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Determination of Some Non-Steroidal Anti-Inflammatory Drug Residues in Industrial Wastewater Effluents in Al-Kharj City using Capillary-Zone Electrophoresis Technique

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Surveillance and quantification of pharmaceutical contaminants in environmental samples is an essential and intricate undertaking because of their potential adverse impacts on human well-being, even at the minimal concentrations. The separation and quantification of three frequently utilized non-steroidal anti-inflammatory (NSAIDs) drugs, namely indomethacin (IND), ketoprofen (KTP) and ibuprofen (IBP), were achieved by the application of capillary-zone electrophoresis (CZE) in the wastewater effluents of a pharmaceutical facilities available in Al-Kharj city of Saudi Arabia. The solid-phase extraction (SPE) technique was optimized and employed as an efficient approach for the sample preparation prior to analysis. In order to optimize the separation profile for the drugs under investigation, the parameters of the capillary electrophoretic method were fine-tuned. The experimental medications exhibited a concentration range spanning from 5 to 100 µg/mL. The developed assay was subsequently utilized in an efficient manner to detect the substances accurately in the real wastewater samples. The proposed method can be efficiently utilized to monitor the extent of environmental contamination caused by the NSAIDs drugs being studied.

Keywords: Non-steroidal anti-inflammatory, Pharmaceutical plant, Pollution, Sustainability, Al-Kharj City.

INTRODUCTION

Drugs are now become as a new emergant environmental pollutants that are widely used in the domains of human and veterinary medicines. After being used, large amounts of these compounds are discharged into the water environment, resulting in their presence being detected in wastewater, surface water, and groundwater [1]. Additionally, medicines can cause harmful effects in the aquatic environments because they can naturally cause certain biological responses. These include the chance of endocrine disruption and severe side effects. Pharmaceutical contamination has emerged as a global environmental issue because of these factors. Pharmaceuticals mostly enter the environment through the release of effluents from wastewater and the disposal of medical items in households [2].

Non-steroidal anti-inflammatory medications (NSAIDs) are widely acknowledged as a class of pharmacological drugs that offer several benefits. Both human and animal populations commonly utilize these compounds as treatments due to their capacity to act as antipyretic, analgesic and anti-inflammatory agents [3]. These medications are based on the cyclooxygenase enzymes, more especially COX-1 and COX-2 as they play a crucial role in the conversion of arachidonic acid (AA) into prostaglandins (PGs). Furthermore, they also helps in the synthesis of thromboxane (TX), subsequently contributing to the control of the inflammatory response. The mode of action of NSAIDs may be further categorized into three subgroups: COX-1 selective, COX-1 non-selective and COX-2 selective [4].

The extensive utilization of NSAIDs in recent years may have contributed to their presence in the environment, particularly in surface water [5,6]. However, there is currently not a specific limit for the tolerance of these contaminants in soil or natural water. Chronic use of NSAIDs drugs has been associated with an elevated susceptibility to peptic ulcer disease, acute renal failure and stroke/myocardial infarction. Furthermore, the prolonged use of NSAIDs might worsen several chronic conditions, such as heart failure and hypertension [7]. It is crucial to identify and monitor the tested medications in the

environment, particularly in wastewater effluents, from this perspective.

Various analytical techniques were employed to quantify the pharmaceuticals under investigation in different sample forms. These techniques included IR spectroscopy [8], high-performance liquid chromatography (HPLC) [9-13], hydrophilic interaction liquid chromatography (HILIC) coupled to tandem mass spectrometry [14], gas chromatography (GC) [15,16] and electrochemistry [17]. Another analytical technique known as capillary electrophoresis (CE) has gained prominence in recent years. People primarily attribute this to its exceptional separation efficiency, minimal use of materials and reagents, rapid analysis time and versatility in handling various types of samples [18]. The study used micellar electro-kinetic chromatography (MEKC) to look at different doses of NSAIDs like ibuprofen (IBP) and ketoprofen (KTP). The method involved the use of 15% methanol in borate buffer (pH 9) and 100 mM sodium dodecyl sulfate as the developing liquid [19]. On the other hand, Bonvin *et al.* [20] used non-aqueous CE along with electrospray ionization mass spectrometry to analyze NSAIDs like indomethacin (IND) and ibuprofen (IBP) in human urine.

