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Development and Validation of Electrochemical Method for Quantification of Palbociclib (Anticancer Agent) in Biological Matrices Using Square Wave- Adsorptive Stripping Voltammetry

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The electrochemical reduction behavior of palbociclib (PLB) was studied using square wave voltammetry, differential pulse polarography and cyclic voltammetry in Britton-Robinson (B-R) buffer of pH 7.0. The mercury electrode was used to accumulate PLB into its surface to give a well-defined reduction wave at -1.05 V potential in the presence of Ag/AgCl reference electrode and Pt auxiliary electrode. There were some analytical parameters which studied to obtain the best reduction signal, such as buffer solutions (types and strength), pH values, scans and stirring rates. Britton-Robinson buffer of pH 7.0, 50 s accumulation time, 0.0 V accumulation potential, 30 Hz frequency, 300 mV s^{-1} scan rate, 50 mV amplitude, 0.6 mm^2 drop area, and 3000 rpm were recorded high sensitivity for the PLB determination, so they were chosen as optimum parameters for the next work. The repeatability, stability, recovery, calibration curve and detection limit were validated to evaluate the analytical performance of the developed method. Repeatability and stability of 5 $x10^{-7}$ mol L^{-1} of PLB were reported 0.0282% relative standard deviation (RSD%) for ten cathodic measurements, and a good stability for 120 min, respectively. Calibration curve was studied over the range $1x10^{-7}$ - 1 x 10^{-6} mol L^{-1} for PLB to be obtained a linear relationship with a 0.992 correlation coefficient (r^2) for sex measurements (n = 6). Lower detection limit (LOD) was calculated to be 8.8 x 10^{-11} mol L^{-1} (0.039 ppb), while lower quantification limit (LOQ) was become 2.9 x 10^{-10} mol L^{-1} (0.131ppb). The square wave voltammetry method was applied for qantification of PLB at the human plasma and urine samples.

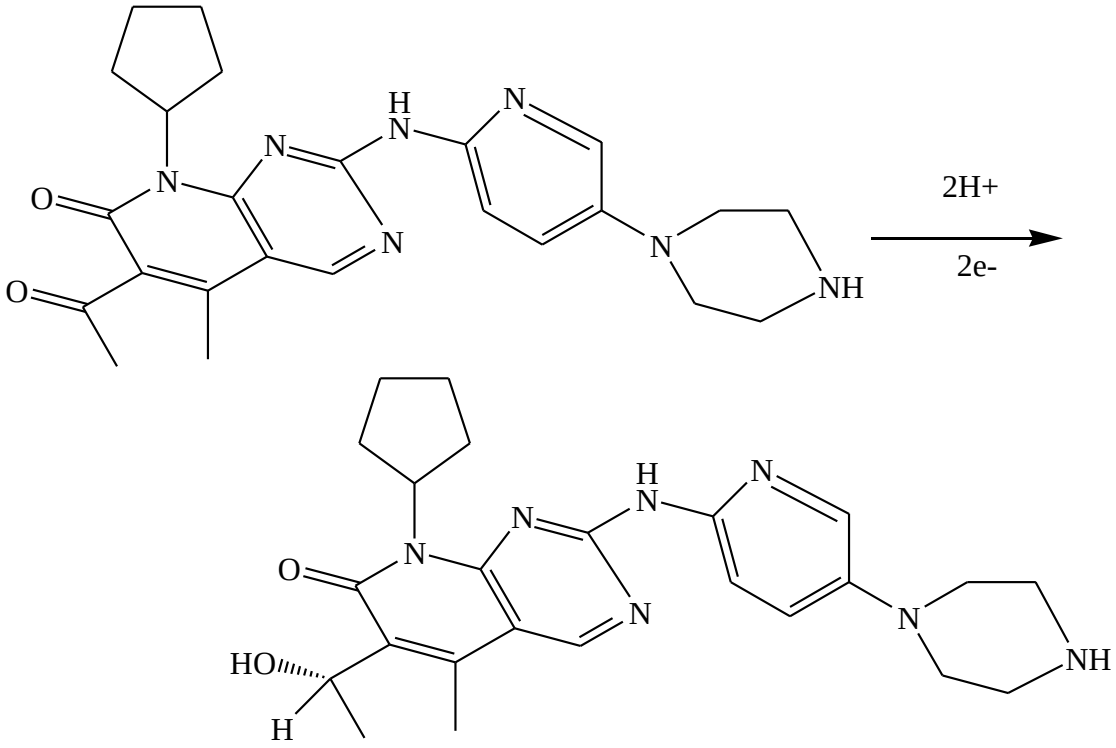
Keywords. Voltammetry, reduction behavior, palbociclib, human plasma, mercury electrode, buffer solution.

1. INTRODUCTION

Electrochemistry has always characterized by instrumental simplicity, cost-effective, selective, reliable and convenient. Electrochemical techniques can straightforwardly be approved to solve many problems of pharmaceutical interest with a high degree of selectivity, sensitivity, accuracy and precision, often in remarkable reproducible ways by employing an analytical method [1-9].

