIS)} = 1790.20\ \text{C—}47.68\ \text{r} = 0.9999 \quad (6)$$

where CPAR is the corrected peak area ratio, PA is the peak area, C is the concentration (µg/mL), and r is the correlation coefficient.

The recovery % was used to assess the assays' accuracy following a total of three runs of the investigation into three distinct concentrations of the medications of concern.

The low RSD values in Table 1 validate the excellent accuracy of the methods. The study evaluated the precision of the measurements, both within a single day and over several days. Table 1 presents the results, which demonstrate a high degree of precision. By making small adjustments to the assay parameters, such as changing the BGE's pH in the CZE technique or the mobile phase's composition in the UHPLC method, the robustness of the methods was also evaluated. The findings imply that these little adjustments had no appreciable impact on the suggested approaches. The analyte concentrations produce peaks with heights that are ten and three times greater than the baseline noise, representing the LOQ and LOD, respectively, as introduced in Table 1 (Supplementary Figures S4–S7). The results obtained validate the acceptable sensitivity of the procedures used.

Table 1. Validation results of the proposed CZE and UHPLC methods.

	CZE Method			**UHPLC Method**		
	RIS	**CPZ**	**HAL**	**HAL**	**RIS**	**CPZ**
Resolution factor (Rs)	-	$R_{RIS/CPZ}$ = 4.60	$R_{RIS/HAL}$ = 9.33	-	$R_{HAL/RIS}$ = 7.00	$R_{RIS/CPZ}$ = 14.20
Number of theoretical plates (N) #	323	408	349	201	861	849
Accuracy (Mean * ± SD)	99.85 ± 0.65	101.44 ± 0.79	102.14 ± 0.89	101.77 ± 0.91	102.01 ± 0.97	99.37 ± 0.66
Precision:						
Intra-day	100.21 ± 0.76	101.55 ± 1.16	101.44 ± 0.56	99.46 ± 0.92	101.33 ± 0.84	98.98 ± 0.85
Inter-day	102.43 ± 0.76	98.79 ± 1,12	102.43 ± 1.12	101.73 ± 0.93	99.83 ± 0.56	101.69 ± 0.80
Robustness:						
BGE pH variation	98.99 ± 0.94	101.22 ± 0.95	100.13 ± 0.45	-	-	-
Elution liquid composition	-	-	-	101.76 ± 0.60	102.93 ± 0.96	101.72 ± 0.55
Linearity						
Concentration range (µg/mL)	0.5–50	0.5–50	0.5–50	0.5–50	0.5–50	0.5–50
Slope	0.1793	0.1199	0.0801	1380.10	1790.20	1590.10
Intercept	−0.0148	−0.0128	−0.0091	68.47	−47.68	−115.44
Correlation coefficient (r)	0.9998	0.9997	0.9998	0.9999	0.9999	0.9999
LOQ	0.5	0.5	0.5	0.5	0.5	0.5
LOD	0.17	0.17	0.17	0.17	0.17	0.17

* Average of three readings. # $N = 16 (t_R)^2/W^2$ for UHPLC, where t_R is the retention time and W is the peak width. $N = 5.54 t_m/W_{0.5}$ for CZE, where t_m is the migration time and $W_{0.5}$ is the peak width at half height of the peak.

3.3. Method Application

Following the optimization of the sample pre-treatment method, the recommended techniques, namely CZE and UHPLC, were implemented for the determination of the studied drugs in wastewater samples spiked with the studied drugs, as shown in Figures 6 and 7. The collected data when applying the proposed methods for the analysis of the wastewater samples spiked with the studied drugs are presented in Tables 2 and 3.

Table 2. Application of the proposed CZE method for the analysis of wastewater samples spiked with the studied drugs.

Sample	**RIS (Rec.% * ± S.D.)**	**CPZ (Rec.% * ± S.D.)**	**HAL (Rec.% * ± S.D.)**
Sample 1	101.44 ± 1.01	99.96 ± 0.	100.54 ± 0.88
Sample 2	100.89 ± 0.67	101.11 ± 0.69	102.09 ± 0.60
Sample 3	99.54 ± 0.65	101.34 ± 0.79	101.90 ± 0.76

* Average of three determinations.

