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Original article

Green inexpensive capillary electrophoretic method for the determination of Eszopiclone in pharmaceutical preparations

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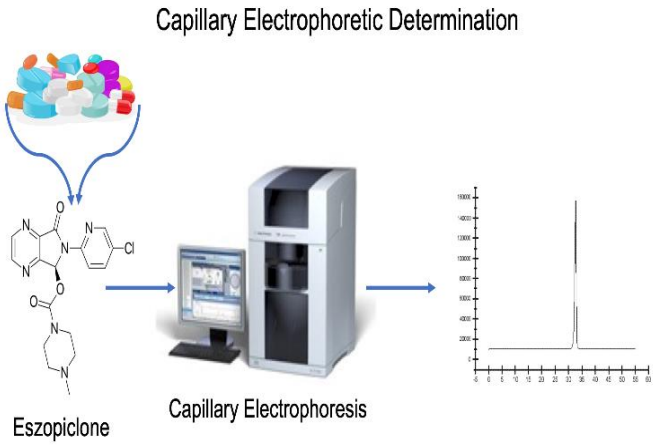
Abstract:

Background: Eszopiclone (EZO) is a newly FDA-approved drug for the treatment of sleeping disorders (insomnia). It is a short-acting non-benzodiazepine sedative hypnotic. Objectives: The development and validation of an environment-friendly, rapid, simple, and inexpensive capillary electrophoresis (CE) for the routine determination of eszopiclone (EZO) in commercial dosage forms. Method: The method was performed using 75 µm × 82 cm fused-silica capillary (70 cm effective length). Detection was performed at 300 nm with a background electrolyte of 10 mM phosphate buffer pH 7.50 with 30kV applied voltage for 10 s at 25°C temperature. Well-shaped peaks of eszopiclone were obtained (at t_m = 4.41 ± 0.18 min). Validation was performed in terms of precision, accuracy, specificity, and robustness in accordance with ICH guidelines. Results: Concentrations within the range of 10–300 µg mL^{-1} were found to be linear with a high correlation coefficient, r = 0.9998. Detection and quantitation limits were 1.36 and 4.66 µg mL^{-1}, respectively. Our proposed method was successfully applied for eszopiclone analysis in its pharmaceutical dosage form. The Analysis of the variance test confirmed that there were no significant differences between the proposed method and the two reported reference methods.



Keywords: Eszopiclone; capillary electrophoresis; tablets; validation; quality control.

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1. Introduction

Eszopiclone (EZO) is a newly FDA-approved drug for the treatment of sleeping disorders (insomnia). It is a non-benzodiazepine short-acting hypnotic sedative. Chemically, EZO belongs to the cyclopyrrolones group of drugs (**Fig. 1**).

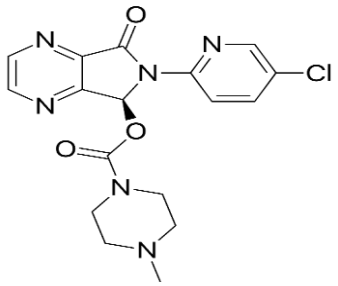

Fig. 1: Chemical structure of eszopiclone.

EZO is the pharmacologically active dextrorotatory stereoisomer of zopiclone. It has less anticholinergic side effects than racemic zopiclone, such as the risk of next-day impairment, tolerance, or rebound effects [(1)]. It is important to have sensitive and selective analytical methods for EZO analysis [(2-8)]. Previous methods reported for the analysis of EZO include spectrophotometric techniques [(9, 10)], HPLC [(11-15)], UPLC [(16)], TLC [(17, 18)] and HPTLC [(19)] techniques that have recently been reported. However, literature reviews lack any Capillary Electrophoresis (CE) method for EZO analysis in its pharmaceutical formulation. CE is becoming a routine analytical technique even with complex samples[(19-23)] due to the advantages of high efficiency, high speed, minimum sample pretreatments, and low running cost, therefore, would be most feasible for high throughput analysis.

Different Pharmacopoeias support the development of novel eco-scale methods for simple and fast analysis of various drugs. Such methods are favorable as they use low quantities of various materials and reagents resulting in minimizing pollution level. A main benefit of CE is being highly efficient and requires small mobile phase quantities. Much fewer quantities of solvents are used for sample analysis with CE when compared to HPTLC and HPLC methods.

