

Capillary Electrophoresis with Diode Array Detection for the Quantification of Triptorelin and Lanreotide in Pharmaceutical Quality Control: Development, Validation, Greenness and Practicality Evaluation

Research paper

O. Stefanik^{1,2}, P. Mikus^{1,2}, J. Piestansky^{2,3}✉

¹Department of Pharmaceutical Analysis
and Nuclear Pharmacy, Faculty of Pharmacy,
Comenius University in Bratislava, Odbojarov 10,
SK-832 32 Bratislava, Slovak Republic

²Toxicological and Antidoping Center, Faculty of
Pharmacy, Comenius University in Bratislava, Odbojarov
10, SK-832 32 Bratislava, Slovak Republic

³Department of Galenic Pharmacy, Faculty of Pharmacy,
Comenius University in Bratislava, Odbojarov 10,
SK-832 32 Bratislava, Slovak Republic

Received 22 August, 2024, accepted 8 October, 2024

Abstract **Aim:** Capillary electrophoresis (CE)-based methods hold significant potential for routine use in pharmaceutical quality control (QC) laboratories. Therefore, this study aimed to develop a novel, green, and simple hydrodynamically open-system capillary zone electrophoresis method with diode-array detection (CZE-DAD) for the simultaneous analysis of lanreotide and triptorelin in a single electrophoretic run and to objectively evaluate the analytical technique's greenness and practicality for application in the pharmaceutical QC settings.

Materials and Methods: The two therapeutic peptides were analysed using a commercially available CZE-DAD analytical system. The separation process was optimised by changing the composition and concentration of the background electrolyte (BGE). The developed method was validated in accordance with the International Conference on Harmonisation (ICH) Q2(R1) guidelines, and Diphereline® (powdered form for injection, 0.1 mg of triptorelin acetate) was used as a real dosage form of triptorelin. Greenness and practicality were evaluated using Green Analytical Procedure Index (GAPI), Analytical GREEnness (AGREE), and Blue Applicability Grade Index (BAGI) metrics.

Results: The optimised method utilised 250 mmol/L formic acid as the BGE, achieving high separation efficiency and short migration times, where both the peptides were analysed in <5 min. The method showed excellent linearity ($r^2 > 0.99$), precision (relative standard deviation [RSD] <7.1%), and accuracy (92.7%–113.6%). The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.5 µg/mL and 2 µg/mL, respectively. The method was also found to be environmentally friendly, with high scores achieved in both the GAPI and AGREE assessments, while also being practical, with a BAGI score of >60.

Conclusions: The newly developed CZE-DAD method proved to be a reliable, efficient, and environmentally sustainable alternative to liquid chromatography (LC)-based methods for the analysis of lanreotide and triptorelin. The method's acceptable validation parameters and favourable greenness and practicality scores support its high application potential in pharmaceutical QC laboratories.

Keywords capillary zone electrophoresis – green analytical chemistry – lanreotide – pharmaceutical quality control – triptorelin

INTRODUCTION

As of 2024, the pharmaceutical market can be divided into two main categories represented by small molecules and biologics. Small molecules, with a molecular weight of <500 Da, offer excellent oral bioavailability but often suffer from reduced target selectivity, which can lead to significant side effects. By contrast, biologics, with molecular weights >5000 Da, generally lack oral bioavailability and

are metabolically unstable, yet they exhibit much greater specificity and efficacy for their targets (Stefanik et al., 2024a; Wang et al., 2022). In the intermediate molecular weight window of 500–5000 Da, therapeutic peptides emerge as a significant category. These molecules effectively bridge the advantages of small molecules and high-molecular-weight protein therapeutics, combining enhanced target specificity with oral bioavailability (Craik et al., 2013).

* E-mail: piestansky@fpharm.uniba.sk

Open Access. © 2024 Authors.  This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License.

Triptorelin and lanreotide represent small synthetic peptides with important clinical applications, and they currently belong to the group of best-selling peptide therapeutics (Ipsen, 2023). Triptorelin is a synthetic decapeptide, representing a very potent agonist for the human gonadotropin-releasing hormone (GNRH). Since endogenous GNRH is rapidly degraded, analogues like triptorelin with prolonged half-lives were synthesised. This synthetic peptide plays an important role in oncology, and in the treatment of endometriosis, uterine fibroids, premature puberty, and hypersexuality by means of chemical castration (Kumar and Sharma, 2014; Stefanik et al., 2024b). Lanreotide is a synthetic cyclic octapeptide, a long-acting analogue of somatostatin. It has a greater affinity for binding to somatostatin receptors and a substantially longer half-life than somatostatin. It suppresses various hormones and neurotransmitters. Therefore, lanreotide is effective in controlling symptoms and growth of neuroendocrine tumours and treating conditions like acromegaly (Mazziotti and Giustina, 2010).

