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Enhanced Sample Throughput Capillary Zone Electrophoresis with UV Detection in Hydrodynamically Closed System for Determination of Ibuprofen

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Abstract: A simple analytical approach based on capillary zone electrophoresis with ultraviolet detection and repeated sample injection strategy (applied in a hydrodynamically closed separation system for the first time) was developed for the determination of ibuprofen (IBU) in commercially available pharmaceutical preparations. The proposed method was characterized by significantly increased sample throughput and favorable validation parameters, highly demanded in routine quality control laboratories. The limit of detection was predicted at the concentration level of 0.31 µg/mL. Intra-day precision expressed as the relative standard deviation of IBU concentration ranged from 1.9 to 5.6%, and corresponding intra-day accuracy expressed as the relative error was in the interval of 87.1–106.5%. Inter-day precision was in the range of 2.6–15.0%, and inter-day accuracy was 94.9–102.7%. The developed method was able to quantify IBU in complex pharmaceutical matrices represented by commercially available tablets and oral suspension. The determined contents of IBU in the tested dosage forms were in good agreement with the manufacturer's declaration. The analytical performance of the developed method was evaluated according to the innovative RGB Additive Color Model strategy. It was demonstrated that the proposed method is characterized by very good analytical performance parameters, safety and eco-friendliness, and practical effectiveness.

Keywords: capillary zone electrophoresis; repeated sample injection; hydrodynamically closed separation system; ultraviolet detection; ibuprofen; analytical method evaluation; drug quality control

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1. Introduction

Recent strategies in analytical methods development are typically focused on the greenness aspect that is represented by the use of energy-efficient equipment, minimal waste generation, and reduced (or fully avoided) use of toxic chemicals and reagents [1]. Therefore, new tools for greenness evaluation of the analytical methods are introduced more often [2–4]. However, such evaluation strategies bring no information regarding the analytical performance and effectiveness of the investigated methods.

Another very important requirement for new approaches is the speed of the analysis. In recent years, the demands on analytical methods capable of being a part of high throughput experimentation in specific areas of bioanalysis and quality control (e.g., pharmaceutical industry) are constantly increasing [5]. In such areas, the analytical approaches should also be characterized by the appropriate capability to identify and quantify demanded analytes at relatively low concentration levels (especially in the case of bioanalysis). Therefore, the development of new analytical methods that fulfill all the mentioned criteria is very challenging. As a good tool for critical evaluation of newly developed methods can serve the RGB Additive Color Model introduced by Nowak and Kościelniak [6]. The model deals with three main attributes, i.e., analytical performance, green chemistry principles, and productivity. Such an approach is very beneficial because it brings complex information about the analytical methods, and so the choice of the best method in a particular situation can be realized [7].

Approaches based on capillary electrophoresis (CE) typically meet the requirements of fast, sensitive, and green methods. CE is characterized by high separation efficiency, low consumption of samples and chemicals, use of an aqueous separation environment, versatility, simplicity in handling, and broad application potential. All these attributes make it particularly attractive and comparable to preferred liquid chromatography [8]. CE experiments can be performed in hydrodynamically open systems using capillaries with small internal diameters (i.d.)—20–75 μm or in hydrodynamically closed systems using wide bore capillaries with i.d. of 300–800 μm. In the case of the hydrodynamically closed separation systems, the separation capillaries with higher i.d. are separated from the reservoir containing the separation buffer by a mechanical barrier (i.e., semipermeable membrane) that is necessary to prevent a hydrodynamic flow of the solution from the wider capillary. In such a separation system, only ions can be electrophoretically transported across the membrane. Moreover, in a hydrodynamically closed system, the electroosmotic flow (EOF) is an unwanted effect causing electroosmotic dispersion effects and must be suppressed or eliminated. The moderate concentration sensitivity of current CE detection systems constitutes a significant challenge in achieving the required limits of detection (LOD) or quantitation (LOQ) for contemporary industrial applications [9]. From this point of view, CE performed in a hydrodynamically closed system represents a favorable approach because of the possibility of injecting large amounts of the sample into the system.

The quantitative analysis in both systems is usually performed in a single-injection mode, which is time consuming since each analyte or standard sample has to be investigated separately and at least in triplicate [10]. This is contrary to the requirements for high throughput analysis. Therefore, multiple injection [10–21] and multisegment injection [22–28] strategies were implemented in the CE environment. According to our best knowledge, these strategies were used only in the case of a hydrodynamically open separation system. There is no publication that implements this injection approach in a hydrodynamically closed separation system.

