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Ciprofloxacin analysis on SQF- 505 by striping square wave voltammetry

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Abstract
In this study a stripping square wave voltammetric method was developed and validated for the direct determination of ciprofloxacin (CIP) in pharmaceutical formulation using dropping mercury dropping electrode (DMDE) surface. Optimal conditions for ciprofloxacin determination by stripping square wave voltammetry as folows CH_3COONH_4, 0.10M; pH = 7.0; V_{pulse} = 30mV; V_{step} = 10 mV; T_{drop} = 5000ms; $V_{electrolize}$ = -1200 mV; $T_{electrolise}$ = 9s; stiring helps increase current intensity dramatically. Validated stripping voltammetric technique can be recommended for use in aquaculture quality control and pharmacokinetics studies.

Keywords: ciprofloxacin, stripping square wave voltammetry, pharmaceutical formulation dropping mercury dropping electrode, aquaculture, pharmacokinetics.

1. Introduction
Antibiotics are produced by microorganisms secrete or are products made from the original product (through synthetic or semi synthetic) may inhibit the process of life of some species of disease for people, pets, and in therapeutic doses, the less impact the health of people/pet [1].

Quinolones are synthetic antibiotics, has antibacterial properties, is used in cases of severe infection, especially aerobic gram (-). This group includes: 1) I: generation quinolones offer type contains no F (except flumequine), poor absorption and metabolism in the liver into the product doesn't work. The narrow antimicrobial spectrum, only works on some gut bacteria and bladder: E.coli, Proteus, Enterobacter, salmonella, gonorrhea. Being fast resistance, so less current used. 2) this type II: generation quinolones contain fluorine, invented in 1985, commonly known as fluoroquinolones. Antibacterial spectrum extends from gram (+) to the gram (+). Fluoroquinolones have little side effects, no resistance as quickly as the I: generation quinolones [3].

Ciprofloxacin: Molecular formula: C17H18FN3O3; Molecular weight: 331.4; Chemical name: 1-Cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(piperazin-1-yl)-3-quinoline carboxylic acid. Ciprofloxacin can exist under the two forms of the drug is ciprofloxacin and ciprofloxacin-hydrochloride. Both are calculated to be white or pale. Form of ciprofloxacin methanol ethanol, soluble, insoluble in water. Form of ciprofloxacin hydrochloride-soluble in water. The antibacterial properties: Ciprofloxacin is an antibiotic sold synthetic generation quinolones II, has very wide antimicrobial spectrum, including the bacteria gram (-) and gram (+), especially gram (-). a) For persons: Ciprofloxacin is specified mostly in cases of severe infections, such as urinary tract inflammation, prostatitis, inflammation of the intestines of heavy infections, osteomyelitis, sepsis. Brain tissue disease is caused by prophylaxis and bacterial infection in humans of immune deficiency. Side effects: gastrointestinal disturbances such as nausea, vomiting, diarrhea, abdominal pain, indigestion, causing headaches, insomnia and restlessness. b) For aquatic products breeding: breeding in aquiculture ciprofloxacin, is used to prevent and treat the disease carrying black, red body, close follower, swollen brought in shrimp. To prevent and treat the disease fade, losing viscosity, intestinal infections in fish [6]. In breeding at present, antibiotics are used very extensively, difficult to control between advocates and medical treatment for animals as well as for farmed seafood. Special to have high economic efficiency, one can mix antibiotics into animal feed, especially for poor quality food and poor sanitation. Do not tightly controlled conditions for antibiotic residues immediately after each use should lead to pollution of antibiotics, including ciprofloxacin pollution in aquatic products breeding currently.