Ensuring the highest efficacy and safety of drugs depends on the quality of pharmaceutical products. The safety, efficacy, stability, patient acceptability, and regulatory compliance are the key factors determining the quality of any drug formulated in a dosage form (Uddin et al., 2016). The World Health Organization (WHO) states that quality control (QC) in the pharmaceutical industry is an essential part of Good Manufacturing Practices (WHO, 2010). In this regard, QC integrates all those activities involving sampling, developing specifications, conducting analytical testing, and monitoring the various materials used and the environmental conditions prevailing within the facility. These activities are conducted through validated methods by trained and experienced personnel (Eldin et al., 2016). Apart from the pre-marketing phase, it is the post-marketing QC by regulatory agencies at both the national and international levels that predominantly contributes towards ensuring drug quality (Dispas et al., 2022).

Among the primary objectives of QC is the identification and quantitation of active substances while monitoring impurities using analytical methods. QC laboratories favour highly robust, high-throughput analytical methods that are also cost-effective and eco-friendly. Liquid chromatography (LC) represents the optimal balance among the mentioned parameters. Therefore, it accounts, by both European and US Pharmacopeias, as the dominant technique used for QC analysis. Alternative techniques are capillary electrophoresis (CE), gas chromatography, supercritical fluid chromatography, and multidimensional chromatography (Dispas et al., 2022). CE attracts increasing attention within the scientific community due to its alignment with several key performance parameters and its adherence to the principles of green analytical chemistry (GAC). It offers advantages such as high separation efficiency, relatively short analysis time, minimal or no consumption of organic solvents, and setup simplicity. CE can also provide complementary information to LC,

utilizing a different separation principle. However, the lower robustness of CE methods limits their ability to potentially replace LC approaches in the QC setting. Lower sensitivity of CE methods is not a concern in pharmaceutical QC, as extremely high sensitivity is not required for the analysis of commercial pharmaceuticals (Jankech et al., 2024; Kašička, 2024; Stefanik et al., 2024a; Van Schepdael, 2023).

It is important to note that while CE methods are recognised for their lower environmental impact compared with traditional LC methods, recent advancements in LC technology pose a challenge to this advantage. Ultra-high-performance liquid chromatography (UHPLC) and nano-LC have significantly minimised waste generation and enhanced sample throughput, narrowing the environmental gap (Jankech et al., 2024).

In an era where environmental concerns dominate global discourse, the field of analytical chemistry increasingly focuses on sustainability. GAC, a relatively recent subfield, aims to integrate sustainable practices into the analytical process. The 12 principles of GAC provide fundamental guidelines for enhancing the environmental sustainability of analytical procedures. Despite these guidelines, some undesirable practices may still be unavoidable under certain conditions. Therefore, it is essential to evaluate the environmental impact of analytical procedures to minimise their effects on both the environment and human health (Robert, 2023).

Between 2019 and 2023, the most dominant greenness metrics for evaluating CE and LC methods were the National Environmental Index (NEMI), Eco-Scale, Green Analytical Procedure Index (GAPI), and Analytical GREENness (AGREE) (Jankech et al., 2024; Kowtharapu et al., 2023). These tools differ in their approach to greenness, reflecting varying levels of detail and the qualitative or quantitative nature of their evaluations.

GAPI is represented as a pictogram with red, yellow, and green sections and is divided into five categories, namely, (i) sample sourcing, (ii) method type, (iii) sample preparation, (iv) reagents and chemicals used, and (v) instrumentation. The final pictogram also contains information regarding the general method type (Kowtharapu et al., 2023; Płotka-Wasyłka, 2018).

AGREE assesses the method greenness based on the 12 principles of green chemistry. This greenness metric features a circular pictogram divided into 12 segments, each representing a different green principle. This modern and comprehensive tool provides both qualitative and quantitative assessments of analytical method greenness, with results displayed by a circle with colours. Each part values range from 0 to 1, transitioning from red to green based on the input method conditions (Kowtharapu et al., 2023; Pena-Pereira et al., 2020).

Besides greenness metrics, the practicality and applicability of the CE methods were assessed using the Blue Applicability Grade Index (BAGI) (Manousi et al., 2023). BAGI addresses the blue concept of white analytical chemistry, investigating 10

key attributes: the type of analysis, the number of analytes simultaneously determined, the number of samples that can be analysed per hour, the type of reagents and materials used, the required instrumentation, the capacity for simultaneous sample treatment, the need for preconcentration, the degree of automation, the type of sample preparation, and the amount of sample. BAGI produces an asteroid-shaped pictogram with a blue colour gradient, indicating scores ranging from 25 to 100. A method is deemed practical if its overall score is >60. The BAGI index helps to identify the strengths and weaknesses of analytical methods in terms of applicability and facilitates comparisons between different techniques. When combined with greenness assessment tools such as GAPI or AGREE, BAGI provides a comprehensive evaluation of both established and newly developed analytical methods (Jankech et al., 2024).

