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ORIGINAL ARTICLE / ÖZGÜN MAKALE

A VALIDATED ADSORPTIVE STRIPPING DIFFERENTIAL PULSE VOLTAMMETRIC METHOD FOR THE QUANTIFICATION OF LINAGLIPTIN FROM URINE SAMPLE

İDRARDAN LİNAGLİPTİN TAYİNİ İÇİN ADSOPRTİF SIYIRMA DİFERANSİYEL PULS VOLTAMETRİK YÖNTEM GELİŞTİRİLMESİ VE VALİDASYONU

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ABSTRACT

Objective: *In this study, a low-cost, sensitive and practical electroanalytical method was developed and validated for the determination of Linagliptin (LNG), a therapeutic agent used in the treatment of Diabetes Mellitus, one of the most common chronic diseases worldwide, characterized by high blood glucose levels and directly affecting millions of people.*
Material and Method: *Electroanalytical studies were completed using screen-printed carbon electrodes in pH: 8 Britton - Robinson buffer solution. Mechanistic studies were carried out by cyclic voltammetry technique. Adsorptive stripping differential pulse voltammetry technique was used for the electrochemical determination of LNG.*
Result and Discussion: *The developed method exhibited linearity in the range of 0.1 - 7.5 µM and the limit of detection value was calculated as 37 nM. The developed method was applied for the determination of LNG from urine and a recovery value of 100.02% was obtained.*
Keywords: *Adsorptive stripping differential pulse voltammetry, antidiabetic drug, electrochemical determination, linagliptin*

ÖZ

Amaç: *Bu çalışmada, dünya çapında en yaygın kronik hastalıklardan biri olan, yüksek kan şekeri ile karakterize, ve milyonlarca insanı doğrudan etkileyen bir rahatsızlık olan Diabetes Mellitus tedavisinde kullanılan terapötik bir ajan olan Linagliptin (LNG) tayini için düşük maliyetli, hassas ve pratik bir elektroanalitik yöntem geliştirilmiş ve valide edilmiştir.*
Gereç ve Yöntem: *Elektroanalitik çalışmalar pH: 8 Britton - Robinson tampon çözelti ortamında perde baskılı carbon elektrotlar kullanılarak tamamlanmıştır. Mekanistik çalışmalar dönüşümlü voltametri tekniği ile yapılmıştır. LNG'nin elektrokimyasal tayini için de adsorptif sıyırma diferansiyel puls voltametri tekniği kullanılmıştır.*
Sonuç ve Tartışma: *Geliştirilen yöntem 0.1 – 7.5 µM aralığında doğrusallık sergilemiş ve gözlenebilme sınırı 37 nM olarak hesaplanmıştır. Geliştirilen yöntem idrardan LNG tayini için uygulanmış ve % 100.02'lik bir geri kazanım değeri elde edilmiştir.*
Anahtar Kelimeler: *Adsorptive sıyırma diferansiyel puls voltametri, antidiyabetik ilaç, elektrokimyasal analiz, linagliptin*

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INTRODUCTION

Diabetes Mellitus (DM) is one of the most prevalent chronic metabolic diseases on earth that is characterised by high glucose levels because of reduced insulin production from pancreas or insufficient usage of produced insulin. It causes serious problems to the many parts of the body such as heart, kidney, eyes and blood vessels [1-3]. According to data presented by the World Health Organization (WHO), 830 million people were living with DM in 2022, up from 200 million in 1990 [4]. This significant rise in DM prevalence underscores the growing importance of effective treatment strategies. Although factors such as regular and balanced nutrition, exercise and lifestyle are effective in the controlling of glucose level, medications bear the majority of the treatment burden. Linagliptin (LNG), the first member of dipeptidyl peptidase-4 inhibitors, is an antidiabetic drug used for treatment of patients with type II DM (Figure 1) [5,6]. Since it was first licensed in the US, Europe, Japan and elsewhere in 2011 [5,7] it is commonly used to improve glycaemic control in adults with the type II DM.