Classical voltammetry (polarography): The polarographic method invented by Heyrovsky (1890-1967), the Sec, in 1922, received the Nobel Prize in 1959, now called classic polarographic method. This method was applied first in analytical chemistry to define a lot of organic and inorganic substances, which in many cases do not need to segregate them from the mixed ingredients. The polarographic method classic based on the survey, the dependence of the intensity of the line on a linear change in the sound of the pressure on the two electrodes of the electrolytic tank in the physical condition to polar surface analysis mercury drops only by diffusion due to the concentration gradient. Here, the pole drop mercury electrode is being polarized, there is a very small surface area, few mm2; Master electrode electrode is being polarized, often used as electrode Ag/AgCl/KCl, Calomen electrode, or is the bottom of mercury. The latest on ultra drops (also known as cưc work) is a constant and variable E1/2 slowly over time (usually from 0.0 to-2.0 V). The survey results are a universal pole map, often called a polarographic wave which essentially is a street line-up. Diffusion lines limited intensity (i.e. the height of the wave polarography) proportional to the concentration of the substance analysis recommended for quantitative purposes. The value corresponding to ½ the height of the wave polarography called the selling wave E1/2, specific to each substance analysis in an ionic liquid the selected background, used for the purpose of qualitative [2, 4].

Normal pulse voltammetry: In the common pulse polarography (NPP) voltage pulse shape how people had increased the magnitude of the perpendicular to the electrode drops and always returns the value that was originally. A drop put a pulse, and the pulse increases linearly as a voltage is placed on. A drop put a pulse at the end of cycle drops in a very short time (40 ms ÷ 100 ms) [2].

Differential pulse voltammetry: In differential pulse polarography, placed perpendicular pulses super imposed DC voltage varies very slowly over time. A drop put a pulse and pulse is placed just before the drop of Hg pulse amplitude fall with fixed (about 2 ÷ 100mV depending on user) with impulse voltage time about 40 ÷ 100ms [2].

Square wave voltammetry: Square-wave polarography is the speed pulse polarographic analysis for quick and high sensitivity. According to this method, mercury droplet electrode is polarized by a power variation over time shaped ladder and added a pulse power of small amplitude are perpendicular form and kept constant throughout the measurement. Pulse pressure cycles are identical (around 5 ms), the step of the ladder is often worth voltage 10mV Esw pulse amplitude, typically 10-50mV, the conditions corresponding to the pulse frequency is 200 Hz scanning rate, so 200ms/V. for reversible reactions, the pulse amplitude is large enough to oxidation products formed in the positive impulses will occur during the process reverse pulse. Such correspondence shall record the faraday currents twice: first in the last positive impulse is and the second is the end opposite impulses, at this time an area of constant Hg droplets to the capacitor is almost intent. The ΔI is proportional to the concentration, and the top of the corresponding to the haft wave [2, 5].

Stripping square wave voltammetry: Stripping polarography is the polarographic method combined with stripping techniques to improve sensitivity. Also known as: ampe-voltage method of dissolution, the method of voltage-ampe polarographic method peeled, peeled. Originally to improve the sensitivity in the analysis of metals (Cu, Pb, Zn, Sb, Cd, Sn, Bi, etc.) and non-metals (As, halogen etc), then expanded into organic analysis. Stripping technique (for analysis of metals) can be divided into three stages. One is the phase of electrolysis are stirring, conducted at a fixed in a few minutes to tens of minutes, to earn contributions (accumulation) of metals in the Persian form precipitate onto the surface electrodes. Two stirring stops electrolysis phase, but still retains that electrolysis, lasts about 15 ÷ 60s to precipitate the evenly distributed on the electrodes. Three is the dissolved phase precipitate, i.e. "bark peeling precipitate" (stripping); the shelling is done by recording the polarographic measurement map vertically scanned it appropriately. The main area of contribution and dissolve again as above helped for the current peak in polarography map increased very substantially. LOD can estimate the level $10^{-10} \div 10^{-11}$M [2, 5].