To the best of our knowledge, our research team was the first to develop CE methods with comprehensive validation protocols and applications for the determination of lanreotide and triptorelin in real pharmaceutical and biomedical samples. Initially, we developed a CE-mass spectrometry (CE-MS) method for the quantification of triptorelin in a pharmaceutical dosage form and a biological matrix (plasma) (Piešťanský et al., 2021). Subsequently, we established two hydrodynamically closed system (HCS) CZE-UV methods, which were used to analyse triptorelin in its real pharmaceutical matrix and the synthetic urine matrix (Stefanik et al., 2024b). In this study, we aimed to develop a novel hydrodynamically open-system capillary zone electrophoresis with a diode-array detection (CZE-DAD) method that allows the simultaneous analysis of lanreotide and triptorelin in a single electrophoretic run. To demonstrate its potential for the use in pharmaceutical QC laboratories, the method was applied to the analysis of a real pharmaceutical drug. Additionally, we aimed to compare the greenness of these CE methods using the GAPI and AGREE metrics, and their practicality using the BAGI index.

EXPERIMENTAL

Chemicals and samples

Lanreotide acetate (purity $\geq 98\%$) and triptorelin acetate were purchased from Sigma Aldrich (Steinheim, Germany). The commercial formulation Diphereline® from Ipsen Pharma Biotech (Boulogne-Billancourt, France), containing 0.1 mg of triptorelin acetate in a powdered injection form, was purchased from a local pharmacy. Background electrolytes (BGEs) were prepared using LC-MS grade formic acid (HfO) and acetic acid (HAc) obtained from VWR (Radnor, PA, USA). A 1 mol/L solution of sodium hydroxide (NaOH) was obtained from Agilent Technologies (Santa Clara, CA, USA). Demineralised water, used as a solvent for the BGEs, was produced with a Millipore Simplicity 185 UV water purification system (Millipore, Molsheim, France). All the electrolytes

prepared were filtered through 0.22 μm pore size membrane filters (Millipore) before use.

Instrumentation

CZE-DAD analyses were conducted using the Agilent 7100 Capillary Electrophoresis system (Agilent Technologies) with DAD detection. A 36 cm $\times$ 75 μm inside diameter (ID) bare fused silica capillary (MicroSolv Technology Corporation, Eatontown, NJ, USA) was employed for the electrophoretic separations. Detection wavelengths were set at 220 nm for lanreotide and 200 nm for triptorelin. Sample injection was performed hydrodynamically at 50 mbar (5000 Pa) for 10 s. The separations were carried out under normal polarity with an applied voltage of 30 kV. Data acquisition and analysis were performed using the OpenLab v.2.6 software (Agilent Technologies).

Capillary conditioning and treatment procedure

Before use, the new separation capillary was conditioned by flushing it with 1 mol/L NaOH solution for 15 min, followed by demineralised water for 15 min, and with the BGE for another 15 min, all under a pressure of 950 mbar (95,000 Pa). Before each sample injection, the capillary was re-equilibrated by applying a -20 kV voltage for 10 s and flushing with BGE for 2 min. Every day, after the experiments, the capillary was flushed with 0.1 mol/L NaOH solution, demineralised water, and BGE, each for 15 min, and left in the BGE for the entire night.

Protocols for standard and sample solutions preparation

To prepare the stock solutions, triptorelin acetate and lanreotide acetate were each dissolved in demineralised water, resulting in concentrations of 1 mg/mL for triptorelin and 2.5 mg/mL for lanreotide. These stock solutions were then further diluted with demineralised water to create 100 $\mu\text{g/mL}$ working solutions. Calibration solutions for both compounds were prepared in a water matrix with concentrations of 2, 4, 8, 12, and 16 $\mu\text{g/mL}$. Each sample was subjected to four replicate analyses.

The triptorelin pharmaceutical sample (Diphereline® 0.1 mg) was prepared by dissolving the powder from the original ampoule in 1 mL of demineralised water. Gentle shaking of the ampoule ensured the formation of a homogeneous solution. The resulting solution was then diluted 20-fold with demineralised water to reach a concentration of 5 $\mu\text{g/mL}$, which is within the calibration range. The prepared sample was then injected into the CE system for analysis. The sample underwent four distinct analyses. Due to a shortage in Slovakia, a commercial drug containing lanreotide could not be obtained.

RESULTS AND DISCUSSION

Separation process optimization

The initial phase in developing a CE method involves optimizing the separation process. Our objective was to develop a CZE-DAD method without sample preparation for simple and reliable analysis of both triptorelin and lanreotide in a single electrophoretic run. Two critical parameters for the CE separation process were identified, namely, the composition and concentration of the BGE. Given the demand for rapid analyses in pharmaceutical QC, we selected the highest recommended voltage of 30 kV and a short capillary length of 36 cm. This setup enabled the migration times for both lanreotide and triptorelin to be <5 min.

In this study, the composition of the BGE was examined by comparing the separation performance of volatile organic acids, specifically HFo and HAc. The decision to use HFo and HAc was based on previous positive empirical experiences (Kašička, 2022, 2024; Stefanik et al., 2024a) with highly acidic separation conditions in the analysis of peptides and the potential for transitioning the method to advanced MS detection. It was observed that a BGE containing HAc alone exhibited significantly lower separation efficiency, expressed by the number of theoretical plates (*N*), when compared with HFo buffers. Additionally, combining HAc with HFo in a single BGE also demonstrated a decline in separation efficiency. The influence of buffer composition and concentration on the intensity and stability of lanreotide and triptorelin analytical signals is detailed in Table 1.