Table 3. Application of the proposed UHPLC method for the analysis of wastewater samples spiked with the studied drugs.

Sample	RIS (Rec.% * ± S.D.)	CPZ (Rec.% * ± S.D.)	HAL (Rec.% * ± S.D.)
Sample 1	100.76 ± 1.02	101.54 ± 0.89	99.56 ± 0.57
Sample 2	102.11 ± 0.99	100.23 ± 0.78	101.25 ± 0.96
Sample 3	99.78 ± 0.93	102.11 ± 0.76	102.03 ± 1.08

* Average of three determinations.

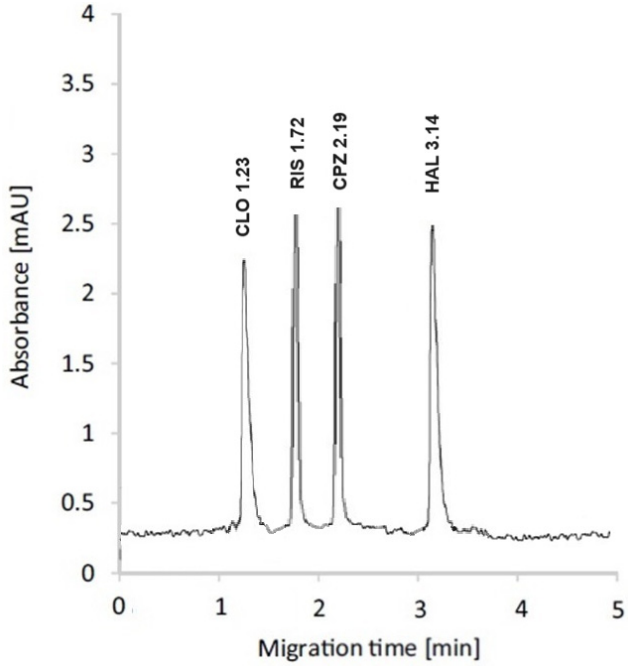

Figure 6. Capillary electropherogram for the studied antipsychotics in spiked wastewater sample. Conditions: fused-silica capillary (64.5 cm total length, 56 cm effective length, 75 µm I.D., 375 O.D.), phosphate buffer (pH 3.0, 30 mM). Voltage 16 kV. UV detection at 238 nm.