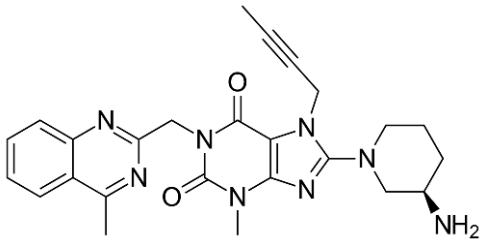

Figure 1. Chemical structure of LNG

Drug analysis is one of the most important fields of analytical chemistry. Drug analysis is performed from different matrices such as pharmaceutical forms, blood, urine and tissue for clinical, quality control, forensic studies, etc. [8]. Different analytical techniques including chromatography [9], spectroscopy [10], and electroanalysis [11] are used in this context. Electroanalytical techniques stand out in the field of analytical methods due to their quick response time, portability, affordability, simple procedures, high sensitivity, and minimal interference from drug excipients. As a result, they have become a crucial tool in pharmaceutical analysis [12-17]. Moreover, these methods offer several benefits over conventional techniques, such as enabling in-situ and real-time analysis without the need for complex sample preparation. This not only accelerates the analysis but also minimizes the risk of sample contamination and loss. Although limited in number, electroanalytical methods developed for LNG analysis can be found in the literature [18-21]. However, these methods are often laborious and costly, involving material synthesis, sensor fabrication and characterization steps. In this study, a rapid and easily applicable adsorptive stripping differential pulse voltammetric (AdSDPV) method for the analysis of LNG, which does not require any material synthesis or sensor fabrication processes, was developed and validated. To evaluate the applicability of the developed method, LNG was analysed from spiked urine.

MATERIAL AND METHOD

Chemicals

LNG as a standard was kindly gifted from Nobel Pharmaceuticals (Türkiye). All chemicals used in the experimental steps were of analytical grade and were used without any purification. Ultra-pure water (Millipore, Merck, Germany) was used for the preparation of aqueous solutions. LNG was dissolved in methanol to obtain stock solution.

Apparatus

All voltammetric measurements were performed using the AUT204NFRA32M model Metrohm-Autolab (Netherlands) potentiostat-galvanostat system controlled with NOVA 2.1 software. Screen-printed carbon (SPC) electrodes (LifeSense, Türkiye) were used during the study.

Voltammetric Measurements

During the study, differential pulse voltammetry (DPV), adsorptive stripping differential pulse voltammetry (AdSDPV) and cyclic voltammetry (CV) were used. Before measurements, SPC electrode surfaces were rinsed with water and ethanol, respectively, to remove dust or any other particles that stuck on to electrode surface. All measurements were carried out in the 0.25 M Britton – Robinson buffer solution (BRbs) as an electrolyte solution. The pH value of BRbs was adjusted with 2 M NaOH and 2 M HCl.

Real Sample Preparation

Firstly, artificial urine sample was filtered from 0.45 PTFE syringe filter. Afterwards, it was diluted 10-fold with BRbs. Finaly, it was spiked with LNG at 1.0 µM concentration.

RESULT AND DISCUSSION

Electrochemical Behaviour of LNG

Electrochemical behaviour of 100 µM LNG on the SPC electrode surface was investigated by DPV in pH: 2 BRbs medium. As seen in Figure 2, a well-defined oxidation peak was observed around 0.95 V with the anodic peak current (I_{pa}) of ~1.8 µA.

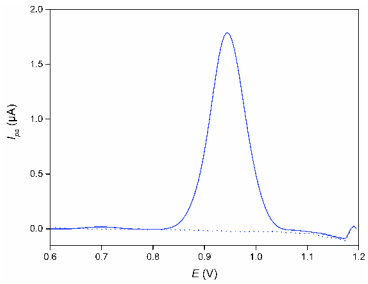

Figure 2. Electrochemical response of LNG on the SPC electrode surface – baseline corrected DPVs of 100 µM LNG in 0.25 M pH: 2 BRbs (Dotted line; 0.25 M pH: 2 BRbs in the absence of LNG)

Optimization of pH

The pH value of the electrolyte solution directly affects both electrodes and methods performance. Therefore, pH optimization of electrolyte solution is one of the most important step in the electroanalytical method development process. In this context electrochemical behaviour of LNG was evaluated in the BRbs at different pH values changing from 2 – 10. Figure 3 shows the I_{pa} of 100 µM LNG in the different pH media. As is seen, increasing analytical responses were observed up to pH: 8. However, after pH: 8 I_{pa} started to decrease. Thus, pH: 8 was chosen as the optimum experimental condition for further experiments.