Peptide analyses are generally employed in buffer systems with high ionic strength (Kašička, 2022, 2024; Scriba, 2016; Stefanik et al., 2024a), and our findings are consistent with this statement. Elevated concentrations of HFo resulted in improved separation efficiencies and slightly longer

migration times. This effect may be attributed to the lower pH values of these electrolytes, resulting in the increased ratio of uncharged silanol groups on the capillary inner wall. This results in decreased peptide adsorption and electro-osmotic flow (EOF) suppression. The highest separation efficiency was achieved with a 250 mmol/L HFo electrolyte. Interestingly, this concentration appeared to reach a plateau, as 500 mmol/L and 1000 mmol/L HFo buffers did not exceed the *N* value of 100,000. Conversely, the lowest concentration tested (50 mmol/L HFo) resulted in significantly distorted peak shapes, insufficient reproducibility of peak area, and inconsistent migration times. Therefore, based on the best separation efficiencies of both peptides, we selected 250 mmol/L HFo as BGE for further analyses in the validation and application studies.

Validation of the optimised CZE-DAD method

The developed method was rigorously validated in accordance with the recommendations stated in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, International Conference on Harmonisation (ICH) Q2(R1) (European Medicines Agency, 2005). The validation process included a thorough examination of various parameters, such as linearity, range, selectivity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, stability, and robustness. The results of validation process are displayed in Tables 2–4. Selectivity characterises the ability of analytical methods to distinguish and quantify specific analytes in the presence of other constituents in a sample. We evaluated this parameter by comparing samples fortified with lanreotide and triptorelin standards at the LOQ concentration level to blank demineralised water samples. No interfering components from the water matrix were observed at the migration

Table 1. Optimization of BGE composition for the determination of lanreotide and triptorelin via CZE-DAD.

BGE	Lanreotide, <i>n</i> =3				Triptorelin, <i>n</i> =3		
	pH	RSD _{tm} (%)	RSD _{area} (%)	<i>N</i>	RSD _{tm} (%)	RSD _{area} (%)	<i>N</i>
50 mmol/L HFo	2.54	16.9	22.1	44,312	17.8	21.6	39,420
100 mmol/L HFo	2.38	1.7	2.8	75,129	1.6	2.6	74,069
250 mmol/L HFo	2.18	0.9	0.9	109,590	0.9	0.4	106,053
500 mmol/L HFo	2.03	0.4	1.8	94,823	0.3	1.6	92,521
1000 mmol/L HFo	1.88	4.9	4.5	95,303	5.1	7.5	97,016
100 mmol/L HFo + 100 mmol/L HAc	2.63	0.5	2.1	38,430	0.5	2.4	31,340
250 mmol/L HFo + 250 mmol/L HAc	2.43	1.1	4.2	71,222	1.0	2.2	63,824

BGE, background electrolyte; CZE-DAD, capillary zone electrophoresis with diode-array detection; HAc, acetic acid; HFo, formic acid; RSD, relative standard deviation. RSD_{area} is the RSD of the peak area, RSD_{tm} is the RSD of the migration time, and *N* is the separation efficiency and it was calculated using the equation $N = 5.545 \times (tm/w_{1/2})^2$ where *tm* is the migration time and *w*_{1/2} is the peak width at half height. The concentration of lanreotide and triptorelin standards was 10 µg/mL.

positions corresponding to the peaks of lanreotide and triptorelin. This indicates favourable selectivity of the method.

Linearity represents the ability of an analytical method to yield test results that exhibit a direct correlation with the concentration levels of the calibration standards for the specific analyte. The developed method demonstrated satisfactory linearity (with r^2 values >0.99) in the concentration range of 2–16 $\mu\text{g/mL}$ for both lanreotide and triptorelin (Table 2). The linearity was confirmed using analysis of variance (ANOVA) tests.

Table 2. CZE-DAD operation and calibration parameters for lanreotide and triptorelin analysis in an aqueous matrix.

Parameter	Lanreotide	Triptorelin
t_m (min)	3.95	4.35
RSD t_m (%), $n = 4$	0.21	0.23
RSD $_{\text{area}}$ (%), $n = 4$	1.64	1.64
Regression equation	$y = 13.427x - 6.0741$	$y = 13.537x - 8.5831$
r^2	0.996	0.991
Linear range ($\mu\text{g/mL}$)	2–16	2–16
LOD ($\mu\text{g/mL}$)	0.5	0.5
LOQ ($\mu\text{g/mL}$)	2	2
N	34,606	29,079

CZE-DAD, capillary zone electrophoresis with diode-array detection; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation. r^2 is the coefficient of determination, and t_m is the migration time. The separation efficiency of lanreotide and triptorelin was calculated at the concentration level of LOQ.

Both LOD and LOQ were determined using model samples with known low concentrations of analytes, compared with blank demineralised water samples. For LOD, a signal-to-noise ratio (S/N) of 3:1 and for LOQ, a S/N of 10:1 was employed. Using this approach, we established LOD values of 0.5 $\mu\text{g/mL}$ and LOQ values of 2 $\mu\text{g/mL}$ for simultaneous analysis of both lanreotide and triptorelin (Fig. 1).