Square wave voltammetry (SWV) is a form of linear potential sweep voltammetry that uses a combined square wave and staircase potential applied to a stationary electrode. It has found numerous applications in various fields, including, food and water analysis [10-12], pharmaceutical and medicinal analysis [13-17], dyes [18,19], and metals [20-22]. SWV is used for both quantitative chemical analysis and study of the mechanism, kinetics, and thermodynamics of chemical reactions. SWV used as an analytical tool offers three major advantages when compared to other electrochemical techniques. It is very sensitive, often allowing direct analyses at the ppb (parts per billion) level and even the low ppt (parts per trillion) level when used in a stripping mode. It requires less time per sweep than older techniques such as differential pulse polarography. A SWV sweep can often be recorded in less than ten seconds, in contrast with a differential pulse polarography (DPP) that typically requires more than two minutes for data acquisition. The square-wave frequency can be used to differentiate between processes with fast and slow kinetics. In some cases, kinetically fast processes can be measured without interference from slower processes that occur in the same potential range [23].

Palbociclib (PLB) is a member of the class of pyridopyrimidines that is [6-Acetyl-8-cyclopentyl-5-methyl-2-{[5-(piperazin- 1-yl) pyrindin-2-l] amino} pyrido [2, 3-d]-pyrimidin-7(8H)-one]. Palbociclib is a unique cyclin-dependent kinase inhibitor that is used in combination with aromatase inhibitors in the treatment of postmenopausal women with metastatic breast cancer. PLB is associated with transient and usually mild elevations in serum aminotransferase during therapy, but has yet to be linked to cases of clinical apparent acute liver injury. So it is used to treat a certain type of breast cancer. It is a chemotherapy drug that works by slowing or stopping the growth of cancer cells [24]. Palbociclib (IBRANCE®) was approved for medical use in the United States in 2017 [25]. The molecular formula of PLB is $C_{24}H_{29}N_7O_2$ and its molecular weight was 447.533 g mol^{-1} (see scheme 1). The aim of this work was to develop a rapid, and sensitive analytical method for a quantitative analysis of PLB in human urine and plasma. So the square wave- adsorptive stripping voltammetry technique was applied to determine the trace levels of palbociclib onto the surface of hanging mercury dropping electrode (HMDE). There is no published article used the same analytical parameters as reported in this research.



Scheme 1. The proposed mechanism for the electrochemical reduction of PLB compound

2. EXPERIMENTAL PART

2.1 Apparatus

A 797 VA instrument (Switzerland made, Metrohm company) was used for the voltammetric determinations of PLB in human plasma and urine fluids. VA instrument is connected with three electrode system, including mercury working electrode, Ag/AgCl (3 mol L^{-1} KCl) reference electrode and platinum auxiliary electrode. The pH measurements were carried out using a digital pH - meter (model pH211, Hanna company). The distilled water was prepared using Millie-Q Plus system water purification system (Milford company, USA). The human plasma and urine biological fluids were centrifuged using a labofuge 200 instrument (Heraeus Sepatech company, Germany).

2.2 Chemicals

Reference standard of palbociclib (PLB) (purity > 99%), was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). The stock solution of PLB was prepared by dissolving PLB in distilled water with some drops from 0.1 mol L^{-1} HCl (pH = 4) at 50 mL volumetric flask. The diluted PLB solutions were prepared in distilled water solvent according to the requested experimental in different concentrations. Britton-Robinson (B-R), phosphate, acetate and carbonate buffers were prepared using the obvious amounts of boric acid, acetic acid, and phosphoric acid and other materials to obtain a well reduction signal [26].

2.3 Procedures

A 10 ml of buffer solution was injected into an electrochemical cell after cleaning by acids and distilled water before any additions. Reduction scan was applied for all measurements over the range of potential - 0.4 to -1.3 V. All solutions were purged using nitrogen gas for 100 s period with stirring. The cathodic voltammograms for PLB were obtained using the optimum parameters; B-R buffer pH 7.0, 50 s accumulation time, 0.0 V accumulation potential, 30 Hz, 300 mVs^{-1}scan rate, 50 mV amplitude, 0.6 mm^2 drop size and 3000 rpm at high sensitivity and selectivity.

2.3.1 Preparation of biological matrixes

The 1.0 mL of 5.0% $ZnSO_4.7H_2O$, 1.0 mL of ethanol and 0.1 mL of NaOH, were added to 0.5 mL of human urine and plasma in a centrifuge tube using 5000 rpm for 8 min centrifuging period [27]. The required concentration of PLB was added to the filtered amount of biological matrixes, then the square wave - adsorptive stripping voltammetry (SW-AdSV) was applied for the determination of PLB by recovery.

3. RESULTS AND DISCUSSION

Extensive literature review indicates that several HPLC [28-34] and LC-MS/MS [35-39] methods have been published for quantification of PLB in bulk powder, dosage forms and biological fluids either alone or with other anticancer drugs. All the reported HPLC methods for determination of PLB in pharmaceutical formulations and/or bulk powder was based on utilizing C18 columns on isocratic mode with mobile phases consisted of different ratio of organic modifier and buffers with UV detection at microgram concentration ranges. Furthermore, Nalanda *et al.* [34] have reported an HPLC-UV method for quantification of PLB in human plasma, and the plasma samples were prepared by solid-phase extraction (SFE). The lower limit of detection was 50 ng/mL. Whereas, Al-Shehri *et al.* [25] has described an HPLC-PDA method for quantification of PLB in combination with letrozole (LTZ) in rat plasma after extraction by protein precipitation (PPT) procedure. The calibration curves were linear over the range of 10-600 ng/mL for PLB and LTZ. There were LC-MS/MS methods mention in phase II clinical studies [35, 36] for determination of PLB in mouse tumor tissue and plasma using two-step extraction procedures, liquid-liquid extraction (LLE) and SPE that lead to low accuracy and precision. Recently, the reported LC-MS/MS methods [37-39] have been developed to estimate PLB with other anticancer drugs in biological matrices by utilizing PPT for sample cleanup. Moreover, the reported LC-MS/MS methods [35-39] have limitations, such as it is expensive, not available in most quality control laboratories, the complexity of the sample matrices, and the tedious manual procedures for purification of sample cleanup by SFE, LLE and PPT that lead to low accuracy and precision. For these reasons, the development of new alternative simple square wave - adsorptive stripping voltammetry (SW-AdSV) with adequate sensitivity for quantification of PLB was seriously needed. SW-AdSV has been widely utilized in pharmaceutical and clinical analysis because of their inherent specificity, great sensitivity, high-throughput, and low cost. As well, SW-AdSV are