The polarographic method of organic matter: To analyze is an organic, the organic matter in it needs to have the functional group can join the electrochemical reaction on the working electrode. With the substance for electrochemical reactions, people conducting indirect analysis: use a chemical reaction to convert the substance for analysis required an electrochemical activity for substances to conduct analysis. In the general case of the reactions on the micro-electrodes with the participation of organic compounds occur more slowly or more complicated than the reaction of inorganic cations. Therefore, the interpretation of the polarographic data theory in this case is much more difficult and perhaps even impossible. However, people still using polarographic measurements to study the structural, qualitative identification of organic compounds and quantitative analysis of the mixture of them. When analysis of carbohydrate, the pole drop of mercury is still kind of pole work is preferred choice because it has a good point is the bềmặt electrode always renewed after every burn. That ensures repeatable. Pole of mercury primarily as a component in the reduction of the substances should facilitate the analysis of the functional group can participate in the electrochemical reaction. Some of the organic functional group for electrochemical reaction as: carbonyl groups (the aldehyde, the cetone, quinones), carboxylic acids some as phumaric acid, maleic acid, phtalic, much of the peroxide, epoxy, nitroso, nitro groups, oxim, azo, most organic compounds containing halogens, hydroquinone [4].

2. Material & Method

2.1 Equipment: Multifunction Analyzer ANALYZER SQF-505 in Tropical Vietnam-Russia Center.

2.2 Chemical: Ciprofloxacin hydrochloride standard from the pharmaceutical Institute-Ministry of health. Solvent: ethanol, Methanol, Acetone (China); Acetonitrile (Merck). Others: CH3COONH4, CH3COONa, NaOH, NH4OH condensed, CH3COOH condensed, H3PO4, H3BO3, borax, etc (China).

2.3 Preparation: The original standard solution are mixed from standard ciprofloxacin hydrochloride 120 mg (purity was 94.18%) in 100 ml of distilled twice (the concentration of cipro is 1018.3 mg/L) and stored in the refrigerator. The smaller concentrations of cipro were mixed from the solution

in the original standard, distilled twice. The other solution are mixed from the solid or liquid concentrate with distilled twice to the concentration required.

3. Result & Discussion

3.1 Effect of scanning to ciprofloxacin appearance:

Choose buffer solution for initial experiment CH3COONH4 (pH=7), and then measure to identify potential E_{peak} = -1326mV.

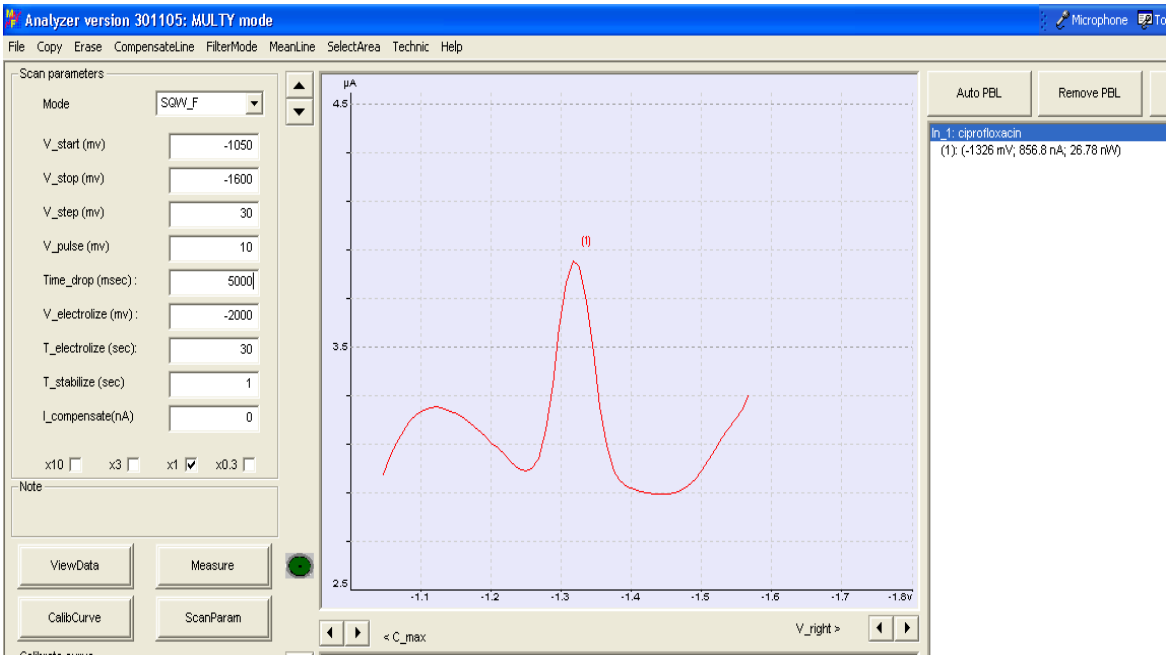

Fig 1: Peak of ciprofloxacin.