To evaluate the precision and accuracy of the developed CZE-DAD method, we analysed a series of samples prepared in a water matrix, encompassing a calibration range of 2–16 $\mu\text{g/mL}$ for both lanreotide and triptorelin (Table 3). Intraday precision and accuracy were verified by performing four analyses of those samples in a single day. Precision, expressed as %RSD, ranged from 1.6% to 7.1%, while accuracy, indicated as %RE, was in the range of 92.7%–106.2%. Interday precision and accuracy were assessed over 3 days, with four analyses conducted each day. Corresponding precision ranged from 2.7% to 6.7%, and accuracy varied from 94.1% to 113.6%.

The recovery of the CZE-DAD method was evaluated by spiking injection solutions of triptorelin commercial pharmaceutical samples with a triptorelin standard at three different concentration levels: 2, 5, and 10 $\mu\text{g/mL}$. Triptorelin recovery fluctuated from 105.9% to 112.5%, suggesting the presence of a matrix effect from the pharmaceutical sample.

To dispose with complex information regarding the studied peptides, the short-term stability of both lanreotide and triptorelin standards was evaluated over a period of 24 h at three different temperatures: 20°C (ambient laboratory conditions with controlled climate), 4°C (refrigerated), and –20°C (frozen). The results are summarised in Table 4. The lanreotide standard showed the highest stability when kept at –20°C. For triptorelin, the stability difference between refrigerated and frozen conditions was insignificant. We conclude that both lanreotide and triptorelin standards are stable up to a 24-h period when stored in a freezer. These findings are consistent

Table 3. Precision and accuracy of the CZE-DAD method for lanreotide and triptorelin quantification.

	Lanreotide				Triptorelin		
	Nominal ($\mu\text{g/mL}$)	Found ($\mu\text{g/mL}$)	RSD (%)	RE (%)	Found ($\mu\text{g/mL}$)	RSD (%)	RE (%)
Intraday, $n = 4$	2	2.07	1.6	3.4	2.12	1.6	6.2
	4	3.96	2.8	–1.0	3.71	2.6	–7.3
	8	7.82	3.5	–2.3	8.13	7.1	1.7
	12	12.25	3.1	2.1	12.18	4.0	1.5
	16	15.90	3.6	–0.6	15.86	5.5	–0.9
Interday, $n = 12$	2	2.19	6.7	9.7	2.27	4.1	13.6
	4	3.94	6.2	–1.4	3.88	4.2	–2.9
	8	7.69	4.5	–3.9	7.53	3.7	–5.9
	12	12.12	4.6	1.0	12.34	3.7	2.9
	16	16.06	2.7	0.4	15.97	2.8	–0.2

CZE-DAD, capillary zone electrophoresis with diode-array detection; RSD, relative standard deviation; RE, relative error.