Therefore, the aim of the present work was to develop a simple CZE method carried out in a hydrodynamically closed system based on a multiple injection strategy (here called "repeated injection") that could be implemented in the quality control of pharmaceutical products. The non-steroidal anti-inflammatory drug (NSAID) ibuprofen was selected as a model analyte, which is one of the most commonly used over-the-counter (OTC) medications against pain, fever, and inflammation [29]. The essential status of ibuprofen was confirmed by its listing in the 2010 Essential Drugs list by the World Health Organization (WHO) [30].

The pharmacological effect of ibuprofen is dependent on its ability to inhibit the fatty acid cyclooxygenase (COX) enzyme, thereby inhibiting the biosynthesis of prostaglandins and thromboxanes. Ibuprofen inhibits COX isoforms but is weakly COX-1 selective. It has immediate analgesic, antithrombotic, and antipyretic effects at lower doses and anti-inflammatory effects at higher doses after at least two weeks of therapy. In general, the risk of unwanted side effects is relatively high, especially if ibuprofen is taken for extended periods of time or by the elderly. The highest incidence of side effects is in the gastrointestinal tract (GIT). Other side effects include, e.g., hypertension, erythema, Stevens–Johnson syndrome, reversible renal insufficiency, analgesic-associated nephropathy, or bronchospasm in aspirin-sensitive asthmatics [31]. Moreover, high consumption of the drug poses an ever-increasing threat to the environment due to its bioactive nature [32].

The pK_a value of ibuprofen is 5.2, which means that it exists as neutral species at $pH < pK_a$, coexists as neutral and anionic species at pH~pK_a, and exists as anionic species at $pH > pK_a$. According to this information, it is possible to affect the electromigration behavior of ibuprofen by optimization of the CE separation environment. Various CZE methods were recently used in combination with UV [33–35] or contactless conductivity—(CD) [36,37] detection to investigate ibuprofen in pharmaceutical preparations. All of them were performed in a hydrodynamically open CE system. CE-CD methods were presented as ultra-rapid because the time of one analysis was lower than 2.5 min. However, the LOD values ranged from 2 to 48 μg/mL [36,37]. The use of CE-UV detection was accompanied by higher times of analysis and LOD values ranging from 0.5 to 1.5 μg/mL [33,35]. Moreover, CE approaches performed in hydrodynamically open separation systems demand preconditioning of the separation capillary before the first analysis and also before each run. This procedure integrates capillary flush with sodium hydroxide, water, and the background electrolyte and application of opposite voltage. It is a time-consuming procedure that takes obviously more than 10 min.

In our work, we developed a new sensitive CE approach with improved sample throughput and no sample preparation (except for simple dilution) suitable for the determination of ibuprofen in pharmaceuticals. A comprehensive view of the usefulness of the method offers its performance, greenness, and productivity evaluation according to the RGB Additive Color Model strategy. Such evaluation of a CE method carried out in a hydrodynamically closed system was considered for the first time.

2. Materials and Methods

2.1. Chemicals and Samples

Ibuprofen analytical standard was obtained from Zentiva (Prague, Czech Republic). Chemicals (analytical grade) used for the preparation of electrolyte systems, i.e., ammonium carbonate—$(NH_4)_2CO_3$, ammonium bicarbonate—NH_4HCO_3, ammonium hydroxide—NH_4OH, 2-(n-morpholino)ethanesulfonic acid—MES, ε-aminocaproic acid—EACA, 2-Amino-2-(hydroxymethyl)propane-1,3-diol—TRIS, and 3-(N-morpholino)propanesulfonic acid—MOPS, methyl hydroxyethylcellulose—m-HEC, were purchased from Merck (Darmstadt, Germany), Sigma Aldrich (Steinheim, Germany) and Serva (Heidelberg, Germany). Ultrapure CE water (Agilent Technologies, Santa Clara, CA, USA) was used for preparation of all solutions. The separation electrolytes were filtered before use through disposable 0.22 μm pore size membrane filters (Millipore, Molsheim, France) and were stored in the fridge before use. The commercial drugs (tablets, oral suspension) containing ibuprofen—Ibalgin® 400 (Zentiva, Prague, Czech Republic), Nurofen 200 mg (Reckitt Benckiser, Prague, Czech Republic), and Nurofen 100 mg/5 mL (Reckitt Benckiser, Prague, Czech Republic), were obtained from a local drug store.