This work aims to establish a novel, green, inexpensive CE analysis for EZO determination in its formulations and its application for routine quality control analysis.

2. Experimental

2.1. Materials and Reagents

Eszopiclone (98.82%),(*S*)-6-(5-Chloro-2-pyridinyl)-7-oxo-6, 7-dihydro-5*H*-pyrrolo[3,4*b*]pyrazin-5yl-4-methyl-1-piperazine carboxylate and NightCalm® tablets (3 mg EZO/tablet with batch number 60264) were offered by Medizen Pharmaceutical Industries, Borg El Arab, Alexandria, Egypt. Phosphate buffer was prepared using NaH_2PO_4 and Na_2HPO_4 (ultrapure bioreagent grade, J.T. Baker, United Kingdom). Freshly prepared deionized distilled water was used throughout all the conducted experiments.

2.2. Instrumentation

CE Agilent 7100 series (Agilent Technologies, Germany) was used. It is supplied with a DAD and Agilent ChemStation Software for data handling. 75 µm fused silica unmodified capillary that is 82 cm in length with 70 cm to the detector was used for the analysis.

2.3. Separation conditions

Phosphate buffer, pH 7.5 (10 mM) was used for the separation. Hydrodynamic injection of the sample was performed at a pressure of 50mbar for 10s. 30 kV (365.8 $V.cm^{-1}$) separation voltage and a 300 nm detection wavelength were used.

Before analysis, the capillary was conditioned by 1M NaOH for 5 min. Prior to each run, 0.1 M NaOH was used for capillary rinsing for 2.5 min, followed by 2.5 min wait time, and then deionized water was used for capillary rinsing for 2.5 min. At the end, the buffer was used to rinse the capillary for 5 min.

2.4. Construction of calibration curve

1 mg mL^{-1} EZO solution in deionized water was prepared. Various concentrations of 10–

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300 µg mL^{-1} were prepared. Triplicate measurements were made for each solution. The peak areas at t_m= 4.41 ± 0.28 min were measured and plotted against EZO concentrations to give a linear calibration curve (**Table 1**).

Table 1: Analytical figures of merits for the determination of EZO with the described CE method

Parameters	Proposed CE method
Linearity range (µg mL^{-1})	10.0 - 300
LOQ (µg mL^{-1})	10.0
LOD (µg mL^{-1})	1.20
Intercept (a)	-5.43
Slope (b)	2.28
Correlation coefficient (r)	0.9998
S_a	1.58
S_b	0.01
$S_{y/x}$	2.33
F	26265
Significance F	$8.69x10^{-9}$

S_a is the standard deviation of intercept, S_b is the standard deviation of slope and $S_{y/x}$ is the standard deviation of residuals

2.5. Assay of pharmaceutical formulations

NightCalm® (3 mg EZO/tablet, batch no. 60264) pharmaceutical formulation was analyzed for the EZO content. Twenty tablets were finely powdered from the dosage form, a mass of 3 mg EZO was weighed, transferred with 10 mL methanol to a volumetric flask (25 mL), and diluted to volume with deionized water. Analysis of the prepared sample was performed using the same procedure mentioned in section 2.4 to determine EZO concentration.

3. Results and discussion

3.1. Optimizing CE parameters

Various parameters were examined for enhancing the CE procedure used in this study.

3.1.1. Buffer and pH

Choice of the electrolyte type, pH, and concentration are requisites for the determination of different analytes in CE. The background electrolyte pH affects the separation resolution. Different buffers of various pHs have been examined, such as acetate (pH 3.6–5.5), borate (pH 8–10), phosphate (pH 6–8), and citrate (pH 3–6). Such trials revealed that with pH < 7, tailed peaks and long migration time were obtained, while buffers with pH > 8 caused very short migration times (less than 2 min). Among all the buffers tested, phosphate buffer at pH 7.5 resulted in well-shaped peaks with short migration time. On the other hand, the buffer concentration showed a pronounced effect on the CE separation because it influences EOF and current developed in the capillary. Subsequently, various phosphate buffer concentrations in the range of 10–60 mM have been tested. Results revealed that upon increasing the buffer concentration, narrower peaks were obtained with longer migration time. Therefore, 10 mM phosphate buffer was selected as sharp-symmetric peaks were obtained with short time of analysis (**Fig. 2a**)