remarkably rapid, easily performed yielding information that would be difficult to obtain by the chromatographic methods. A search of the scientific literature indicates that no SW-AdSV method has been described concerning the bioanalysis of PLB in human urine and plasma. The present study describes, for the first time, rapid, cost-effective, selective, and reliable SW-AdSV method for the determination of PLB in human urine and plasma with much lower LOD and full validation information.

3.1 Preliminary Observations

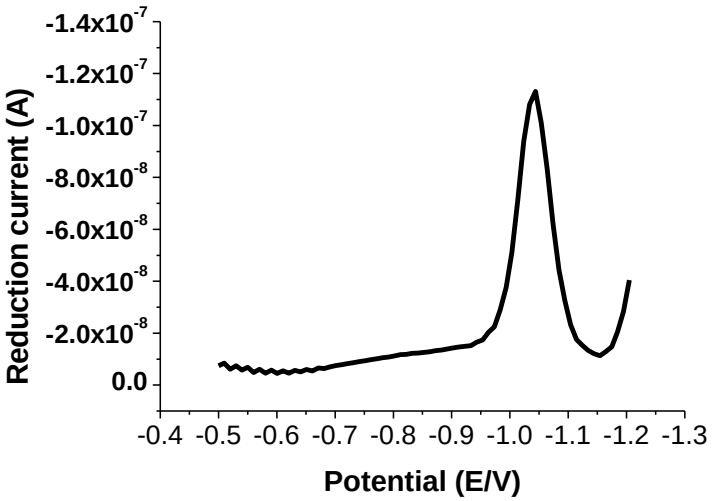


Figure 1. Differential pulse polarographic signal of $2x10^{-5}$ mol L^{-1} of PLB in B-R buffer pH 7.0

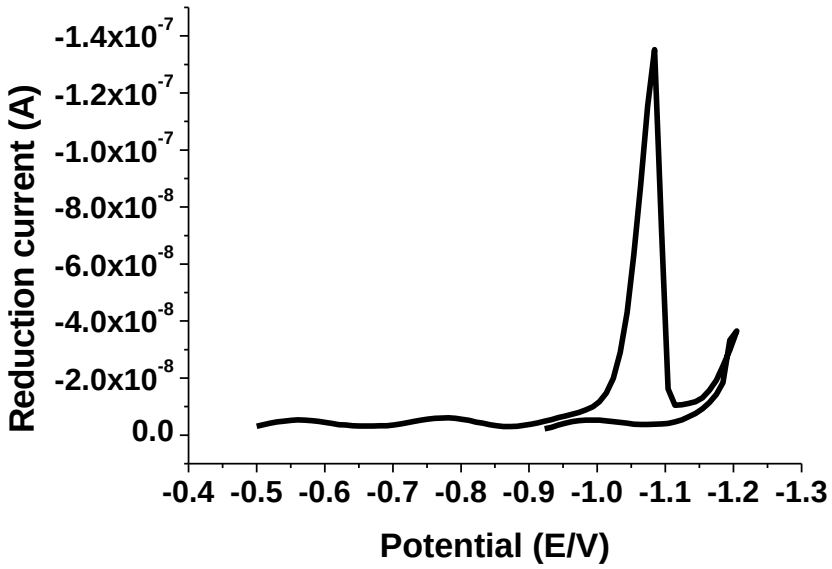


Figure 2. Cyclic voltammogram signal of $5x10^{-7}$ mol L^{-1} of PLB in B-R buffer pH 7.0 at 100 mV^{-1} scan rate

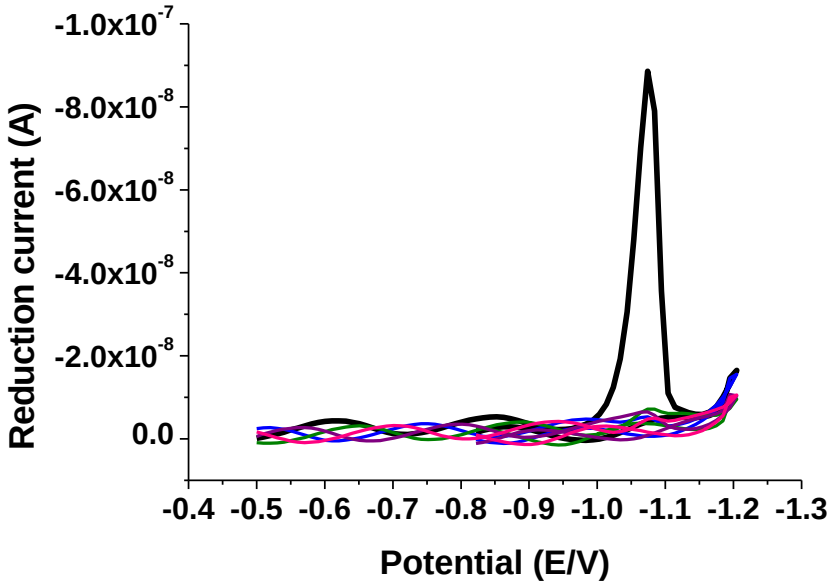


Figure 3. Multi-Cyclic voltammogram signals of $5x10^{-7}$ mol L^{-1} of PLB in B-R buffer pH 7.0 at 100 mV^{-1} scan rate