2.2. Instrumentation

The CZE experiments were performed on the EA 102 apparatus (Villa Labeco, Spisska Nova Ves, Slovakia) in a single column arrangement. A separation column was provided with a 300 μm internal diameter (I.D.) polytetrafluorethylene (PTFE) capillary tube of a 160 mm total length and a contactless detector. Prior to the use, the capillary was not treated by any rinsing procedure to suppress an EOF. A dynamic coating of the capillary wall by means of m-HEC present in the background electrolyte served this purpose. An ultraviolet (UV) spectrophotometric absorbance detector ECD 2600 UV-VIS (ECOM, Prague, Czech Republic) was connected to an on-column photometric detection cell via optical fibers. The detector was set at the wavelength of 220 nm. The background electrolyte (BGE) in the capillary was replaced by the fresh one between each run. The experiments were performed under anodic movement of the analytes. The sample was injected by a 200 nL internal sample loop of the injection valve of the EA 102 apparatus. All experiments were performed in a constant current mode. The driving current was 50 μA.

2.3. Procedures for Standard Solution and Sample Preparation

The ibuprofen stock solution was prepared by dissolving 10 mg of the standard in 10 mL of ultra-pure CE water. The working solutions were prepared by appropriate dilution of the stock solution with ultra-pure CE water to provide the desired analyte concentration. Calibration standards in the concentration range of 1.25–50 μg/mL (1.25, 2.5, 5, 10, 20, 50 μg/mL) were prepared by dilution of the stock solution with ultra-pure CE water. Each sample was measured three times.

Two different sample preparation procedures were performed depending on the investigated pharmaceutical formulation. Tablet dosage forms containing ibuprofen were prepared as follows: five tablets of each investigated preparation (Ibalgin® 400 and Nurofen) were weighted and grounded in a mortar. The quantity of powder equivalent to one tablet was weighted and dissolved in a 100 mL volumetric flask with ultra-pure CE water. Then, the solution was sonicated in an ultrasonic bath for 30 min and filtered using Whatman filter paper No. 1. Finally, the solution was diluted with ultra-pure CE water to reach a concentration order inside the calibration range. The samples were directly injected into the CE analyzer and measured in three replicates.

Oral suspension (Nurofen 100 mg/5 mL) was prepared as follows: 1 mL of the suspension was transferred into 20 mL volumetric flask and dissolved with the water/methanol (50/50, *v*/*v*) mixture. The solution was sonicated in an ultrasonic bath for 30 min. The obtained solution was diluted with ultra-pure CE water to reach a concentration inside the calibration range and directly injected into the CE analyzer. The sample was measured three times.

3. Results and Discussion

3.1. Optimization of the CZE-UV Method

The working conditions such as buffer constituents, the concentration of carrier cations, pH, and driving current were optimized for the CZE separations performed in a hydrodynamically closed system. Three types of buffer solution were tested: BGE—$(NH_4)_2CO_3$, NH_4HCO_3, and a mixture of MOPS and TRIS, EACA and NH_4OH, or MES and NH_4OH (see Table 1). The use of NH_4HCO_3 electrolyte and more basic electrolytes composed of 10–50 mM $(NH_4)_2CO_3$, or a mixture of EACA with NH_4OH, was accompanied by an unstable baseline, overheating of the buffer in the separation capillary, and excessive generation of the system peaks, which often disables quantitative analysis of the analyte. Moreover, the relative standard deviation (RSD%) of the ibuprofen peak area was relatively high (>17.5%) in the case of the most basic BGE tested (i.e., 5 mM EACA + 10 mM NH_4OH). The use of such BGE was also accompanied by the lowest IBU peak height. On the contrary, the BGE composed of MOPS and TRIS was able to perform stable analysis without the negative effect observed with previously tested buffers. Therefore, a more detailed optimization procedure focused on such BGE composition. Various concentrations of MOPS (10–25 mM) and TRIS (10–50 mM) were investigated. It was demonstrated that higher concentrations of both components in the mixture were accompanied by higher migration times and significantly lower separation efficiency expressed as the number of theoretical plates (N). The intensity (peak height) of the analyte was also significantly decreased. Best results were obtained with the BGE composed of 10 mM MOPS and 20 mM TRIS (pH 8.29) with 0.05% m-HEC, which was selected as the optimal one and used in further experiments. The increase in the peak height obtained with the use of 10 mM MOPS + 20 mM TRIS buffer can be explained by appropriate ionization of the analyte under these separation conditions and also as an effect of sufficient ion strength of the electrolyte.

Table 1. Optimization of the BGE composition.

Electrolyte	pH	t_m (min)	Peak Height	$RSD_{peak\ height}$ (%)	N
10 mM $(NH_4)_2CO_3$	9.21	18.16	70.6	3.5	10,152
10 mM NH_4HCO_3	7.80	19.53	80.8	1.2	11,949
10 mM NH_4OH + 20 mM MES	5.76	12.26	41.9	2.5	4894
5 mM EACA + 10 mM NH_4OH	9.75	6.56	25.2	7.9	1707
10 mM MOPS + 20 mM TRIS	8.29	11.24	186.1	1.6	20,775
25 mM MOPS + 50 mM TRIS	8.44	15.04	80.1	3.4	9070

Concentration of the injected IBU sample was 10 µg/mL. N—separation efficiency expressed as number of theoretical plates.