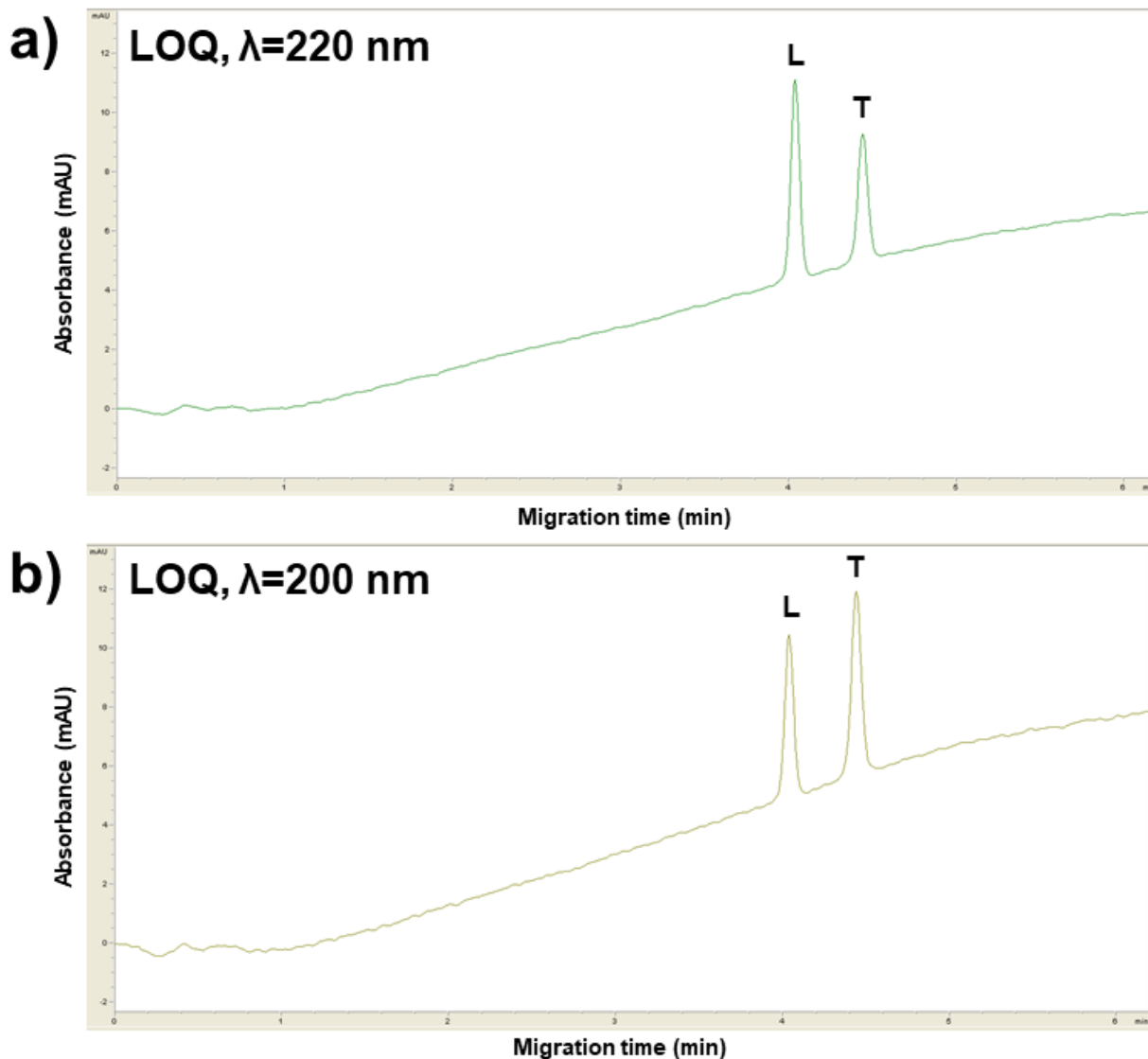


Figure 1. Electropherograms illustrating the simultaneous analysis of lanreotide (L) and triptorelin (T) at their LOQ concentration level (2 µg/mL) in the aqueous matrix, comparing wavelengths: (a) 220 nm and (b) 200 nm. LOQ, limit and quantification.

Table 4. Short-term stability assessment of lanreotide and triptorelin standards in aqueous solutions.

Storage conditions (°C)	Sample/fresh solution peak area ratio	
	Lanreotide, <i>n</i> = 4	Triptorelin, <i>n</i> = 4
20	0.81 ± 0.08	0.83 ± 0.12
4	0.87 ± 0.06	1.02 ± 0.08
−20	0.94 ± 0.10	1.05 ± 0.02

with our earlier assessments of lanreotide and triptorelin short-term stability (Stefanik et al., 2024b).

Robustness was tested in the final step of the validation protocol by intentionally modifying the 250 mmol/L BGE

concentration by ±1 mmol/L HFO. These variations had a minimal impact on both migration time (<4%) and peak area (<9%). Thus, we can state with confidence that the developed CZE-DAD approach demonstrates sufficient robustness.

Application of the CZE-DAD technique

After the optimization and validation phases, the developed method was applied for the quantification of triptorelin in a real pharmaceutical matrix. For this purpose, the commercially available drug Diphereline® 0.1 mg in the dosage form of a powder for injection was selected. The analysis required minimal sample preparation, and a simple 20-fold dilution of the drug was sufficient. The manufacturer declared a

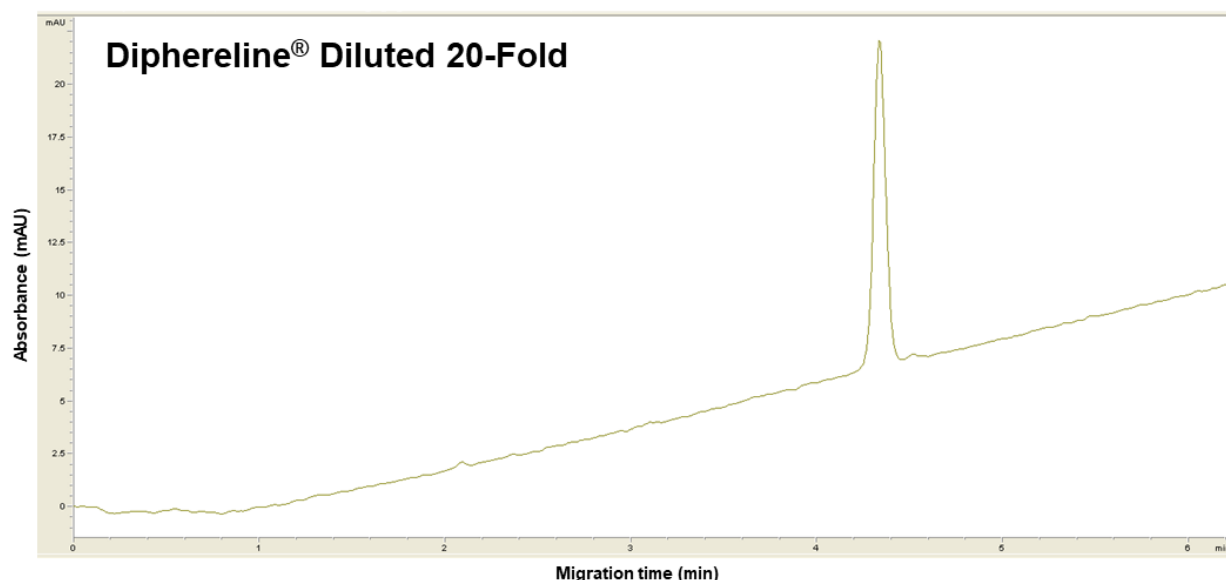


Figure 2. Electropherogram depicting the analysis of Diphereline® with expected triptorelin content at the concentration level of 5 µg/mL after dilution.