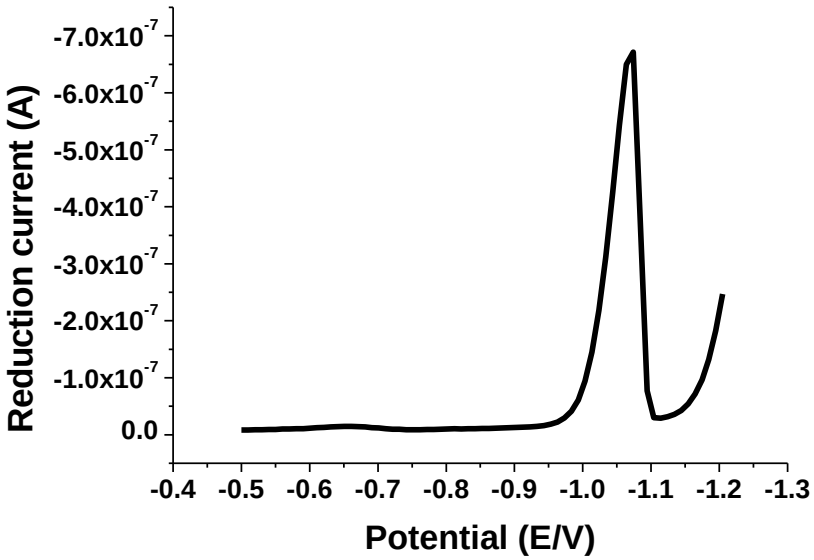


Figure 4. Square wave- Adsorptive stripping voltammogram signal of $5x10^{-7}$ mol L^{-1} of PLB in B-R buffer pH 7.0

The differential pulse polarography behavior was investigated for analysis of $2x10^{-5}$ mol L^{-1} of PLB in B-R buffer pH 7, a broad polarographic wave at -1.05 V potential was observed (see figure 1). The obtained polarographic wave is probably due to the electrochemical reduction of the peripheral carbonyl group (out of rings) to hydroxyl alcohol group. Scheme 1 was explained the proposed

mechanism for the electrochemical reduction of this electroactive group in PLB compound. The electrochemical reaction is an irreversible process, an assumption which was confirmed by cyclic voltammetric approach at 100 mV^{-1} scan rate for $5x10^{-7}$ mol L^{-1} of PLB in B-R buffer pH 7(see figure 2). So no anodic peak was observed on the measured cyclic voltammogram, indicating the irreversibility nature of the cathodic reduction process for PLB. On the other hand, the multi-cyclic voltammetry approach was applied for monitoring the cyclic voltammetric signal of PLB for five sweeps rate (see figure 3), yielded the reduction current was suddenly decreased after the first sweep. The decreased measurements were confirmed the HMDE surface would be saturated by PLB material from the first scan.

In order to obtain a voltammetric peak at high sensitivity, a HMDE was used to study the adsorptive prosperities of PLB drug. So, the SW-AdSV behavior of PLB was investigated in various buffer solutions at different pH values and other conditions, to record a well-developed and defined SW-AdSV peak corresponding to the carbonyl electroactive group at peak potential of –1.05V . A typical square wave- adsorptive stripping voltammogram for $5x\ 10^{-7}$ mol l^{-1} of PLB in B-R buffer pH 7 is shown in figure 4, which illustrates a well observed SW-AdSV signal that indicating a strong and readily adsorption process at the surface of the working electrode (HMDE).

3.2 Analytical parameters

Many analytical parameters were affected on the voltammetric signals such as buffers, pH, scan rate, time and potential of accumulation, frequency, amplitude voltage step, drop size and convection rate. According to high current and sensitivity for every measurement, the square wave adsorptive stripping voltammogram was selected and reported in this work**.**

3.2.1 Influence of buffer and pH

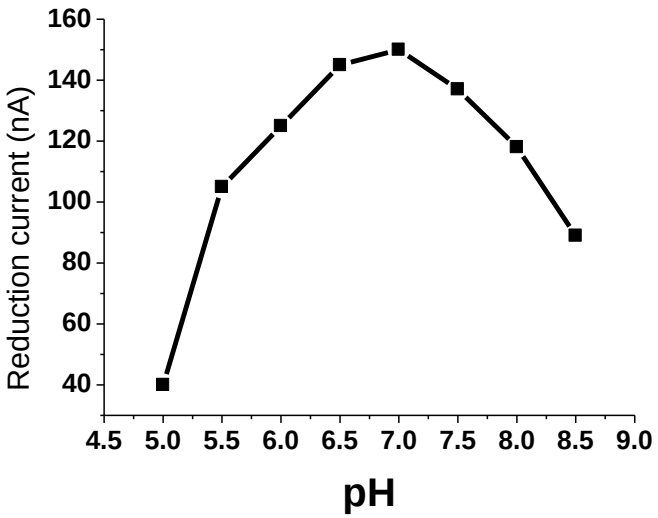


Figure 5. Influence of pH on reduction peak for $5x10^{-7}$ mol L^{-1} of PLB at B-R buffer