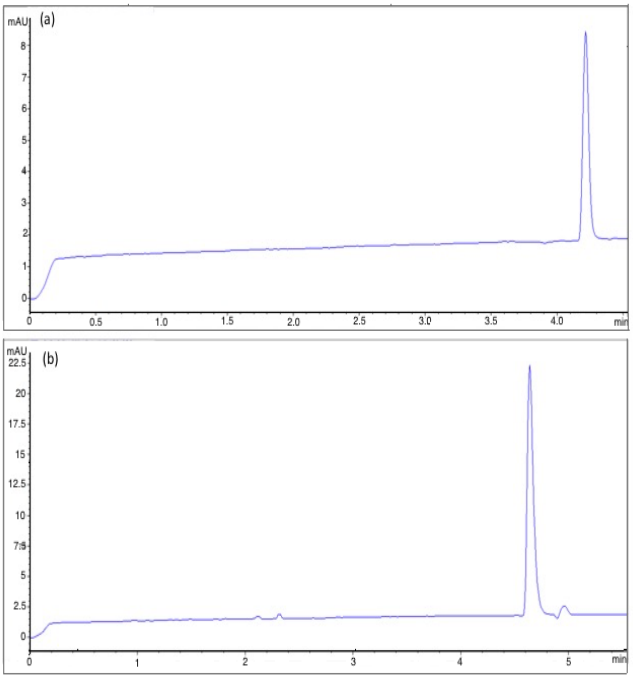

Fig. 2: CE electropherogram of (a) standard eszopiclone (50 µg mL^{-1}); (t_m: 4.23 ± 0.02), and (b) eszopiclone (EZO) extracted from NightCalm® tablets (t_m: 4.56 ± 0.02).

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3.1.2. Capillary Temperature, applied voltage, injection time and detection wavelength.

Temperature influence was examined in the range of 15–30 ˚C. Greater temperatures produced sharper peaks and lower times of migration. Thus, 25˚C was selected for this study as good efficiency and reasonable analysis time were obtained. Also, an applied voltage in the range of 5–30 kV was studied for EZO CE determination. It is expected that as the applied voltage increases, the EOF will increase, minimizing the analysis time and increasing efficiencies. However, applied voltage elevation results in producing higher current and more joule heating. So, to determine the highest possible voltage causing the smallest joule heating, Ohm's plot was constructed. With 25°C temperature and 30 kV applied voltage; no deviations appeared in Ohm's plot. Thus, 30 kV was selected for EZO determination. Also, in order to optimize the injection time as it affects peak height and peak width, a 50 mbar hydrodynamic injection of EZO solutions with changing injection time of 5 - 40 s was performed. At injection times greater than 10 s, the peak widths increased with deformed peak shapes. Thus, 10 s injection time was selected for the analysis. Finally, a 300 nm detection wavelength was selected for the selective determination of EZO without any interferences of excipients present in the matrix of pharmaceutical formulation.

The migration time of EZO obtained using the proposed method was tm = 4.23 ± 0.02 min and displayed a sharp symmetric peak for EZO (Fig. 2a) with a short run time of 5 min, allowing this proposed method to suit the high throughput analysis required in various QC laboratories.

3.2. Method Validation

According to the ICH guidelines [24], the parameters studied are as follows:

3.2.1. Linearity

Various concentrations of EZO were measured. The calibration graph showed a linear relationship. Table 1 presents all the statistical parameters and linearity results [25, 28]. Results show high F-values (variance ratio) indicating that the regression mean of squares will increase, while residuals will decrease, increasing the steepness of the line and lowering data scatter around the line [26, 29].

3.2.2. Detection and Quantitation Limits

LOD and LOQ were estimated experimentally using the signal/noise ratio approach. The obtained LOD and LOQ (Table 1) showed that the proposed method is sufficiently sensitive.

3.2.3. Accuracy and Precision

Three replicates at three different EZO concentration levels (50,100 and 200 µg mL^{-1}) were measured. The proposed method was done three times during one day to assess intra-day precision and on three separate days to assess the inter-day precision for each concentration. The obtained results are reported in **Table 2** showing low %RSD and % Er < 2%. This reflects the good accuracy and high precision of the studied method.