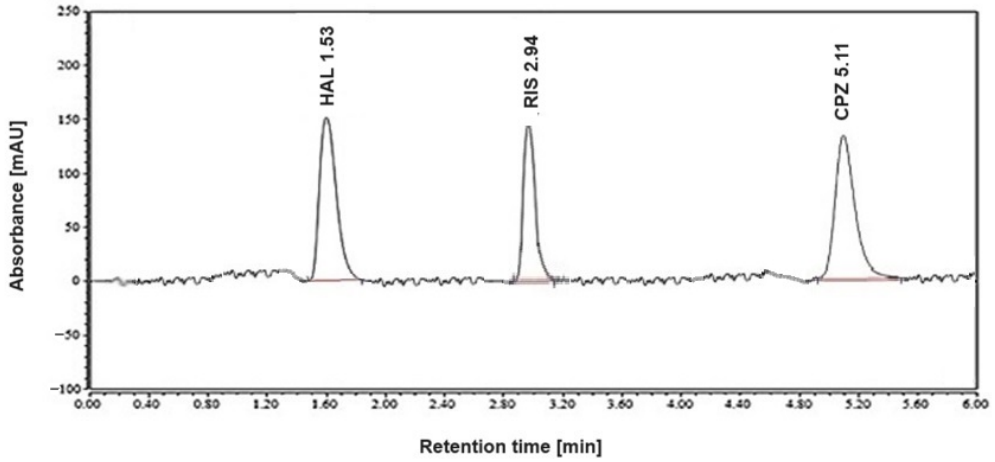

Figure 7. UHPLC chromatogram for the separation pattern of the studied antipsychotics in spiked wastewater sample. Conditions: stationary phase, C8 reversed-phase Waters™ column (100 × 2.1 mm, 1.7 µm, 130 Å). Mobile phase is a blend of potassium dihydrogen phosphate buffer containing 0.5% *v*/*v* trimethylamine (with pH adjusted to 3.0 using o-phosphoric acid) and acetonitrile in a ratio of 7:3, *v*/*v*. Flow rate 1.0 mL/min. UV detection at 238 nm.

4. Discussion

Ensuring the protection of human health requires careful monitoring of environmental contamination. The present study demonstrated the feasibility of quantifying different residues of antipsychotic drugs in wastewater effluents. This was achieved through the application of highly sensitive, fast, and accurate capillary zone electrophoresis (CZE) and ultra-high-performance liquid chromatography (UHPLC) techniques. The primary objective was to monitor the environmental levels of the investigated drugs and mitigate their potential negative impacts on human health.

The proposed methods were optimized to obtain the best separation pattern with maximum resolution either in the CZE or UHPLC method. Regarding the CZE method, the effect of electrolyte concentration in the BGE was studied, and it was found that the optimum phosphate ion concentration is 30 mM. This concentration confirms the lowest migration time and maximum peak area. On the other hand, the pH of the BGE greatly affects the EOF during the electrophoretic analysis. The impact of pH variation on the electrophoretic separation was studied, and a pH of 3.0 was found to be the optimum value. At the same time, the applied voltage was chosen to be 16 kV, which affords the best compromise between migration time, separation efficiency, and resolution.

Regarding the UHPLC method, the mobile phase was originally prepared by combining phosphate buffer and acetonitrile at a ratio of 80:20, v/v. The current mobile phase achieved complete separation of all analytes; however, it resulted in high retention, leading to prolonged analysis times (Supplementary Figure S3). Also, broad peaks were noticed, which lead to decreased resolution. Consequently, increased concentrations of acetonitrile were tested, namely within the range of 20–40%. The optimum percentage of acetonitrile that achieved full analyte separation with acceptable retention times was 30%. This percentage was employed in all subsequent tests to minimize analysis times without relying on the flow rate or composition gradients.

All of the parameters that were chosen for the method validation were included in a thorough validation sheet. The acquired data are shown in Table 1. The proposed methods were praised for their exceptional precision and accuracy. In addition, the validation sheet verified that the methods used were highly robust, as evidenced by the fact that even little changes in the experimental parameters did not have any noticeable impact on the performance of the methods. The techniques' sensitivities were noticeable due to the linearity range, detection, and quantification limits. They were highly proficient in accurately identifying the requisite drugs at amounts that were anticipated to be found in the actual wastewater samples.

When utilizing a technique like CZE or UHPLC, which are extremely reliable and have wide application but are prone to interference from a wide spectrum of interferents, a properly selected sample pre-treatment process is frequently required. The selection of solid-phase extraction (SPE) was based on its advantages over liquid–liquid extraction and protein precipitation techniques. SPE is a more efficient purification method compared to protein precipitation and is also less time consuming and environmentally harmful than liquid–liquid extraction. Moreover, SPE enables the samples to be concentrated, therefore achieving the appropriate level of sensitivity.

The selection of cyanopropyl (CN) cartridges for the sample pre-treatment stage was based on their hydrophilic, lipophilic, and hydrogen-bond-forming characteristics. The initial tests conducted with this absorbent yielded favorable outcomes in terms of both the amount of substance extracted and the degree of specificity. The initial washing stages used only water twice. To enhance selectivity, methanol was subsequently used to provide adequate purification of the sample while maintaining excellent extraction yields.