3.2. Repeated Injection Procedure

The next step in the development of a new CZE-UV method for the determination of ibuprofen was focused on the possibility of improving the sample throughput. In the hydrodynamically open CZE systems, it is common to perform repeated injections of the sample in one run, which has resulted in multiple injection and multisegment injection strategies. However, such an approach has never been investigated in the case of hydrodynamically closed CE separation systems. The same principle of multiple-injection and/or multisegment injection strategy is not applicable because of differences in experimental instrumentation. Therefore, we decided to explore the strategy based on repeated injection of the sample after pausing the separation procedure at a defined time point and its subsequent turn on. We have focused on the optimization of the time period between repeated sample injections. Time intervals of 40, 60, 80, and 100 s were investigated. Logically, higher time intervals were associated with improved resolution between the peaks. This can be beneficial when some other sample constituents (e.g., impurities, excipients, etc.) migrate close to the peak of ibuprofen under the same separation conditions. It is necessary to ensure that no overlapping peaks of the substances will be present in the electropherogram after repeated injection of the sample. By keeping this in mind, the time of 100 s was selected as the optimal one. Selected experimental conditions enabled the analysis of three samples within one electrophoretic run (approx. 16 min). This is illustrated by the record in Figure 1 obtained from the analysis of ibuprofen standard at the concentration level of 1.25 µg/mL (representing the limit of quantification—LOQ) that compares results obtained from the single sample and repeated sample injection techniques. The efficiency of the separation was not affected by the voltage breaks, which were necessary for the repeated injection of the sample. Moreover, the results (peak area) obtained from the analysis of standard samples at the LOQ level performed by the classical and repeated sample injection provided a high level of similarity (differences were lower than 1%).

A complete overview of optimized separation and detection conditions of the developed CZE-UV method for the determination of ibuprofen are summarized in Supplementary Material, Table S1.

3.3. Validation

Validation of the developed CZE-UV method for the determination of ibuprofen was performed according to the ICH Q2(R1) guideline recommendations [38]. Parameters such as linearity, range, selectivity, the limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness were investigated. The results obtained from the validation procedure are summarized in Tables 2 and 3.

Linearity as an ability to obtain test results directly proportional to the concentration of analyte in the sample was evaluated using calibration standards in the concentration range of 1.25–50 µg/mL. A linear calibration curve was obtained for ibuprofen with a correlation coefficient (r^2) of 0.9984 (Table 2).