Table 2: Evaluation of accuracy and precision of the proposed CE method for the determination of EZO (n=9)

Concentration	Mean ± SD[b]	RSD %[c]	Er %[d]
(a) Accuracy and intra-day precision			
50	49.8± 0.99	1.99	-0.32
100	100.5± 1.6	1.61	0.45
200	198.9± 2.08	1.05	-0.55
(b) Accuracy and inter-day precision			
50	49.1± 0.47	0.95	-1.86
100	96.3± 1.15	1.20	-3.70
200	191.1± 1.51	0.79	-3.94

[a] Final concentration in µg mL^{-1}

[b] Mean ± standard deviation of three determinations.

[c] Percentage relative standard deviation.

[d] Percentage relative error.

3.2.4. Robustness

The method robustness was accomplished by the determination of three different EZO concentrations (50, 100, and 200 µg mL^{-1}). The studied parameters were buffer

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concentration, buffer pH, and the detection wavelength (**Table 3**). Small variations in such parameters had no significant effect on EZO analysis with the developed method reflected by the obtained small %RSD values (**Table 3**) indicating the good method robustness.

Table 3: Robustness of the proposed CE method for the determination of EZO

	Mean % Found of peak areas ± RSD[a]	Migration Time ± SD
1) Buffer conc. (10 mM ± 1)	99.38±1.50	4.64±0.112
2) pH value (7.5 ± 0.2)	98.14±2.38	4.57±0.095
3) Wavelength (300 ± 2 nm)	100.18±2.07	4.56±0.266

[a] Mean ± %RSD of the average of three concentrations; 50, 100, and 200 µg mL^{-1}

3.2.5. Specificity

EZO standard and the pharmaceutical dosage form of EZO were analyzed and compared. The EZO peak was determined by comparing the spectra and t_m of the peak to standards. In addition, the superimposed UV spectra of EZO in samples of dosage form without any interference from tablet excipients indicated a good correlation between the spectra of EZO samples (**Fig. 3**).

3.3. Analysis of the pharmaceutical dosage forms

Determination of EZO in its commercial preparations was applied (Fig. 2b). The obtained data was in good agreement with the label claims. **Table 4** shows the obtained results demonstrating good % recovery and standard deviation and showing no interferences from excipients and other components that may be present in the dosage form. Such as sucrose, lactose-monohydrate, starch, hypermellose, sodium alginate, PEG 6000, povidone K30, boric acid, purified talc, titanium dioxide, magnesium stearate, rose-caramellose sodium, brilliant blue-lake, colloidal silicon hydroxide, carboxy methyl cellulose and citric acid (Fig. 2b). Comparison of the results with two other reported methods [15, 20] was accomplished using the variance ratio F-test and Student's t-test in order to compare (**Table 4**). The values obtained were less than the theoretical ones, which indicate that there is no significant difference in precision and accuracy for the performance of the compared methods.

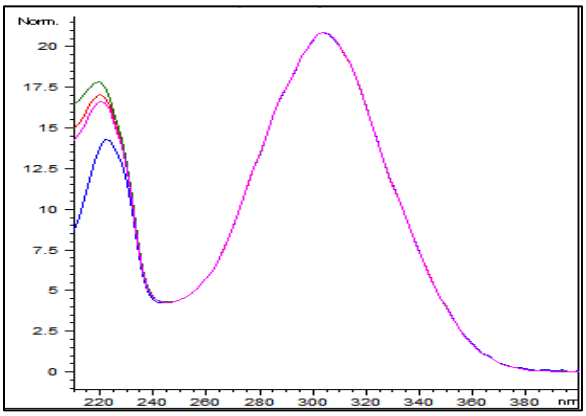

Fig.3: EZO peak purity spectrum obtained from pharmaceutical formulations.

3.4. Assessment of Method Greenness

Two comprehensive approaches were applied for assessing the greenness of the applied analytical methodology; GAPI and analytical Eco-scale [27, 28]. Using such meters, we compare different parameters and different steps of our developed analytical method to those applied in previous reports including; HPLC [11], HPTLC [19], and spectrophotometric methods [10].