Literature indicates that LC-MS/MS is the most commonly used technique for concurrently determining the particular medicine. However, capillary-zone electrophoresis (CZE) stands out for its simplicity and cost-effectiveness when considering operating expenses [21] in comparison to LC-MS/MS technique. The primary objective of this work is to validate and employ a straightforward CZE technique for quantifying the targeted drugs in wastewater originating from the pharmaceutical facilities.

EXPERIMENTAL

Capillary-zone electrophoresis (CZE) analysis was conducted using Agilent 7100 CE instrument (Waldbronn, Germany), which also includes a UV-Vis detector and an automated injector. The separation technique utilized a fused silica capillary manufactured by Polymicro Technologies in Phoenix, Arizona, USA. The capillary had a length of 64.5 cm and an inner diameter of 75 µm. Peak areas and retention times, along with other pertinent data, were measured using the Agilent Chem-Station software. A pH meter (Mettler Toledo, Switzerland), was used to measure the pH of the solutions.

Standard solutions: Pure NSAIDs *viz*. ibuprofen (IBP, CAS no. 15687-27-1), ketoprofen (KTP, CAS no. 22071-15-4) and indomethacin (IND, CAS no. 53-86-1) were procured from Cymit Quimica S.L., Barcelona, Spain. The purities of IBP, KTP and IND were 100.32%, 100.86% and 100.91%, respectively. The solvents utilized were of the highest quality for HPLC analysis. Merck (Darmstadt, Germany) provided analytical grade methanol, sodium hydroxide and boric acid. The Milli-Q Plus system, manufactured by Millipore, Bedford, USA, was employed to get the distilled water required for the present investigation. A background electrolyte (BGE) consisting of 40 mM solution of boric acid, which was adjusted to pH 8.5 using NaOH, was employed. The running buffer was prepared by adding methanol to BGE as an organic modifier at a concentration of 10%.

The samples were processed using Oasis® HLB cartridges. Acrodisc nylon membrane syringe filters (0.2 µm, 13 mm; Pall Corp., Washington, NY, USA) were used to filter the BGE and final extract. The wastewater samples were filtered using a Millipore solvent filter system and nylon membranes (0.2 µm, 47 mm; Supelco, Bellefonte, PA, USA).

In order to prepare stock solutions with a concentration of 500 µg/mL for each compound, an appropriate quantity was dissolved in the BGE. The stock solutions were stable for at least 45 days when stored at -20 ºC. Every time, the working-standard solutions were prepared freshly and the solutions were stored in a dark environment.

Collection of wastewater samples: Wastewater samples were collected from a pharmaceutical plant in Al-Kharj city, Kingdom of Saudi Arabia (KSA). To prevent any degradation, the samples were stored in opaque glass vials and kept under refrigeration.

Pre-conditioning of capillaries: The preconditioning procedure for new capillaries entailed a series of flushing steps. Initially, a 20 min flush was performed using a 1 M NaOH solution followed by another 20 min flush with same solution. Deionized water was then used for 2 min followed by 30 min flush with a BGE solution. On the subsequent day, the capillary was subjected to a 20 min flush with a 0.1 M NaOH solution, followed by a 5 min flush with a 1 M NaOH solution, a 2 min rinse with water and a 30 min rinse with BGE. The capillary ends were immersed in water overnight following a daily 20 min cleansing process.

Conditions for capillary electrophoresis: To achieve the optimum separation, a voltage of 20 kV was applied at room temperature to separate the medicines under investigation. The solution was injected hydrodynamically for 10 s at 60 mbar. The substances under investigation were identified at the wavelength of 222 nm.

Validation of the method: The ICH-Q2B recommendations [22] were followed for the assay validation.

Linearity: Precisely different portions of the examined NSAIDs (ranging from 50 to 1000 µg) were transferred to a series of 10 mL of volumetric flasks. In order to attain a concentration ranging from 5 to 100 µg/mL for each medicine under investigation, the final capacity of each flask was filled with BGE. The elution procedure was conducted by employing a running solution consisting of 50 mM borate buffer with a pH of 8.5, supplemented with 10% methanol as organic modifier.

Accuracy: It can be defined as the proportion of an analyte recovered from the specified amount [22]. The linearity conditions were employed to analyze nine samples with 10, 30 and 50 µg/mL for each medication being investigated.