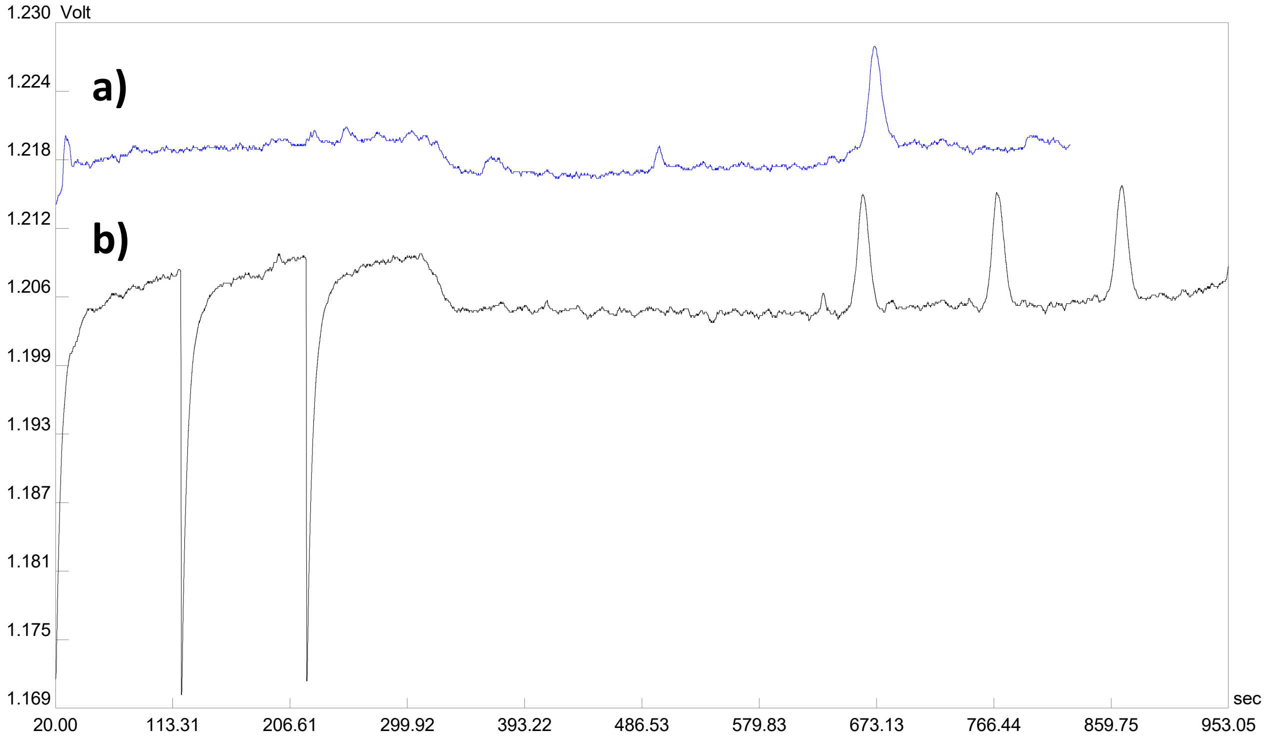

Figure 1. Illustrative electropherograms obtained from the analysis of ibuprofen standard at the LOQ concentration level 1.25 µg/mL under conventional single sample injection (**a**) and repeated sample injection (**b**).

Table 2. Selected operation and calibration parameters of the CZE-UV method.

Parameter	Ibuprofen
t_m [min]	11.61
RSD_{tm} [%], $n = 6$	0.54
RSD_{area} [%], $n = 6$	5.51
Regression equation	y = 139.23x − 22.09
r^2	0.9984
Linear range [µg/mL]	1.25–50
LOD [µg/mL]	0.31
LOQ [µg/mL]	1.25
N	20,944

Table 3. Accuracy and precision of the developed CZE-UV method from analysis of QC samples.

QC Sample	Intra-Day, *n* = 3			Inter-Day, *n* = 9		
Nominal (µg/mL)	Found (µg/mL)	RSD (%)	RE (%)	Found (µg/mL)	RSD (%)	RE (%)
1.25	1.09	3.4	−12.9	1.25	15.0	0.3
2.5	2.35	2.1	−6.0	2.46	9.9	−1.4
5	4.60	1.9	−7.9	4.84	5.6	−3.2
10	9.15	5.6	−8.5	9.49	6.6	−5.1
20	21.30	3.4	6.5	20.53	4.9	2.7
50	49.68	1.9	−0.7	49.89	2.6	−0.3

The limit of detection (LOD) was calculated according to the signal-to-noise (S/N) ratio, which should be 3:1. Here, the samples at known low concentration levels of the analyte were compared with those of blank samples. According to this procedure, the concentration of 0.31 µg/mL was evaluated as the LOD. A similar procedure was applied for the investigation of the limit of quantification (LOQ), where the S/N ratio should be 10:1. Finally, the concentration level of 1.25 μg/mL that represents the first point of the calibration line was predicted as LOQ (see Figure 1). The obtained results are the best of the previously published ones for the determination of ibuprofen in pharmaceutical matrices.

Precision and accuracy of the CZE-UV method were tested by the analysis of six quality control (QC) samples in the concentration range of 1.25–50 μg/mL (Table 3). Repeatability represents intra-day precision and is characterized as the precision under the same operating conditions over a short interval of time—typically within one day (run by run). The intermediate precision (inter-day precision) was evaluated by repeated analysis of the samples in three replicates per day for three consecutive days. The intra-day precision (%RSD) ranged from 1.9 to 5.6%, and the corresponding accuracy (expressed as relative error, %RE) was in the interval of 87.1–106.5%. The inter-day precision varied from 2.6 to 15%, and the accuracy from 94.9 to 102.7%. The obtained results were satisfactory; however, better results were obtained for inter-day accuracy. This can be explained by the demands on skilled personnel operating the device.

Selectivity is the ability of the method to determine the investigated analyte in the presence of interferences. The comparison of electropherograms of ibuprofen standard and real drug dosage form (Figures 1 and 2) indicates that no interferences from the excipients in the sample solution were found in the migration position of ibuprofen. All substances present in the pharmaceutical matrices were separated from the ibuprofen peak. This is clearly illustrated in the analysis of ibuprofen present in the oral suspension drug Nurofen (Figure 2c). Therefore, the proposed method is suitable for its application in the routine analysis of ibuprofen in its dosage forms.