3.2 Effect of different buffer solutions to analysis

3.2.1 Effect of CH_3COONH_4

Various technical parameters of machine operated: V_{start} = -1050mV, V_{stop} = -1600mv, V_{step} = 10mV, V_{pulse} = 30mV, T_{drop} = 5000ms. The resuts are as follows:

Table 1: Ciprofloxacin in CH_3COONH_4 at various pH values

pH	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
6.0	-1316	834.9	835.9	831.7	834.2
7.0	-1326	856.8	859.5	860.7	859.0
8.0	-1346	822.3	822.4	828.8	824.5
9.0	-1384	456.2	463.8	450.7	456.9

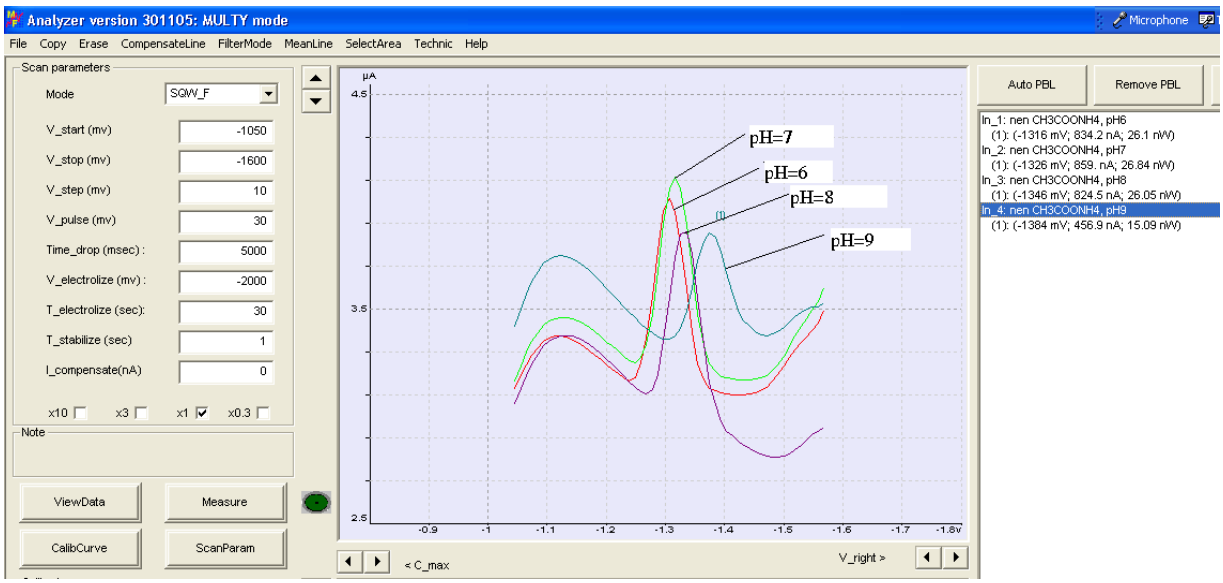

Fig 2: Peak of ciprofloxacin in CH_3COONH_4 at various pH values.