Accuracy: The percentage relative standard deviation for a set of statistically significant trials, both between and within a single day, quantifies precision. Three different concentrations of each medication (10, 30 and 50 µg/mL) were subjected to three separate tests, either on the same day (intra-day) or on three consecutive days (inter-day).

Limit of detection (LOD) and limit of quantification (LOQ): The limit of quantification (LOQ) refers to the minimum amount of a drug that can be quantified with accuracy and

consistency. Conversely, the limit of detection (LOD) represents the minimum quantity that surpasses background noise. These parameters were obtained in accordance with the guidelines stated by the US Pharmacopeia (USP) [23]. To determine the LOQ and LOD, the concentrations must be identified that generate peaks which are ten times and three times greater than the baseline noise, respectively.

Robustness: Assessing the effects of minor modifications on the proposed methodology allows for the evaluation of robustness. The achievement was attained by manipulating the methanol concentration (± 1%) inside the elution solution. Furthermore, a modification of 1 kV in the applied voltage was implemented.

Applications: Within the framework of the solid-phase extraction (SPE) method, Oasis® HLB cartridges were utilized. The SPE sorbent was initially pre-conditioned using a solution consisting of 6 mL of methanol and 5 mL of deionized water. The wastewater sample was agitated for 1 min with a volume of 10 mL. Following this, the specimen was allowed to remain in a light-free environment for at least 0.5 h. Subsequently, the sample underwent filtration in order to remove any suspended particles. A precise amount of drugs (10 µg of each drug) was added to the sample and then put onto the pre-conditioned cartridge. To eliminate any unbound chemicals and minimize interference, the cartridge was washed with 5 mL of water. Next, the sample was eluted using 5 mL of methanol at a flow rate of 1 mL/min. The eluate was subjected to vacuum evaporation using a rotary evaporator and then it was reconstituted using 1 mL of BGE. The optimized procedures were ultimately implemented.

RESULTS AND DISCUSSION

CZE optimization: The separation quality is significantly influenced by the electrolyte concentration due to its propensity to affect electro-osmotic flow (EOF), joule heating, ionic strength and the current produced in the capillary. Hence, the electrolyte concentration will exert an impact on both the peak area and migration time. An investigation was conducted to examine the impact of different amounts of borate (20 mM to 70 mM) on the BGE (Fig. 1). The concentration of the studied NSAIDs was established at 50 µg/mL, the pH was modified to 8.5 and the methanol percentage in the running buffer was set at 10%. Analyte peak areas increased linearly with borate concentrations, ranging from 20 mM to 50 mM. When the concentration of borate exceeded 50 mM, an increase in the migration time, a decrease in the peak area and a significant rise in the analysis time were observed. Thus, the optimal concentration of borate was determined to be 50 mM.

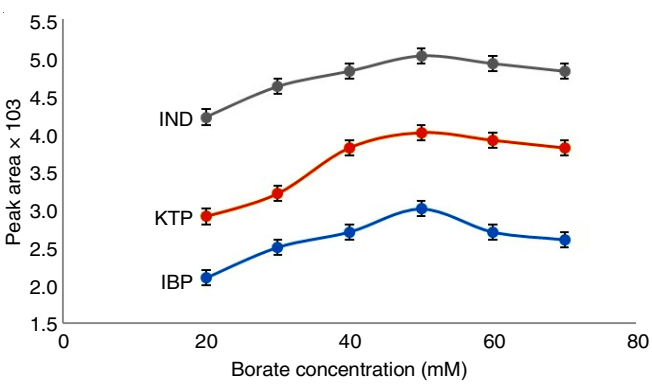

Fig. 1. Effect of borate concentration on the capillary electrophoretic performance

The buffer pH has a significant impact on the CZE analysis of the pharmaceuticals under investigation, as it directly impacts the electro-osmotic flow (EOF). A concentration of 50 mM of borate was used, with a methanol concentration of 10% and the analytes were fixed at a concentration of 50 µg/mL. The results demonstrated that when the pH of BGE increased from 7.0 to 8.5, there was a corresponding increase in the peak area (Fig. 2). However, when pH exceeded 8.5, there was an rise in the migration time, but the peak area remained rather stable. Therefore, a BGE pH of 8.5 was chosen for further investigation.