5. Conclusions

In this study, the CPZ, HAL, and RIS in actual wastewater effluents were simultaneously determined through the use of CZE and UHPLC methods in a comparative study for environmental pollution monitoring. The above procedures were optimized to

provide compromised sensitivity, accuracy, and selectivity. The utilization of SPE served as a method for preparing the sample, guaranteeing efficient purification and optimal extraction efficiency.

The novelty of the presented work originates from the fact that no previous studies have been published on the analysis of the examined drugs in pharmaceutical wastewater effluents utilizing the CZE and UHPLC procedures. The studied drugs were previously quantified in the wastewater effluents using GC-MS/MS and LC-MS/MS techniques. This article presents a solution to the limitations faced when using the GC-MS/MS technology, namely the issue of low repeatability [28]. Also, the proposed techniques (CZE and UHPLC) do not need the complex and tedious derivatization procedure needed for the GC analysis. Similarly, the proposed study resolves the issues associated with the implementation of LC-MS/MS analysis, including the limited sample throughput due to the elevated expenses for operation and maintenance.

Compared to liquid–liquid extraction, SPE is a more efficient and environmentally friendly sample pre-treatment method that requires less time and produces less pollution. The suggested methods demonstrate good recoveries when used for the simultaneous measurement of the drugs under study in wastewater effluents. Both the CZE and UHPLC methods can be effectively applied for the environmental monitoring of the studied drugs in wastewater samples and used as effective tools for controlling environmental pharmaceutical pollution.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors12070123/s1, Figure S1: Capillary electrophoretic diagram of the studied antipsychotics and the IS. Conditions: fused-silica capillary (64.5 cm total length, 56 cm effective length, 75 µm I.D., 375 O.D.), phosphate buffer (pH 3.0, 30 mM). Voltage 20 kV. UV detection at 238 nm. Each drug concentration 10 µg/mL. Figure S2: Capillary electrophoretic diagram of the studied antipsychotics and the IS. Conditions: fused-silica capillary (64.5 cm total length, 56 cm effective length, 75 µm I.D., 375 O.D.), phosphate buffer (pH 3.0, 30 mM). Voltage 16 kV. UV detection at 238 nm at 35 °C. Each drug concentration 10 µg/mL. Figure S3: UHPL chromatogram for the separation pattern of the studied antipsychotics. Figure S4: Capillary electrophoretic diagram of the studied antipsychotics at the LOQ. Figure S5: Capillary electrophoretic diagram of the studied antipsychotics at the LOD. Figure S6: UHPL chromatogram for the studied antipsychotics at the LOQ. Figure S7: UHPL chromatogram for the studied antipsychotics at the LOD.

Author Contributions: Conceptualization, S.A.A.-G.; methodology, R.A., N.F.S. and S.A.A.-G.; software, R.S. and S.L.; validation, I.E.O. and S.A.A.-G.; formal analysis, A.B.A.; investigation, A.Z., N.F.S. and S.A.A.-G.; resources, A.B.A. and S.L.; data curation, R.S., I.E.O. and A.Z.; writing—original draft, N.F.S. and S.A.A.-G.; writing—review and editing, A.B.A.; project administration, R.A. All authors have read and agreed to the published version of the manuscript.

Funding: This project is sponsored by Prince Sattam Bin Abdulaziz University (PSAU) as part of funding for its SDG Roadmap Research Funding Programme project number PSAU-2023/SDG/33.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sweetman, S.C. *Martindale: The Complete Drug Reference*, 36th ed.; The Pharmaceutical Press: London, UK, 2009.
2. Lemke, T.L.; Williams, D.A. *Foye's Principles of Medicinal Chemistry*; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2008.
3. Trawiński, J.; Skibiński, R. Studies on photodegradation process of psychotropic drugs: A review. *Environ. Sci. Pollut. Res.* **2016**, *24*, 1152–1199. [CrossRef]
4. Goff, D.C.; Falkai, P.; Fleischhacker, W.W.; Girgis, R.R.; Kahn, R.M.; Uchida, H.; Zhao, J.; Lieberman, J.A. The Long-Term Effects of Antipsychotic Medication on Clinical Course in Schizophrenia. *Am. J. Psychiatry* **2017**, *174*, 840–849. [CrossRef]