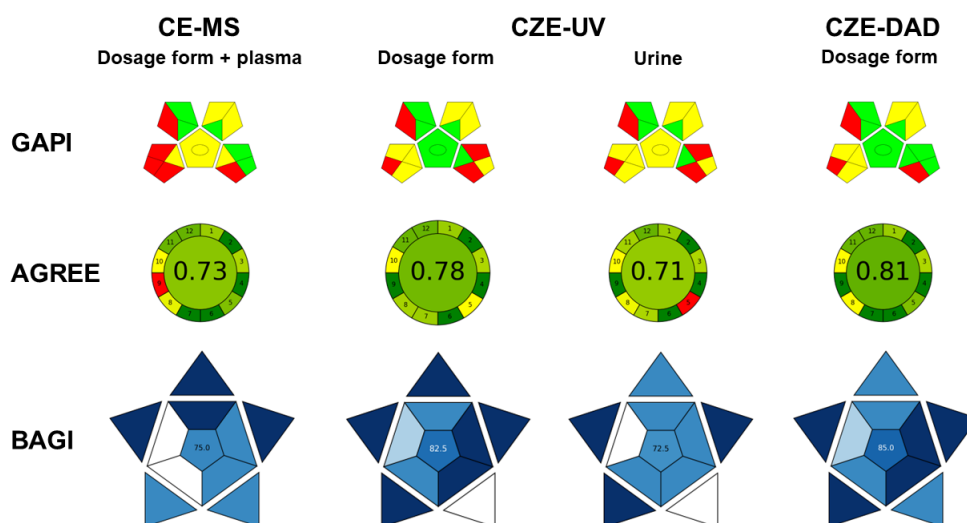


Figure 3. Greenness and applicability comparison of CE methods developed for lanreotide and triptorelin analysis: CE-MS (Piešťanský et al., 2021), CZE-UV (Stefanik et al., 2024b), and CZE-DAD (present study) using GAPI, AGREE, and BAGI metrics. AGREE, analytical GREENness; BAGI, Blue Applicability Grade Index; CE-MS, capillary electrophoresis-mass spectrometry; CZE-DAD, capillary zone electrophoresis with diode-array detection; GAPI, Green Analytical Procedure Index.

triptorelin content of 100 µg/dose. Using the suggested CZE-DAD methodology, we quantified the triptorelin content in Diphereline® to be 96.19 ± 0.09 µg, demonstrating both high repeatability and acceptable concordance with the manufacturer's specification. An illustrative electropherogram of the pharmaceutical sample analysis is displayed in Fig. 2.

Greenness and applicability: Evaluation and comparison

The greenness of the CZE-DAD method developed in this study was confirmed by favourable GAPI assessment (6 green,

5 yellow, and 3 red regions in the pictogram and green general method type in the centre) and achieved an AGREE score of 0.81. Compared with the CE methods previously developed by our research group (Piešťanský et al., 2021; Stefanik et al., 2024b), the presented CZE-DAD method demonstrated the highest greenness in both metrics, as illustrated in Fig. 3. The primary greenness advantages of the proposed CZE-DAD method, when compared with the CE-MS method, include significantly lower energy consumption and no sample preparation. As for the experimental HCS CZE-UV method, the CZE-DAD method offers a higher degree of automation using an autosampler, hermetic sealing of the analytical

process, and reduced waste of the BGE electrolyte.

The method's applicability was evaluated using the BAGI index metric, yielding a score of 85, showing excellent practicality. The newly developed CZE-DAD method thus outperformed the other CE methods in terms of applicability, achieving the highest BAGI score (see Fig. 3). Notable applicability advantages over the other CE methods discussed include (i) simultaneous analysis of both lanreotide and triptorelin in a single electrophoretic run, (ii) the use of commercially available semi-automated CE-DAD instrumentation, (iii) simultaneous preparation of over 95 samples, and (iv) no sample preparation or preconcentration.

CONCLUSIONS

In this study, we successfully developed and validated a simple CZE-DAD method for the simultaneous quantification of two short therapeutic peptides, namely, lanreotide and triptorelin. A thorough validation process confirmed the high selectivity, reliability, and reproducibility of the presented method, without the need for sample pretreatment or preconcentration. The method was tested by analysing a real

pharmaceutical dosage form of triptorelin, making it a viable green option for routine pharmaceutical QC applications. Furthermore, the greenness and practicality of the method were evaluated using the AGREE, GAPI, and BAGI metrics. The method scored highly in all of these assessments, which affirms its potential to improve the sustainability of analytical practices in QC laboratories. The presented CZE-DAD method offers a green, cost-effective, and efficient alternative to traditional LC techniques, in line with emerging trends and the focus on the development of eco-friendly analytical methods.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

ACKNOWLEDGMENTS

This work was supported by the projects UK/3023/2024 and Slovak Research and Development Agency under the contract no. APVV-23-0508.