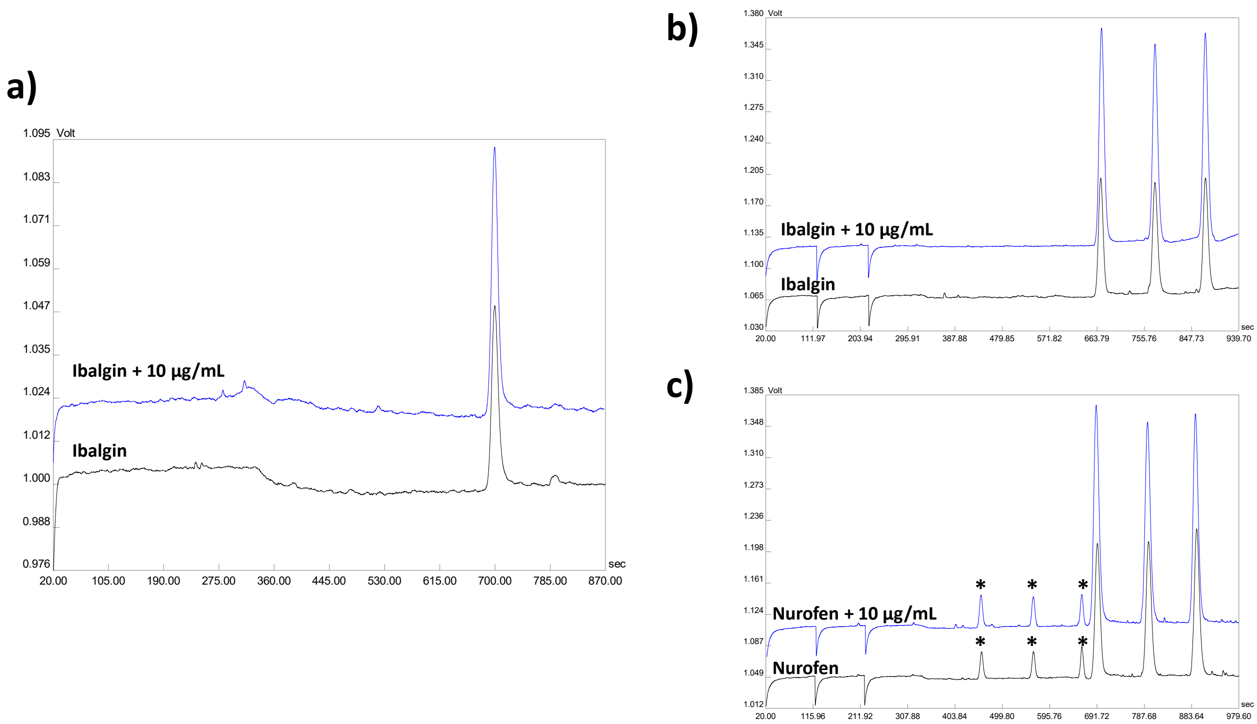

Figure 2. Illustrative electropherograms obtained from the selectivity and recovery tests in pharmaceutical matrices. (**a**) Analysis of drug sample (Ibalgin tablets) and fortified drug sample with the ibuprofen standard at a 10 μg/mL concentration level performed with the conventional single sample injection. (**b**) Analysis of drug sample (Ibalgin tablets) and fortified drug sample with the ibuprofen standard at a 10 μg/mL concentration level performed with the repeated sample injection. (**c**) Analysis of drug sample (Nurofen oral suspension) and fortified drug sample with the ibuprofen standard at a 10 μg/mL concentration level performed with the repeated sample injection. *—an unidentified component present in the oral suspension sample matrix.

Recovery of the method was investigated by spiking real pharmaceutical samples (tablets, oral suspension) with ibuprofen standard at three concentration levels—5, 10, and 25 µg/mL, namely. For illustrative electropherograms obtained from the analysis of tested sample matrices and fortified sample matrices with the ibuprofen standard at the concentration level of 10 µg/mL, see Figure 2. The calculated recovery of ibuprofen was in the range of 92.7–100.9%. This indicated just a slight effect of the pharmaceutical dosage form matrix on the analyte signal.

Robustness was evaluated from the small and deliberate variations to the selected optimum separation conditions—i.e., the concentration of the BGE (±1 mM MOPS) and pH of the buffer (±0.1 unit). The effect of these changes on the migration time and peak area was negligible (<2.5%), and it can be stated that the method is robust enough.