Britton-Robinson (B-R), phosphate, acetate and carbonate buffers at different values of pH (3, 7 and 10), were used for the quantificational determination of PLB using SW-AdSV method. B-R buffer of pH 7.0 was given a high voltammetric current and it was selected for the next conditions. B-R buffer was used to analysis PLB over the pH range 5 to 8.5, yielded pH 7.0 was given a high reduction current (see figure 5).

3.2.2 Influence of accumulation time and potential

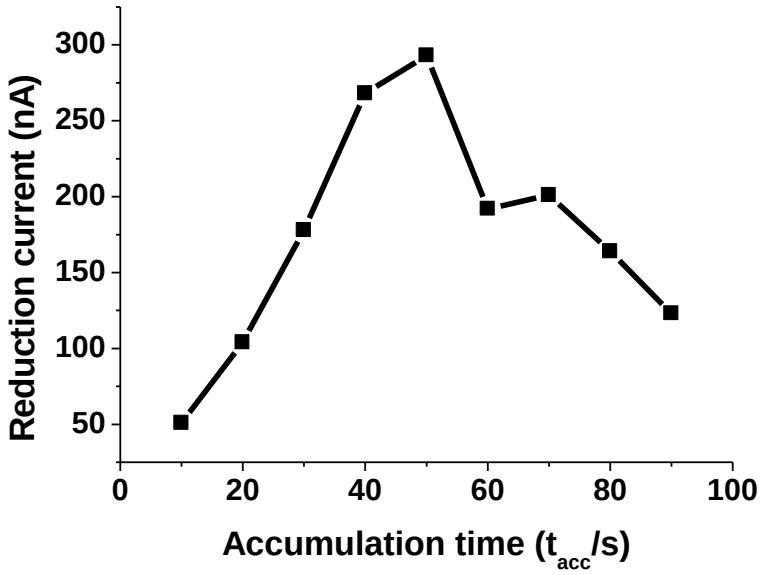


Figure 6. Influence of accumulation time on reduction peak for 5x10^{-7} mol L^{-1} of PLB at B-R pH 7.0

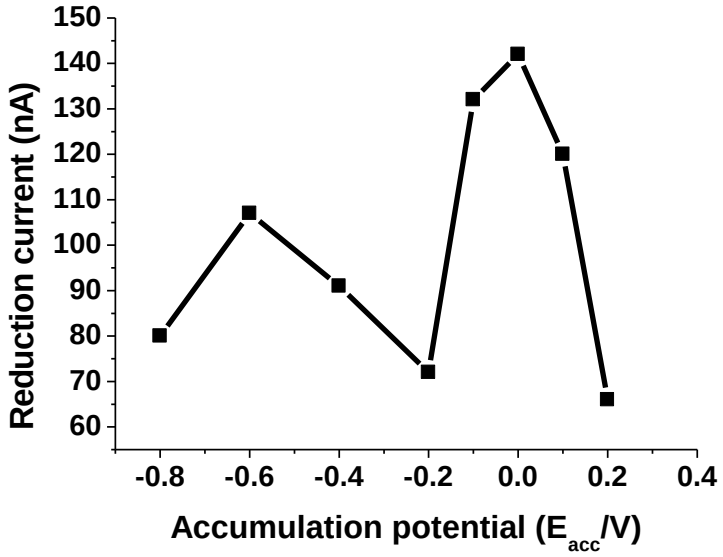


Figure 7. Influence of accumulation potential on reduction peak for 5x10^{-7} mol L^{-1} of PLB at B-R pH 7.0 and 50 s t_{acc}

The accumulation time and potential were known important parameters which affected on the reduction peak for the PLB determination. Accumulation time (t_{acc}) was monitored over the range 10 to 90 s (see figure 6), yielded 50 s recorded a high current, so it was chosen for the future procedures. On the other hand, accumulation potential (E_{acc}) was used over the range -0.8 to +0.4 V (see figure 7) at 50 s t_{acc} for studying the reduction behavior of PLB compound. The cathodic current was reached a high sensitivity with 0.0 V E_{acc}, so this potential was selected as optimum value for the next experiments.

3.2.3 Influence of scan rate and frequency

The scan rate was considered a very important parameter for reporting high sensitivity voltammetric signal. So, scan rate was studied over the range 50 to 400 mVs^{-1} for analysis of PLB solution (see figure 8). It was recorded a linear relation between reduction current and scan rate from 50 to 300 mVs^{-1}, thereafter, the voltammetric current was decreased gradually. So, 300 mVs^{-1} scan rate was given a high reduction current and selected for the subsequent procedures. On the other hand, the frequency of reduction wave was monitored over the range 10 to 60 Hz (see figure 9). The frequency of 30 Hz was given a high reduction current for the PLB determination. A 30 Hz was chosen for the next work.