References

- [1] Craik DJ, Fairlie DP, Liras S, Price D. The future of peptide-based drugs. *Chem Biol Drug Des.* 2013;81(1):136–147.
- [2] Dispas A, Sacré PY, Ziemons E, Hubert P. Emerging analytical techniques for pharmaceutical quality control: Where are we in 2022? *J Pharm Biomed Anal.* 2022;221:115071.
- [3] Eldin AB, Ismaiel OA, Hassan WE, Shalaby AA. Green analytical chemistry: Opportunities for pharmaceutical quality control. *J Anal Chem.* 2016;71(9):861–871.
- [4] European Medicines Agency. ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology. Step 4 Version. 2005. (<https://www.ich.org/>). Accessed October 16, 2023.
- [5] Ipsen. Press release: Ipsen Delivers Solid Sales Growth in the First Nine Months of 2023 and Confirms its Full-Year Guidance. 2023. (<https://ml-eu.globenewswire.com/Resource/Download/20586a11-7fdd-4501-b4c5-d500312ae738>). Accessed August 19, 2024.
- [6] Jankech T, Gerhardtova I, Stefanik O, et al. Current green capillary electrophoresis and liquid chromatography methods for analysis of pharmaceutical and biomedical samples (2019–2023) – A review. *Anal Chim Acta.* 2024;1323:342889.
- [7] Kašička V. Recent developments in capillary and microchip electroseparations of peptides (2019–mid 2021). *Electrophoresis.* 2022;43(1–2):82–108.
- [8] Kašička V. Recent developments in capillary and microchip electroseparations of peptides (2021–mid-2023). *Electrophoresis.* 2024;45(1–2):165–198.
- [9] Kowtharapu LP, Katari NK, Muchakayala SK, Marisetti VM. Green metric tools for analytical methods assessment critical review, case studies and crucify. *TrAC Trends Anal Chem.* 2023;166:117196.
- [10] Kumar P, Sharma A. Gonadotropin-releasing hormone analogs: Understanding advantages and limitations. *J Hum Reprod Sci.* 2014;7(3):170–174.
- [11] Manousi N, Wojnowski W, Płotka-Wasyłka J, Samanidou V. Blue Applicability Grade Index (BAGI) and software: A new tool for the evaluation of method practicality. *Green Chem.* 2023;25(19):7598–7604.
- [12] Mazziotti G, Giustina A. Effects of lanreotide SR and Autogel on tumor mass in patients with acromegaly: A systematic review. *Pituitary.* 2010;13(1):60–67.
- [13] Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—analytical GREEnness metric approach and software. *Anal Chem.* 2020;92(14):10076–10082.
- [14] Piešťanský J, Čížmárová I, Štefánik O, et al. Capillary electrophoresis–mass spectrometry with multisection injection and in-capillary preconcentration for high-throughput and sensitive determination of therapeutic decapeptide triptorelin in pharmaceutical and biological matrices. *Biomedicines.* 2021;9(10):1488.
- [15] Płotka-Wasyłka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. *Talanta.* 2018;181:204–209.
- [16] Robert M. Green analytical chemistry: A sustainable approach to chemical analysis. *Adv Appl Sci Res.* 2023;14(7):107–108.
- [17] Scriba GKE. Separation of peptides by capillary electrophoresis. In: Schmitt-Kopplin P. *Capillary Electrophoresis: Methods and Protocols.* Methods in Molecular Biology. Human Press; New York, 2016, p. 365–391.
- [18] Stefanik O, Majerova P, Kovac A, Mikus P, Piestansky J. Capillary electrophoresis in the analysis of therapeutic peptides-A review. *Electrophoresis.* 2024a;45(1–2):120–164.

- [19] Stefanik O, Mikus P, Piestansky J. Quantitative analysis of therapeutic peptides by CZE using multiple sample injection in hydrodynamically closed separation system. *Electrophoresis*. 2024b. in press
- [20] Uddin Md, Mamun A, Rashid M, Asaduzzaman Md. In-process and finished products quality control tests for pharmaceutical capsules according to pharmacopoeias. *Br J Pharm Res*. 2016;9(2):1–9.
- [21] Van Schepdael A. Capillary electrophoresis as a simple and low-cost analytical tool for use in money-constrained situations. *TrAC Trends Anal Chem*. 2023;160:116992.
- [22] Wang L, Wang N, Zhang W, et al. Therapeutic peptides: Current applications and future directions. *Signal Transduct Target Ther*. 2022;7(1):481–27.
- [23] WHO. Annex 1: WHO Good Practices for Pharmaceutical Quality Control Laboratories. 2010. (https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/quality-control/trs957-annex1-goodpractices-pharmaceuticalquality-control-laboratories.pdf?sfvrsn=ca0c211c_0). Accessed August 19, 2024.