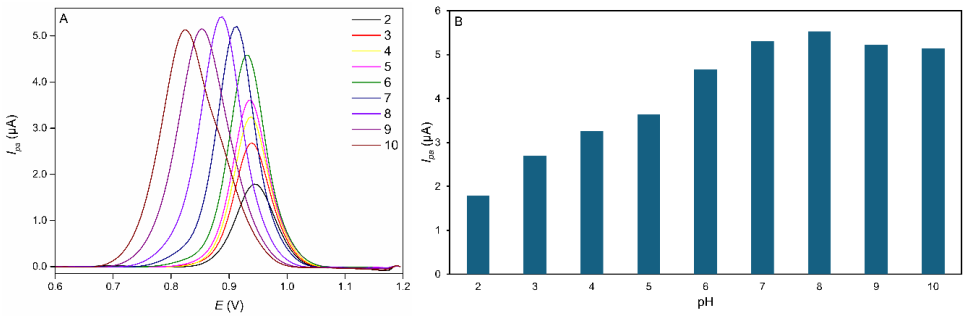

Figure 3. (A) Baseline corrected DPVs of 100 µM LNG in 0.25 M BRbs at different pH values, (B) Electroanalytical response of 100 µM LNG at different pH media

Influence of Scan Rate

In electroanalytical studies, it is important to determine the reaction mechanism by which the target analyte moves on the sensor surface. For this purpose, CVs of 100 µM LNG in 0.25 M pH: 8 BRbs on the SPC electrode surface at different scan rates (v) from 0.01 – 0.50 V·s^{-1} were recorded (Figure 4). A linear relationship between the logarithm of the scan rate (log v) and logarithm of the I_{pa} (log I_{pa}) was observed. The slope of this plot indicates the type of reaction that occurs on the electrode surface. The slope of 0.6685 (Seen in Figure 4B) which is between 0.5 and 1 indicates that the electro-oxidation of LNG on the SPC electrode surface is adsorption as well as diffusion controlled.

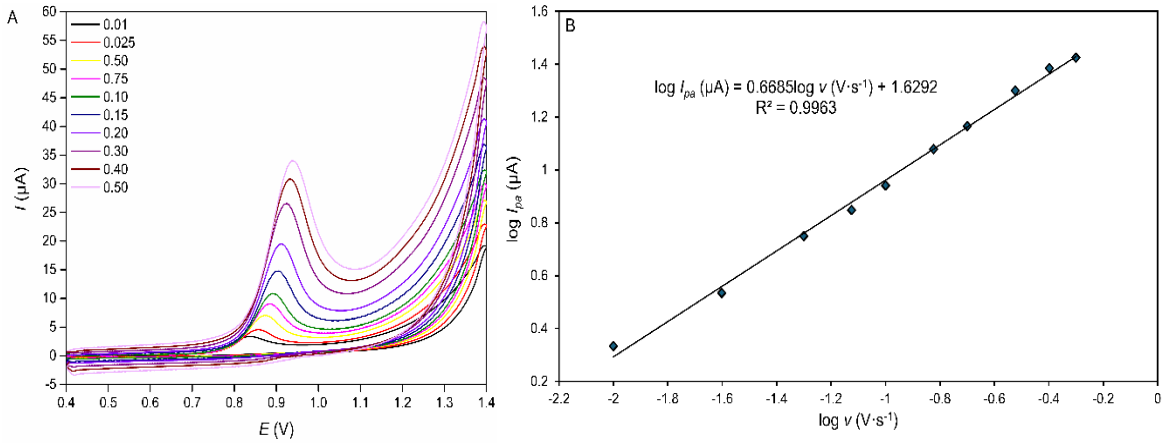

Figure 4. (A) CVs of 100µM LNG in 0.25 M, pH: 8 BRbs at different scan rates, (B) plot of log v vs. log I_{pa}

Optimization of Accumulation Step

Since the electro-oxidation mechanism of LNG on the SPC electrode surface is affected by adsorption as well as diffusion, the accumulation steps need to be optimized to get the best performance from the developed method. In this context, voltammetric responses of 100 µM LNG in 0.25 M pH: 8 BRbs at different accumulation potentials (E_{acc}) and different accumulation times (t_{acc}) were compared. Obtained results are exhibited in Figure 5. As can be seen, an increase in I_{pa} was observed with an increase in potential up to 0.4 V, after which the I_{pa} started to decrease. For this reason, 0.4 V was chosen as the optimum E_{acc}. Similarly, it is also observed that the I_{pa} increases with increasing t_{acc}. However, after 480 s, the rate of increase decreased very much and became insignificant. Therefore, 480 s was chosen as the optimum t_{acc} in order not to keep the analysis time too long.