5. Daniel, D.; Gutz, I.G.R. Spectroelectrochemical determination of chlorpromazine hydrochloride by flow-injection analysis. *J. Pharm. Biomed. Anal.* **2005**, *37*, 281–286. [CrossRef]
6. Yasir, M. Development and Validation of UV Spectrophotometric Method for the Estimation of Haloperidol. *Br. J. Pharm. Res.* **2014**, *4*, 1407–1415. [CrossRef]
7. Kulkarni, S.; Chhabra, G.; Shivani, M. Development and validation of UV spectrophotometric method for the determination of risperidone in bulk and tablets formulation. *Int. J. Pharm. Chem. Res.* **2010**, *1*, 1–5.
8. Varak Neshin, M.; Sanavi Khoshnood, R.; Sanavi Khoshnoud, D. Surfactant assisted magnetic dispersive micro solid phase extraction-HPLC as a straightforward and green procedure for preconcentrating and determining Caffeine, Lidocaine, and Chlorpromazine in biological and water samples. *Int. J. Environ. Anal.* **2010**, *103*, 9661–9678. [CrossRef]
9. Yamini, Y.; Faraji, M. Extraction and determination of trace amounts of chlorpromazine in biological fluids using magnetic solid phase extraction followed by HPLC. *J. Pharm. Anal.* **2014**, *4*, 279–285. [CrossRef] [PubMed]
10. Ali, I.; Aboul-Enein, H.Y. Fast Determination of Haloperidol in Pharmaceutical Preparations Using HPLC with a Monolithic Silica Column. *J. Liq. Chromatogr. Relat.* **2005**, *28*, 3169–3179. [CrossRef]
11. Jones, T.; Van Breda, K.; Charles, B.; Dean, A.J.; McDermott, B.M.; Norris, R. Determination of risperidone and 9-Hydroxyrisperidone using HPLC, in plasma of children and adolescents with emotional and behavioural disorders. *Biomed. Chromatogr.* **2009**, *23*, 929–934. [CrossRef]
12. Zhang, L.; Wu, P.; Zhang, Y.; Jin, Q.; Yang, D.; Wang, L.; Zhang, J. A GC/MS method for the simultaneous determination and quantification of chlorpromazine and diazepam in pork samples. *Anal. Methods* **2014**, *6*, 503–508. [CrossRef]
13. Zhang, G.; Terry, A.V.; Bartlett, M.G. Sensitive liquid chromatography/tandem mass spectrometry method for the determination of the lipophilic antipsychotic drug chlorpromazine in rat plasma and brain tissue. *J. Chromatogr. B* **2007**, *854*, 68–76. [CrossRef]
14. Hempenius, J.; Steenvoorden, R.J.J.M.; Lagerwerf, F.M.; Wieling, J.; Jonkman, J.H.G. 'High throughput' solid-phase extraction technology and turbo ionspray LC-MS-MS applied to the determination of haloperidol in human plasma. *J. Pharm. Biomed. Anal.* **1999**, *20*, 889–898. [CrossRef]
15. Abounassif, M.A.; Hefnawy, M.M.; Hassanien, M.M.; Mostafa, G.A.E. Polyvinyl Chloride Membrane Sensors for Potentiometric Determination of Chlorpromazine in Some Pharmaceutical Formulations. *Sens. Lett.* **2012**, *10*, 966–973. [CrossRef]
16. Frag, E.Y.Z.; Zayed, M.A.; Omar, M.M.; Elashery, S.E.A.; Mohamed, G.G. Potentiometric Determination of Chlorpromazine HCl Using Carbon Paste Electrode in Pure and Pharmaceutical Preparations. *Int. J. Electrochem. Sci.* **2012**, *7*, 650–662. [CrossRef]
17. Purushothama, H.T.; Arthoba Nayaka, Y.; Manjunatha, P.; Yathisha, R.O.; Vinay, M.M.; Basavarajappa, K.V. Electrochemical determination of Chlorpromazine using l-Cysteine modified carbon paste electrode. *Chem. Data Collect.* **2019**, *23*, 100268. [CrossRef]