3.2.2 Effect of NH_4^+/NH_3

Table 2: Ciprofloxacin in NH_4^+/NH_3 at various pH values

pH	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
8.0	-1336	691.7	694.3	696.5	694.2
9.0	-1404	442.2	445.8	446.5	444.8
10.0	No peak signal				

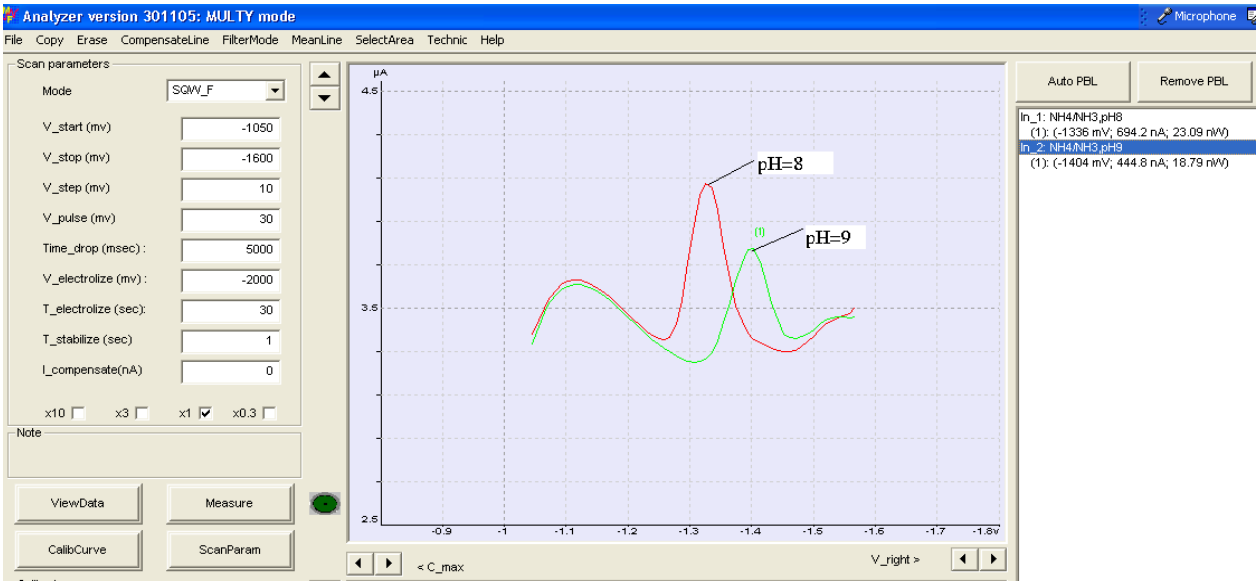

Fig 3: Peak of ciprofloxacin in NH_4^+/NH_3 at various pH values

3.2.3 Effect of CH_3COONa/CH_3COOH

Table 3: Ciprofloxacin in CH_3COONa/CH_3COOH at various pH values

pH	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
4.0	No peak signal				
5.0	No peak signal				
6.0	-1326	816.8	816.0	818.8	817.2