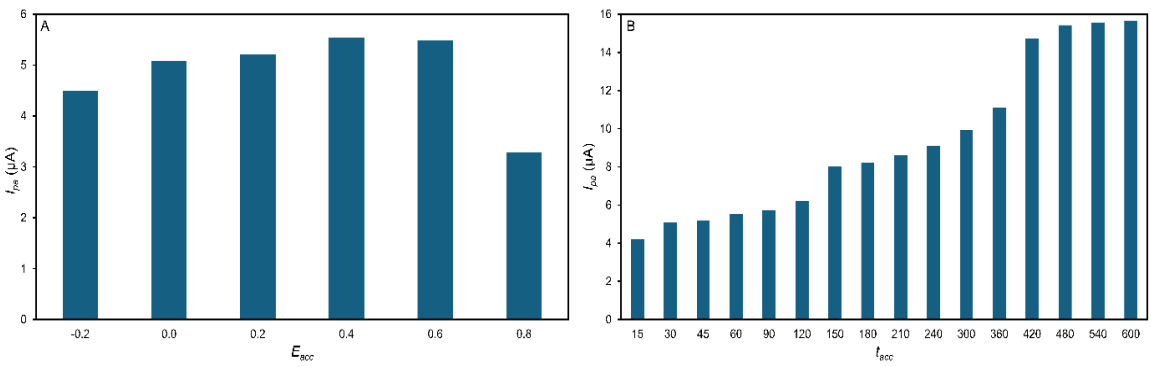

Figure 5. (A) I_{pa} of 100 µM LNG in 0.25 M pH: 8 BRbs at different E_{acc}, (B) I_{pa} of 100 µM LNG in 0.25 M pH: 8 BRbs at different t_{acc}

Analytical Performance

Before the electroanalytical measurements, the experimental parameters that affect the AdSDPV responses were optimized as shown in Table 1. Under the optimized conditions, the I_{pa} values was found to be linear against the increasing concentration of LNG from 0.1 to 7.5 µM with the related regression equation of I_{pa} (µA) = 0.3524C_{LNG} (µM) + 0.2163 (R^2 = 0.9961) (Figure 6). The limit of detection (LOD) and limit of quantification (LOQ) values were calculated according to the formula *k.SS/m*, where *k* is 3 for LOD and 10 for LOQ, *SS* is the standard deviation of the I_{pa} of 6 consecutive measurements recorded at the lowest concentration of the linear working range, and *m* is the slope of calibration curve. Using this formula, LOD and LOQ values were calculated as 37 nM and 123 nM, respectively. Table 2 shows the results of previously reported studies in the literature for LNG analysis. As it is seen, even without any modification of the SPC electrode, highly sensitive results were obtained compared to existing studies, which proves the superiority of this study.

Table 1. Optimized experimental parameters affecting the AdSDPV responses

Parameter	Value
Start potential (V)	0.4
Stop potential (V)	1.4
Deposition potential (V)	0.4
Deposition time (s)	480
Step (V)	0.005
Modulation amplitude (V)	0.025
Modulation time (s)	0.05
Interval time (s)	0.1
Scan rate ($V \cdot s^{-1}$)	0.05

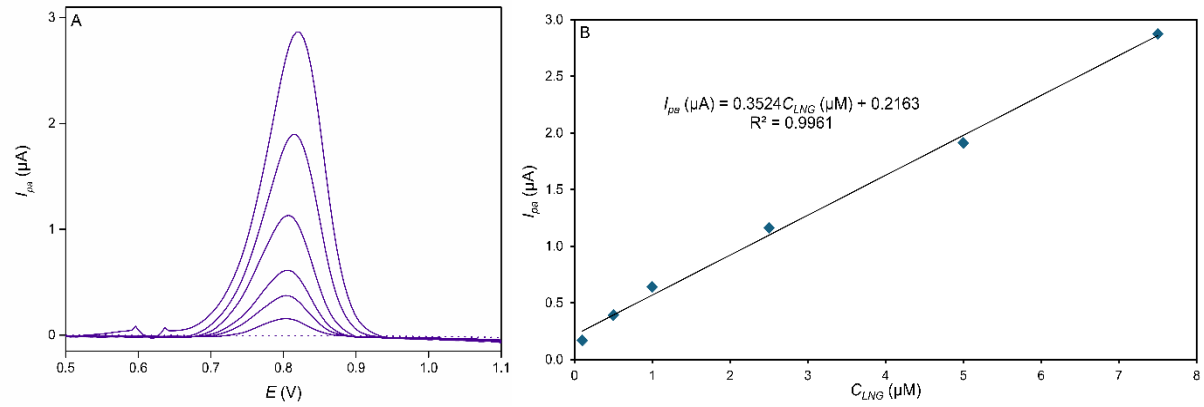

Figure 6. (A) AdSDPVs of LNG at increasing concentrations (Dotted line; 0.25 M pH: 8 BRbs in the absence of LNG), (B) Calibration plot of LNG

Table 2. Comparison of previously reported electroanalytical methods for LNG detection