In the GAPI scale approach, specific symbols composed of five pentagrams can be used as a tool to assess the environmental impact and greenness of the developed analytical methodology involved in all steps starting from sample collection and ending with sample determination. A pentagram scale composed of three colors; green, yellow, and red is considered a visual representation for judging the environmental impact of each step of the procedure as low, medium to high impact, respectively [27]. **Fig. 4** shows the

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GAPI pictograms constructed according to data collected in **Table 5.** Both pictogram and data confirm that the proposed electrochemical method has the lowest environmental hazards compared to the other reported methods.

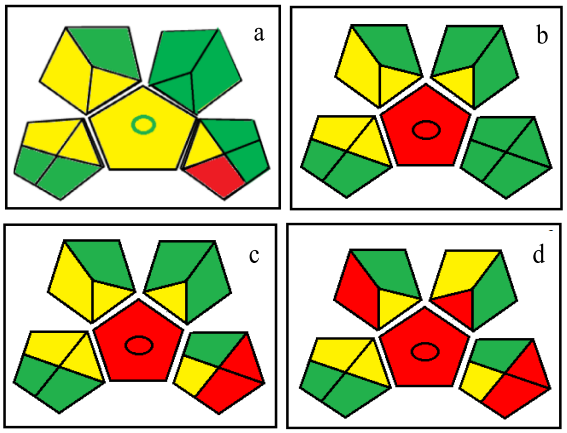

Fig. 4: Green Analytical Procedure Pictograms for (a) proposed CE method, (b) reported spectrophotometric method, (c) reported HPTLC method, and (d) reported HPLC method.

The analytical Eco-scale approach [28], provides information about the greenness of the analytical method more quantitatively concerning the type and amount of reagents and solvents, instrument energy, occupational hazards, and generation of wastes. Penalty points are assigned for each parameter to calculate the final eco-scale score by subtraction of the total penalty points from 100. The higher the Eco-scale value (closer to 100), the greener the analytical method. **Table 6** demonstrates that our proposed electrochemical method has the highest Eco-scale score of 94 compared to other reported spectrophotometric, HPTLC, and HPLC methods of 88, 82, and 73 scores, respectively. Therefore, the developed method is the most eco-friendly compared to the other reported methods.

3.5. Comparison to Other Reported Methods

Few methods have been reported for the determination of EZO pharmaceutical dosage forms in literature. Pandya et. al.[10] has reported a simple spectrophotometric method by direct measurement of maximum absorbance of EZO peak at 305 nm, El-Yazbi *et al.*[19] reported an HPTLC method for EZO determination analytical tool applicable for quality control using methanol–water (60:40 v/v) as a mobile phase. While the HPLC method was reported by Ravi *et al.* [11] with a mobile phase consisting of phosphate buffer of pH 3.5 and acetonitrile in the ratio of 50:50 (v/v), **Table 7.**

4. Conclusions

For the first time, an environment-friendly, inexpensive, simple, and rapid CE analysis was presented for the analysis of EZO in pharmaceutical dosage forms. Statistical analysis indicated the suitability of the proposed method for the analysis of EZO in pharmaceutical preparations. Upon comparison of the described CE method with the reported HPLC and HPTLC methods for the analysis of EZO in pharmaceutical formulations, our method showed many advantages such as being a fast inexpensive method that requires minimum sample pretreatment. Also using water as the diluting solvent and aqueous running buffer promotes our method to be a greener alternative to other non-environment friendly methods. Moreover, upon comparison with the spectrophotometric method previously reported, the proposed CE method showed higher selectivity and specificity. This permits the described method to be selected for the routine analysis of EZO in commercial tablets during high-throughput analysis in QC laboratories and bioavailability studies.

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Table 4: Statistical comparison of EZO determination

Dosage form	Mean found± RSD% [a]		Mean found± RSD% [a]	
	Proposed CE method	Reported method [b]	Proposed CE method	Reported method [c]
NightCalm® tablets (3 mg EZO/tablet)	99.06±1.38	99.95±1.19	99.06±1.38	99.07±1.19
t[d]	0.304		0.989	
F[d]	1.34		1.33	

[a] Percentage recovery from the label claim amount, Percentage relative standard deviation for five determinations.
[b] HPTLC method [21].
[c] HPLC method [15]
[d] Theoretical values of t and F for p=0.05 are 2.31 and 6.39, respectively.