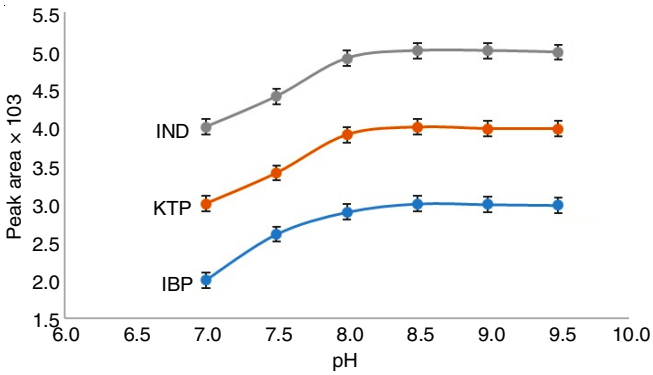

Fig. 2. Effect of pH on the capillary electrophoretic performance

The inclusion of an organic modifier in the developing liquid also significantly impacts the efficiency of separation. Methanol has the ability to enhance separation efficiency by altering the zeta potential and decreasing the EOF. This study investigates the impact of varying concentrations of methanol (0, 10, 15 and 20%, v/v) in the borate buffer on the separation of the drugs under investigation. The analytes were attained with optimal baseline resolution by the utilization of a buffer solution consisting of 10% (v/v) methanol. Hence, the employed running buffer consisted of borate buffer (50 mM, pH 8.5) supplemented with 10% methanol. The electroosmotic and electrophoretic velocities exhibit a direct proportionality to the intensity of electric field. Consequently, employing the maximum voltage achievable will yield the shortest migration time. The primary constraint in this context is heat. Thus, the study focused at four different voltages *viz.* 10, 15, 20 and 25 kV. The voltage for separation was established at 20 kV, resulting in an optimal equilibrium between resolution, peak area and migration time. After modifying the experimental parameters, a mixture of the examined pharmaceuticals was analyzed, whereby each drug was administered at a concentration of 10 µg/mL. Fig. 3 displays the separation profile.

Validation of the method: The validation process was conducted following the requirements outlined in ICH-Q2B [22]. A linear relationship was observed between the peak area and drug concentration within the concentration range of 5 to

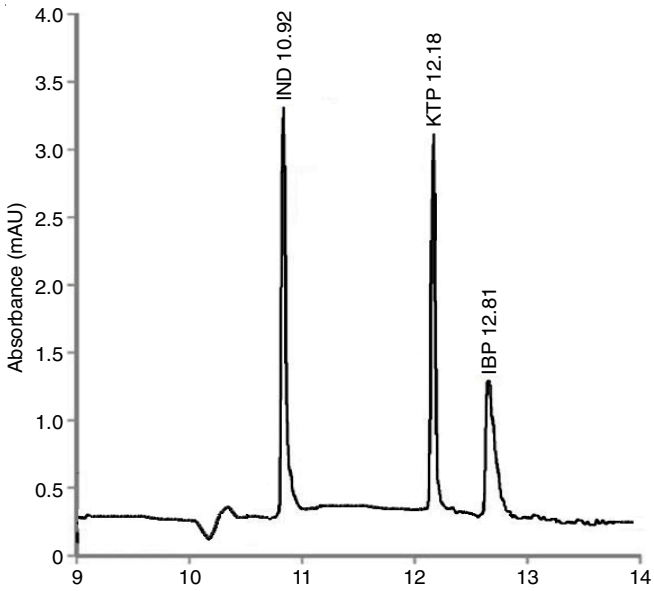

Fig. 3. Capillary electrophoretic separation pattern of IND, KTP and IBP

100 µg/mL. The subsequent regression equations were determined to be as follows:

$$\text{Peak area (IND)} = 99.80\ C - 31.30 \quad r = 0.9999 \quad (1)$$

$$\text{Peak area (KTP)} = 79.50\ C - 16.60 \quad r = 0.9999 \quad (2)$$

$$\text{Peak area (IBP)} = 60.50\ C - 12.50 \quad r = 0.9996 \quad (3)$$

where C is the concentration (µg/mL) and r is the correlation coefficient.