18. Zhang, G.; Terry, A.V.; Bartlett, M.G. Simultaneous determination of five antipsychotic drugs in rat plasma by high performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B* **2007**, *856*, 20–28. [CrossRef]
19. Mercolini, L.; Grillo, M.; Bartoletti, C.; Boncompagni, G.; Raggi, M.A. Simultaneous analysis of classical neuroleptics, atypical antipsychotics and their metabolites in human plasma. *Anal. Bioanal. Chem.* **2007**, *388*, 235–243. [CrossRef]
20. Reichert, J.F.; Souza, D.M.; Martins, A.F. Antipsychotic drugs in hospital wastewater and a preliminary risk assessment. *Ecotoxicol. Environ. Saf.* **2019**, *170*, 559–567. [CrossRef]
21. Khelfi, A.; Azzouz, M.; Abtroun, R.; Reggabi, M.; Alamir, B. Determination of Chlorpromazine, Haloperidol, Levomepromazine, Olanzapine, Risperidone, and Sulpiride in Human Plasma by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS). *Int. J. Anal. Chem.* **2018**, *2018*, 5807218. [CrossRef]
22. Miroshnichenko, I.I.; Baymeeva, N.V. Simultaneous Determination of Antipsychotic Drugs and Their Active Metabolites by LC–MS-MS and its Application to Therapeutic Drug Monitoring. *J. Chromatogr. Sci.* **2018**, *56*, 510–517. [CrossRef]
23. Pucci, V.; Raggi, M.A.; Kenndler, E. Quality Control of Pharmaceutical Formulations of Neuroleptic Drugs by Capillary Zone Electrophoresis. *J. Liq. Chromatogr. Relat.* **2000**, *23*, 25–34. [CrossRef]
24. Gao, Z.T.; Zhong, W.W. Recent (2018–2020) development in capillary electrophoresis. *Anal. Bioanal. Chem.* **2022**, *414*, 115–130. [CrossRef] [PubMed]
25. Wang, M.Y.; Gong, Q.; Liu, W.F.; Tan, S.W.; Xiao, J.; Chen, C.P. Applications of capillary electrophoresis in the fields of environmental, pharmaceutical, clinical, and food analysis (2019–2021). *J. Sep. Sci.* **2022**, *45*, 1918–1941. [CrossRef] [PubMed]
26. D'Atri, V.; Fekete, S.; Clarke, A.; Veuthey, J.L.; Guillarme, D. Recent Advances in Chromatography for Pharmaceutical Analysis. *Anal. Chem.* **2019**, *91*, 210–239. [CrossRef] [PubMed]
27. Fekete, S.; Schappler, J.; Veuthey, J.L.; Guillarme, D. Current and future trends in UHPLC. *Trends Anal. Chem.* **2014**, *63*, 2–13. [CrossRef]
28. Logarinho, F.; Rosado, T.; Lourenço, C.; Barroso, M.; Araujo, A.R.; Gallardo, E. Determination of antipsychotic drugs in hospital and wastewater treatment plant samples by gas chromatography/tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2016**, *1038*, 127–135. [CrossRef]
29. Massano, M.; Salomone, A.; Gerace, E.; Alladio, E.; Vincenti, M.; Minella, M. Wastewater surveillance of 105 pharmaceutical drugs and metabolites by means of ultra-high-performance liquid-chromatography-tandem high resolution mass spectrometry. *J. Chromatogr. A* **2023**, *1693*, 463896. [CrossRef]

30. Teasdale, A.; Elder, D.; Nims, R.W. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. In *ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (B)*; PharmaLogica, Inc.: Charlotte, NC, USA, 2005.
31. Convention USP. *The United States Pharmacopeia*; U.S. Pharmacopeia: Rockville, MD, USA, 2013.