Table 5: Comparison between analytical procedure index parameters of the proposed method and other reported methods for EZO determination

Parameters	Proposed Electrochemical Method	Reported Spectrophotometric Method(10)	Reported HPTLC Method(19)	Reported HPLC Method(11)
Sample Preparation				
Collection (1)	At-line	At-line	At-line	At-line
Preservation (2)	None	None	None	None
Transport (3)	None	None	None	None
Storage (4)	Normal conditions	Normal conditions	Normal conditions	Normal conditions
Type of method: direct or indirect (5)	Simple procedures	Filtration	Filtration	Filtration
Scale of extraction (6)	Micro-extraction	Micro-extraction	Micro-extraction	Micro-extraction
Solvents/reagents used (7)	Green solvents/ reagents used	Green solvents/ reagents used	Green solvents/ reagents used	Non-green solvents/ reagents used
Additional treatments (8)	None	None	None	None
Reagent and solvents				
Amount (9)	<10 mL	10-100 mL	10-100 mL	>100 mL
Health hazard (10)	Highest NFPA Score = 1	Highest NFPA Score = 1	Highest NFPA Score = 1	Highest NFPA Score = 3
Safety hazard (11)	Highest NFPA Instability Score = 0	Highest NFPA Instability Score = 0	Highest NFPA Instability Score = 0	Highest NFPA Instability Score = 0
Instrumentation				
Energy (12)	≤1.5 kWh per sample	≤0.1 kWh per sample	≤1.5 kWh per sample	≤1.5 kWh per sample
Occupational hazard (13)	Hermetic sealing of analytical process	Hermetic sealing of analytical process	Hermetic sealing of analytical process	Hermetic sealing of analytical process
Waste (14)	No waste	No waste	>10 mL	>10 mL
Waste treatment (15)	-	-	No treatment	No treatment
Quantification	Yes	Yes	Yes	Yes

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Table 6: The penalty points to calculate analytical Eco-scale

Hazards / Methods	Penalty Points			
	Proposed Electrochemical Method	Reported Spectrophotometric Method(10)	Reported HPTLC Method(19)	Reported HPLC Method(11)
Solvents/Reagents				
Disodium hydrogen phosphate	4	-	-	-
***O*-Phosphoric Acid**	2	-	-	2
Methanol	-	12	12	-
Water	-	-	0	0
Sodium lauryl sulphate	-	-	-	6
Sodium dihydrogen phosphate monohydrate	-	-	-	1
Acetonitrile	-	-	-	12
Instruments				
Instruments Energy	0 (<0.1 kWh per sample)	0 (<0.1 kWh per sample)	1 (<1.5 kWh per sample)	1 (<1.5 kWh per sample)
Occupational Hazards (Emission of vapors)	0	0	0	0
Waste	0 (None)	0 (None)	5 (>10 mL)	5 (>10 mL)
Total Penalty Points	Σ 6	Σ 12	Σ 18	Σ 27
Analytical Eco-Scale Total score	94	88	82	73

Table 7: Comparison of the proposed method for EZ determination with other reported methods

Point of comparison	Proposed CE method	Reported Spectrophotometric Method(10)	Reported HPTLC Method(19)	Reported HPLC Method(11)
Linearity range (µg/mL)	10.0 - 300	4-24	2–12	49.85 -179.46
LOD (µg/mL)	1.20	0.43	0.60	0.05
Correlation Coefficient (r)	0.9998	0.999	0.996	0.999
Applications	Determination of drug in pharmaceutical dosage form	Determination of drug in pharmaceutical dosage form	Determination of drug in pharmaceutical dosage form and investigation of its water-induced degradation kinetics	Determination of drug in pharmaceutical dosage form

Declaration of competing interest

The authors declare that they have no conflict of interest.

Availability of data and materials

Data and materials are presented in detail in the manuscript. For further information, please contact the authors.

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Authors' contributions

Authors have equally shared the method development, data analysis, manuscript writing, and revisions.

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Highlights

- A capillary electrophoretic method is developed for eszopiclone analysis.
- The developed method is highly sensitive, rapid, simple, economical, and eco-friendly.
- It is applied for the determination of eszopiclone in commercial formulations.

Supplementary Information

Supplementary Information (1).docx

5. References

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