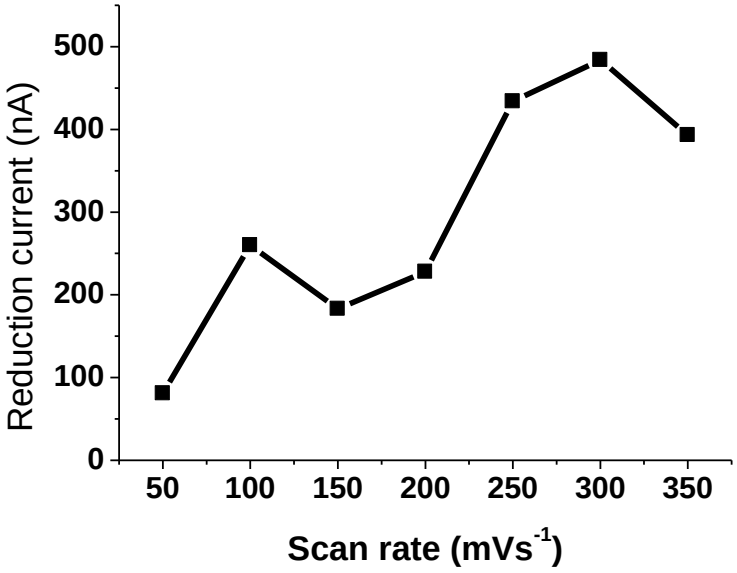


Figure 8. Influence of scan rate on reduction peak for $5x10^{-7}$ mol L^{-1} of PLB at B-R pH 7.0, 50 s t_{acc} and 0.0 V E_{acc}

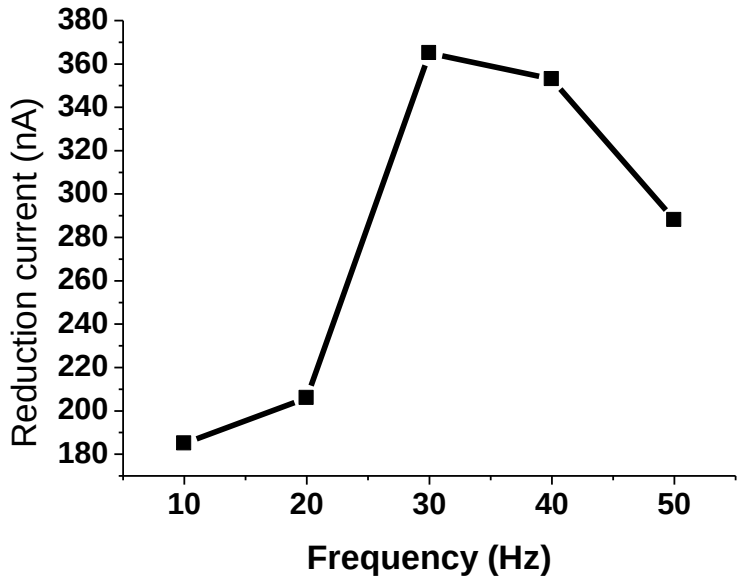


Figure 9. Influence of frequency on reduction peak for $5x10^{-7}$ mol L^{-1} of PLB at B-R pH 7.0, 50 s t_{acc}, 0.0 V E_{acc} and 300 mVs^{-1} scan rate

3.2.4 Influence of pulse amplitude, convection rate and drop size

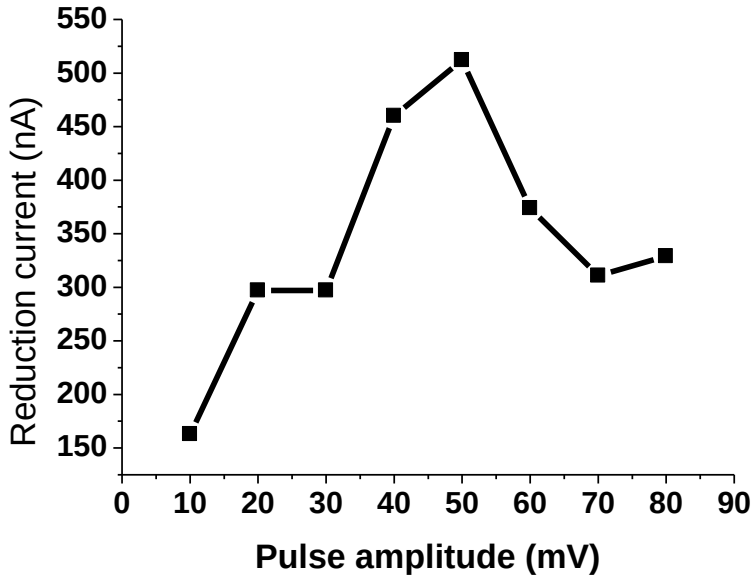


Figure 10. Influence of pulse amplitude on reduction peak for $5x10^{-7}$ mol L^{-1} of PLB at B-R buffer pH 7.0, 50 s t_{acc}, 0.0 V E_{acc}, 300 mVs^{-1} scan rate and 30 Hz