3.4. Application and Evaluation of the Analytical Method

The proposed CZE-UV method was finally applied for the determination of ibuprofen in three various pharmaceuticals containing ibuprofen. Two of them were formulated as tablet dosage forms and one as an oral suspension. The obtained results summarized in Table 4 were in good accordance with the declared content by the manufacturer. The declared content for Ibalgin tbl. was 400 mg per tablet (determined content 372.8 ± 10.8 mg/tablet), for Nurofen tbl. 200 mg per one tablet (determined content 181.9 ± 2.7 mg/tablet), and for Nurofen oral susp. 100 mg/5 mL (determined content 93.1 ± 3.1 mg/5 mL). Moreover, the electropherogram obtained from the analysis of a real pharmaceutical sample Nurofen oral suspension in Figure 3 clearly demonstrates the effectivity of the developed method via resolving the peak of ibuprofen from the peak of an unknown UV absorbing pharmaceutical excipient present in the sample even when using repeated sample injection. As it can be seen, there was no overlapping peak observed in the electropherogram.

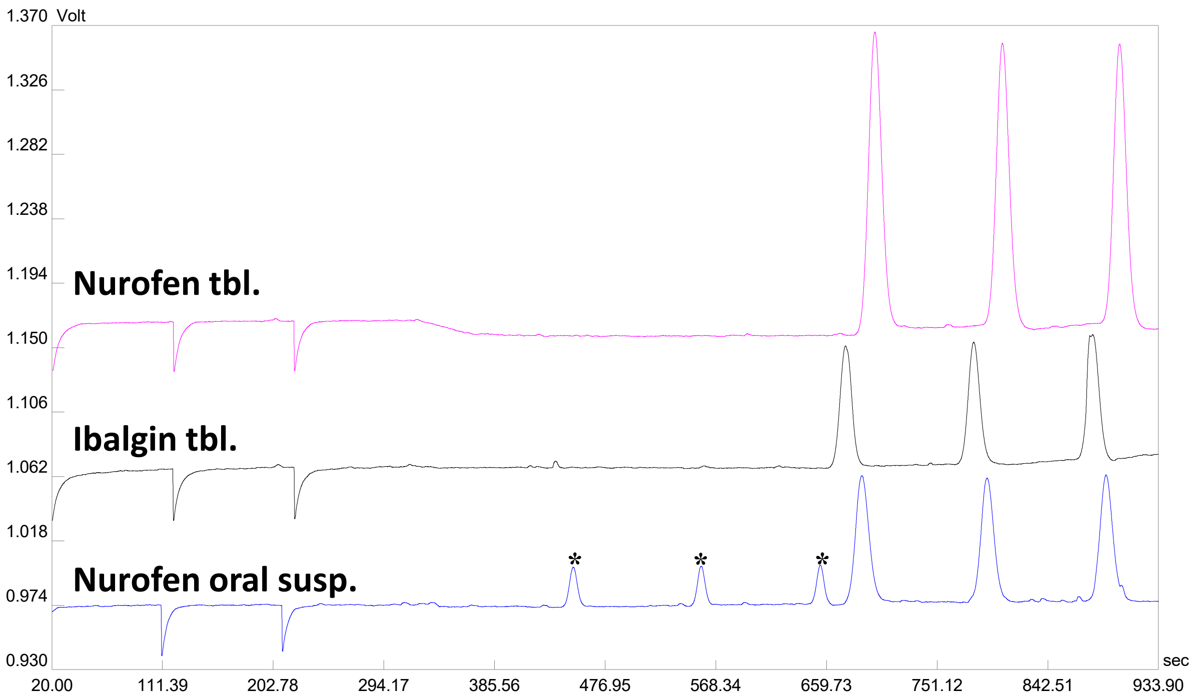

Figure 3. Illustrative electropherograms obtained from the CZE-UV analysis with repeated sample injection of ibuprofen in real pharmaceutical samples—drug Ibalgin (tablets), drug Nurofen (tablets) and drug Nurofen (oral suspension). *—an unidentified component present in the oral suspension sample matrix.

Table 4. Assay results for the determination of ibuprofen in its commercial drugs.