As presented in Table-1, the acceptable levels of repeatability, accuracy and intermediate precision of the method is shown. The robustness of the approach was further assessed by introducing a minor modification to the applied voltage and the composition of the elution liquid. The recommended robustness of the approach was validated by all the data, demonstrating that implementing these minor modifications had no significant effect on the proposed method. The calculated LOD and LOQ values proved the method's sensitivity.

Method application: When employing a method such as CZE, which is highly dependable and has extensive use but is susceptible to interference from a diverse range of interferents, it is often necessary to implement a well-chosen sample pretreatment procedure. Solid-phase extraction (SPE) was chosen due to its superiority over liquid-liquid extraction and protein precipitation methods. In this work, the real wastewater samples contaminated with the specific amounts of the pharmaceuticals was investigation using the optimized sample preparation approach. After employing the same sample pretreatment methodology (Table-2), the results were compared to those of the reference method [12] used to quantify the examined NSAIDs. The technique employed for the examination of the medicines under investigation was determined to possess characteristics of simplicity, sensitivity and suitability for environmental analysis.

Conclusion

This study aims to assess the presence of nonsteroidal anti-inflammatory drugs (NSAIDs) in wastewater effluents using

TABLE-1
VALIDATION PARAMETERS OF THE PROPOSED METHOD

Parameter	Ibuprofen	Indomethacin	Ketoprofen
Resolution factor (Rs)	–	$R_{IND/KTP}$ = 12.60	$R_{KTP/IBP}$ = 4.20
Accuracy (Mean* ± SD)	99.34 ± 0.94	100.75 ± 0.47	101.17 ± 1.02
Precision:			
Intra-day (Mean* ± RSD%)	101.35 ± 0.98	101.31 ± 1.56	102.47 ± 0.99
Inter-day (Mean* ± RSD%)	101.93 ± 0.55	99.67 ± 1.39	102.44 ± 1.45
Robustness:			
BGE pH variation	98.96 ± 0.98	101.43 ± 0.95	100.13 ± 0.57
Applied voltage variation	100.46 ± 1.62	101.45 ± 1.04	98.49 ± 1.13
Linearity:			
Range (µg/mL)	5-100	5-100	5-100
Slope	99.80	79.50	60.50
Intercept	-31.30	-16.60	-12.50
Correlation coefficient (r)	0.9999	0.9999	0.9996
LOQ (µg/mL)	5.0	5.0	5.0
LOD (µg/mL)	1.7	1.7	1.7

*Average of three readings.

TABLE-2
DETERMINATION OF THE STUDIED NSAIDs IN SPIKED INDUSTRIAL WASTEWATER SAMPLES USING THE CAPILLARY ZONE ELECTROPHORESIS METHOD AND THE REFERENCE METHOD [12]•

Sample	Ibuprofen (Rec.%* ± S.D.)	Ref. method (Rec.%* ± S.D.)	Indomethacin (Rec.%* ± S.D.)	Ref. method (Rec.%* ± S.D.)	Ketoprofen (Rec.%* ± S.D.)	Ref. method (Rec.%* ± S.D.)
Sample 1	101.78 ± 1.23	100.97 ± 0.88	99.96 ± 1.14	99.56 ± 0.78	100.89 ± 0.66	100.54 ± 0.85
Sample 2	101.89 ± 0.58	100.59 ± 0.92	100.11 ± 0.87	100.33 ± 0.87	101.45 ± 0.75	101.76 ± 0.45
Sample 3	99.54 ± 0.65	99.76 ± 0.65	101.55 ± 0.45	100.54 ± 0.77	102.90 ± 0.78	101.67 ± 0.61

*Average of three determinations.
•RP-HPLC method using Kinetex Evo C18 column (150 × 4.6 mm × 5 µm) as a stationary phase and acetonitrile: 10 mM phosphate buffer pH 2.5 (50:50, v/v), as a mobile phase. The flow rate is 0.8 mL/min. and detection using photo-diode array detector.

the capillary-zone electrophoresis (CZE) approach for measuring environmental contamination. The optimized methodology has been thoroughly refined to achieve the optimal levels of sensitivity, accuracy and selectivity. When compared to liquid-liquid extraction, SPE is a more effective and eco-friendly process for pre-treating samples. It consumes less time and generates fewer pollutants. The proposed methodology exhibits favourable recoveries and confirms the effectiveness of optimal sample extraction techniques when employed for the concurrent quantification of the medicines being investigated in wastewater effluents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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