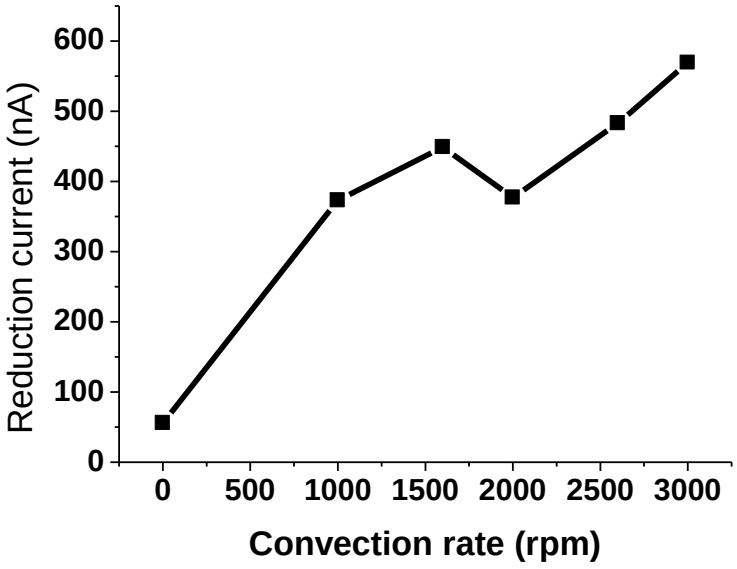


Figure 11. Influence of convection rate on reduction peak for $5x10^{-7}$ mol L^{-1} of PLB at B-R pH 7.0, 50 s t_{acc}, 0.0 V E_{acc}, 300 mVs^{-1} scan rate, 30 Hz and 50 mV amplitude

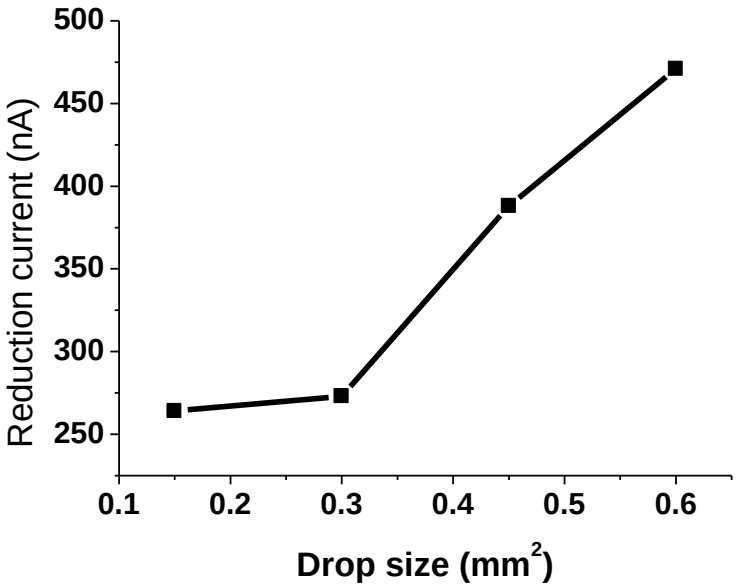


Figure 12. Influence of HMDE drop size on reduction peak for $5x10^{-7}$ mol L^{-1} of PLB at B-R pH 7.0, 50 s t_{acc}, 0.0 V E_{acc}, 300 mVs^{-1} scan rate, 30 Hz , 50 mV amplitude and 3000 rpm

The pulse amplitude was measured over the range 10 to 70 mV (see figure 10), yielded the linear relation was obtained from 10 to 50 mV, next the reduction current was gradually decreased. A 50 mV was given a high current and selected for the next analytical studies. The convection rate was studied over the range 0.0 — 3000 rpm(see figure 11). It was recorded a linear relation between cathodic current and stirring rate. A 3000 rpm was given a high current so, it selected as optimum

parameter. Finally, The size of mercury drop was studied over the range 0.15 to 0.6 mm^2(see figure 12). It was obtained a linear relationship over the studied range, so 0.6 mm2 was given a high current and selected as optimum parameter.

3.5 Method validation

Square wave – adsorptive stripping voltammetric method was validated for linearity, limit of detection, limit of quantification, recovery, repeatability, and stability**.**

3.5.1 Linearity and detection limit

According to the US-FDA guidance for bioanalytical method [40] validation, the linearity of SW-AdSV method was evaluated using calibration curve and the linear regression analysis, which was calculated by least square equation as shown in table 1.

Table 1. Analytical information for calibration curve, LOD and LOQ for PLB determination under optimum parameters

Parameters	**PLB**
Range (mol mL^{-1}) [a]	$1x10^{-7}$- $1x10^{-6}$
LLOQ (ppb) [b]	0.131
LLOD (ppb) [c]	0.039
Correlation coefficient®^{2}	0.992
Equation	Y = $9.6x10^8$ C+153.73
Y	I (nA)
n (number of measurements)	6
Slope (b)	9.6 $x10^8$
Intercept (a)	153.73

[a] n = 6
[b] LLOD (S/N = 3)
[c] LLOQ (S/N = 10)