Method	Sensor	Linear range (µM)	LOD (nM)	Reference
SWV	Co_3O_4NPs/MWCNT	39.8 – 1530	11300	[18]
DPV	gCN-βCD/CPE	0.01 – 50	3	[19]
SWV	Fe_2O_3NPs/CPE	0.063 – 182	17	[20]
DPV	L-cys-MoS_2/GCE	1 – 153.4	190	[21]
SWV	PGE	0.5 – 11	210	[22]
SWV	GCE	0.4 – 4.8	110	[23]
AdSDPV	SPC electrode	0.1 – 7.5	37	This study

SWV; square wave voltammetry, DPV; differential pulse voltammetry, NPs; nanoparticles, MWCNT; multi-walled carbon nanotube, CPE; carbon paste electrode, PGE; pencil graphite electrode, GCE; glassy carbon electrode

Repeatability and Selectivity

In order to evaluate the repeatability of the method, 1 µM LNG was measured 6 times consecutively and the relative standard deviation (*RSD*%) of the electroanalytical responses was calculated. The *RSD*% was found to be 1.66%. This value proves that the method is valid in terms of repeatability. Another important parameter in analytical methods is selectivity. Thus, the changes on the electroanalytical response of LNG in the presence of possible 100-fold interference such as glucose, urea, Ca^{2+}, Na^{+}, K^{+}, CO_3^{2-}, Cl^{-}, and NO_3^{-} were evaluated. The difference of I_{pa} (ΔI_{pa}) and the difference of E_{pa} (ΔE_{pa}) between in the absence and presence of interferences were calculated as 0.71% and 0.003 V respectively (Presented in Table 3). These results are clear evidence that the developed method is able to selectively detect LNG in the presence of possible interferences.

Table 3. AdSDPV responses of 1 µM LNG in the absence and in the presence of various interferences (N = 3)

Analyte	C (µM)	I_{pa} (µA)	ΔI_{pa} (%)	E_{pa} (V)	ΔE_{pa} (V)
LNG	1	0.14203	0.71	0.815	0.003
LNG	1	0.14102		0.818	
Glucose	100				
Urea	100				
$CaCO_3$	100				
NaCl	100				
KNO_3	100				

Real Sample Analyses

The suitability of the method was evaluated by analysing the LNG content of urine sample using standard addition method [24]. The average recovery value was found 100.02% with the *SD* of 0.52 (N = 3). It is clearly seen that the developed method can detect LNG from complex biological matrix with high recovery values (Table 4).

Table 4. Recovery values of LNG from spiked urine sample

Added (µM)	Found (µM)	Recovery (%)
1.0000	0.9981	99.81
1.0000	1.0063	100.63
1.0000	0.9961	99.61
	Mean	100.02
	SD	0.54

Conclusion

DM is a chronic disease characterized by high blood glucose levels that directly affects more and more people in the world, is directly and indirectly responsible for many deaths, and has many social and economic impacts on society. Although it cannot be completely cured, it is possible for DM patients to keep their blood sugar levels under control with various treatment methods and lead a life like healthy individuals. Undoubtedly, medication plays the biggest role in these treatment methods. LNG is a dipeptidyl peptidase-4 inhibitor used in the treatment of DM.

Electroanalytical methods stand out among analytical methods because they are cheap, fast and easy to apply. They are increasingly preferred in drug analysis, especially because they can determine the target drug without the need to separate excipients in pharmaceutical forms. Screen-printed electrodes are very easy to procure and very practical to use systems that include traditional triple electrode systems consisting of working electrode, reference electrode and counter electrode on a single platform.

In this study, AdSDPV method was developed for LNG determination using SPC electrodes and

validated in terms of selectivity, repeatability and recovery parameters. The developed method exhibited linearity in the concentration range of 0.1 - 7.5 µM and reached a LOD of 37 nM. In repeatability studies, the RSD% value was calculated as 1.66%. The developed method was able to analyse LNG in the presence of possible interferences without being affected by these interferences. The method proved its suitability by determining LNG from urine with a recovery of 100.02%.

The developed method has been introduced to literature as a cheaper and practical method compared to existing methods involving material synthesis and characterization in terms of using the SPC electrode surface without any modification process. It also stands out as a more sensitive method than most of the existing methods. As a result, it is considered an innovative alternative in areas where both quality control and clinical analysis of LNG are needed.

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AUTHOR CONTRIBUTIONS

Concept: A.K.A.; Design: A.K.A.; Control: A.K.A.; Sources: A.K.A.; Materials: A.K.A.; Data Collection and/or Processing: A.K.A.; Analysis and/or Interpretation: A.K.A.; Literature review A.K.A.; Manuscript Writing: A.K.A.; Critical review: A.K.A.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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