Preparation	Parameter			
	Found ± SD (μg/mL)	**RSD (%), *n* = 3**	**Declared (μg/mL)**	**RE (%)**
Ibalgin tbl.	9.32 ± 0.27	2.9	10	−6.8
Nurofen tbl.	18.19 ± 0.27	3.6	20	−9.1
Nurofen susp.	9.31 ± 0.31	3.3	10	−6.9

Finally, the developed CZE-UV method with repeated sample injection was evaluated in terms of analytical performance, green chemistry principles, and productivity with the use of the RGB Additive Color Model strategy (Figure 4). The levels of satisfaction for the RGB Model evaluation were selected according to the recommendations presented in the pioneering paper dealing with the RGB strategy [6].

			w=3	w=3	w=2	w=2
REDNESS (analytical performance)	W=4		Accuracy	Precision (RSD%)	Linearity range	LOD
CS: 70.8%		LAV=33.3	80 - 120%	15	1 order (1 - 10)	50 μg/mL
		LSV=66.6	90 - 110%	5	2 orders (1 - 100)	5 μg/mL
		Result	87 - 107%	3.4	1.5 order	0.31 μg/mL
		Score (0-100)	66.6 66.6 66.6	78 78 78	50 50	95 95
			w=3	w=3	w=2	w=2
GREENNESS (safety and eco-friendliness)	W=2		Chemical consumtion	Chemical safety/hazards	Additional risk factors	Energy
CS: 88.7%		LAV=33.3	200 mL/100 runs	10 hazard pictograms in total	10 independent hazards	>1.5 kWh
		LSV=66.6	100 mL/100 runs	3 hazard pictograms in total	3 independent hazards	0.5 kWh
		Result	30 mL/100 runs	0 hazard pictograms in total	1 independent hazard	0.5 kWh
		Score (0-100)	95 95 95	100 100 100	89 89	66.6 66.6
			w=3	w=3	w=2	w=2
BLUENESS (productivity / practical effectiveness)	W=3		Cost-effectiveness	Time-effectiveness	Sample destruction	Service
CS: 85.3%		LAV=33.3	10 €/sample	1 sample/16 min	5 μL	after 20 samples
		LSV=66.6	5 €/sample	2 samples/16 min	1 μL	after 50 samples
		Result	1.5 €/sample	3 samples/16 min	0.2 μL	after 100 samples
		Score (0-100)	90 90 90	85 85 85	75 75	90 90

FINAL COLOR:	REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):	81.2%
WHITE	≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%		
	yes	yes	yes	yes	yes	yes		
Short annotation: 81.2white	Long annotation: 81.2white(70.8/8red-88.7/8green-85.3/8blue)							

Figure 4. Evaluation of the developed CZE-UV method with repeated injection for determination of ibuprofen according to the RGB Additive Color Model.

The analytical performance dealt with convenient validation processes and was evaluated according to four parameters—i.e., accuracy, precision, linearity range, and limit of detection (LOD). The color score (CS) of this part was 70.8%, which means that the method gained its primary color (here red). In the second step, the safety and eco-friendliness of the CZE-UV method were investigated. Four main criteria were evaluated—chemical consumption, chemical hazard, additional risk factors, and energy consumption. The resulting CS was 88.7%, which represents an excellent result from the greenness aspects of the method. The last investigated area includes cost- and time-effectiveness, the extent of sample destruction, and some operation-related aspects accompanied by the service frequency of the instrument. The CS achieved a value of 85.3%, and the productivity parameter retained its primary color (here blue). Finally, the method brilliance was evaluated. This parameter integrates all the three previously described scores and offers a quantitative parameter that can be used as a between method comparison tool. The brilliance value of our CZE-UV method was 81.2%, and the final method color was white. According to these results, the method represents an effective, eco-friendly, and low-cost tool for the determination of ibuprofen in pharmaceutical samples.

4. Conclusions

In conclusion, we developed and validated an innovative approach for capillary zone electrophoresis performed in hydrodynamically closed separation systems based on a repeated sample injection strategy. The introduced approach was advantageously used for quantification of ibuprofen in two commercially available drug dosage forms—i.e., tablets and oral suspension. Favorable performance parameters of the CZE-UV method with the repeated sample injection strategy enabled the determination of ibuprofen at low concentration levels over a short period of time. These parameters are beneficial from the practical point of view, as sensitive determination and enhanced sample throughput are highly demanded in current drug quality control. Moreover, it was demonstrated that the developed method is able to analyze highly diluted samples without the loss of separation efficiency and sensitivity. It is the result of enhanced sample loading capacity in comparison to the convenient hydrodynamically open CE systems.

The complex analytical potential of the developed CZE-UV method with the repeated sample injection strategy was evaluated according to the RGB Additive Color Model. The model confirmed the analytical excellence, greenness, and practical effectiveness of the method.

Further improvement of the method applicability can be seen in the transfer of the method from the manual electrophoretic apparatus EA 102 (used in this work) to fully automated instruments (working in hydrodynamically closed systems), such as EA 202 M. The use of such automated instrument can solve the problems with the variability of the results obtained by manual injection of the sample which demands experienced laboratory personal.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations9050118/s1, Table S1: Optimized conditions of the developed CZE-UV method for the determination of ibuprofen.

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