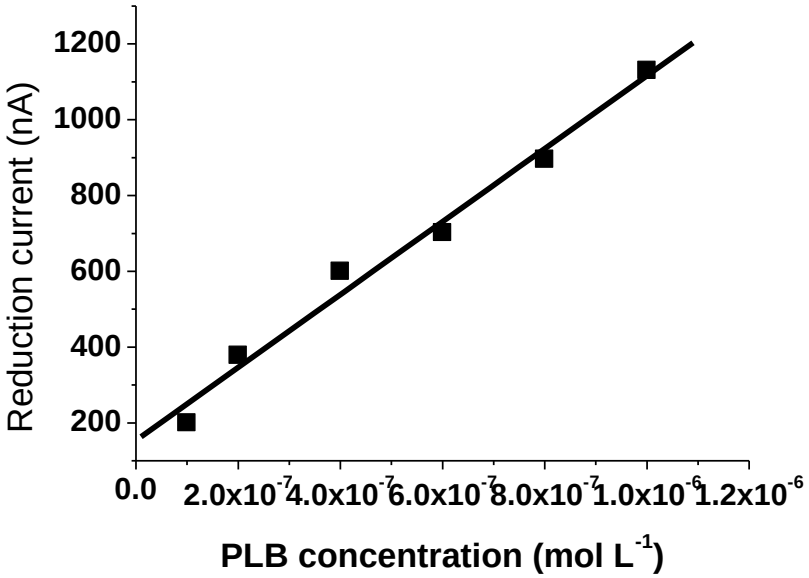


Figure 13. SW-AdSV Calibration curve over the range $1x10^{-7}$ — $1x10^{-6}$ mol L^{-1} of PLB at B-R buffer pH 7.0 , 50 s t_{acc}, 0.0 V E_{acc}, 300 mVs^{-1} scan rate, 30 Hz, 50 mV amplitude, 3000 rpm and 0.60 mm^2 drop size

The calibration curve was studied over the concentrations range $1x10^{-7}$ — $1x10^{-6}$ mol L^{-1} of PLB. It was given an excellent linear relation between the cathodic current and PLB concentrations as presented in figure 13.

On the other hand, the low values of LOQ and LOD obtained for PLB ensure the applicability of the developed SW-AdSV method for the determination of trace concentrations PLB (table 1). The LOD and LOQ results were confirmed a high sensitivity of the SW-AdSV technique for the determination of PLB at the biological matrixes. There are no published articles reported LOD or LOQ lessen than the used SW-AdSV for determination of PLB, as obtained in the currently research.

3.5.3 Recovery, repeatability and stability

Table 2. Repeatability and recovery results for PLB determination under optimum parameters

PLB Conc.	Current (nA)	Current Average ± S.D.	RSD (%)	Recovery (%) ($2x10^{-7}$ mol L^{-1})
	537			106
	538			103
	542			105

$5x10^{-7}$ mol L^{-1}	539	539.2±1.5	0.0282	106	
	540			105	
	538			Mean	105
	541			SDV	±1.1
	539.5				
	540				
	537.5				

The monitored stability for the analysis of PLB was very well for 120 min.

The analytical performance of the SW-AdSV method was investigated through study of some parameters such as recovery, repeatability and stability. The recovery was studied for $2x10^{-7}$ mol L^{-1} of Palbociclib, while the repeatability and stability were studied for $5x10^{-7}$ mol L^{-1} of PLB. The recovery was reported 105% ± 1.1, where the repeatability was recorded 0.0282% (RSD%) for ten reduction measurements as listed in table 2.

3.6 Application of method

The reliability of the developed SW-AdSV method was assessed by recovery of PLB in biological fluids (human urine and plasma). The added PLB content in these samples, was quantized via the optimized SW-AdSV procedure for $2x10^{-7}$ mol L^{-1} of PLB by adding 2 ml of human sample to 10 ml B-R buffer pH 7.0, which injected in the electrochemical cell and deoxygenated for 5 minutes. In order to minimize matrix effects, the voltammetric quantities were done by the standard addition method. Six aliquots of this sample were analyzed by the developed SW-AdSV method.

Table 3. The developed SW-AdSV results for determination of PLB in biological matrixes

Recovered PLB Conc. (2×10^{-7} mol L^{-1})	Recovery %	
	Urine	Plasma
	90 93 95 92 95	85 88 87 85 87
Mean	93	86.4
Standard Deviation	±1.90	±1.20

Table 3 summarizes the analytical results that obtained by the developed SW-AdSV method for PLB determination. These results reported by recovery of $2x10^{-7}$ mol L^{-1} PLB in human urine and plasma. The recoveries recorded 93% and 86.4% with standard deviations of ± 1.90% and 1.20%, respectively for urine and plasma. The results obtained were favorably compared with a reference method [34] based on determination of PLB in human plasma after SFE using HPLC-UV technique. Statistical analysis [41] of the results obtained by both methods using the Student *t*-test and Variance Ratio *F*-test, shows no significant difference between the performance of the two methods (Table 4).

Table 4. Statistical analysis of the results obtained by the proposed and reference method for pure sample of palbociclib

Method	Reference method [34]	SW-AdSV method
Number of experiments	3	6
Mean found (%)	74.51	86.40
Variance	2.25	1.44
Students *t*-value	1.17 (2.365)	1.38 (2.365)
Variance ratio *F*-test	3.24 (4.74)	2.75 (4.74)

NB: Figures in parentheses are the tabulated *t*- and *F*-values, respectively, at $P = 0.05$ [41].

4. CONCLUSION

In this research, a WS-AdSV method has been developed for the determination of PLB in urine and plasma samples for the first time. The limit of detection (LOD) of the assay was 8.8×10^{-11} mol L^{-1} (0.039 ppb) and the linear range of $1x10^{-7}$ - $1x10^{-6}$ mol L^{-1} for PLB. The advantage of the developed WS-AdSV method over the reported methods are more selective, faster, more cost-effective, involve no sample preparation and specificity. The used HMDE working electrode gave an added advantage for the determination of the PLB. The possibility of monitoring of the PLB in human urine and plasma makes the proposed WS-AdSV method useful for pharmacokinetic and pharmacodynamic purposes.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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