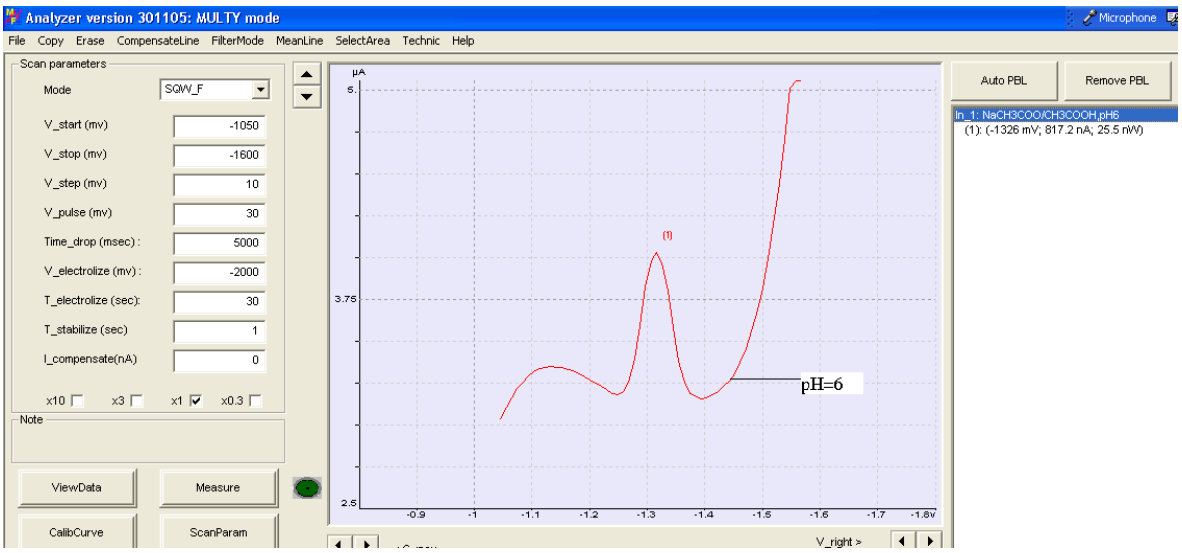

Fig 4: Peak of ciprofloxacin in CH_3COONa/CH_3COOH at various pH values

3.2.4 Effect of $H_2PO_4^-/HPO_4^{2-}$

Table 4: Ciprofloxacin in $H_2PO_4^-/HPO_4^{2-}$ at various pH values

pH	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
5.0		No peak signal			
6.0	-1316	839.7	831.1	834.4	835.1
7.0	-1326	847.5	846.1	847.2	846.9
8.0	-1336	806.1	803.7	808.3	806.0

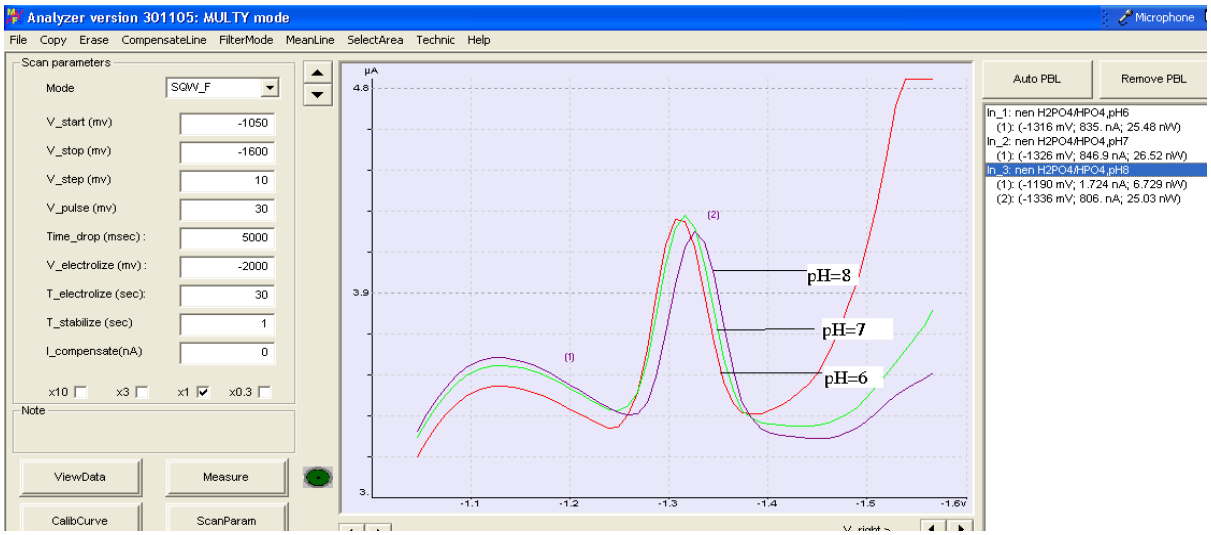

Fig 5: Peak of ciprofloxacin in $H_2PO_4^-/HPO_4^{2-}$ at various pH values

3.2.5 Effect of Britton - Robinson

Table 5: Ciprofloxacin in Britton-Robinson at various pH values.

pH	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
6.0	-1336	815.3	812.0	812.9	813.4
7.0	-1346	832.3	837.8	837.3	835.8
8.0	-1354	827.3	827.1	825.3	826.6
9.0	-1394	380.3	385.8	386.5	384.2

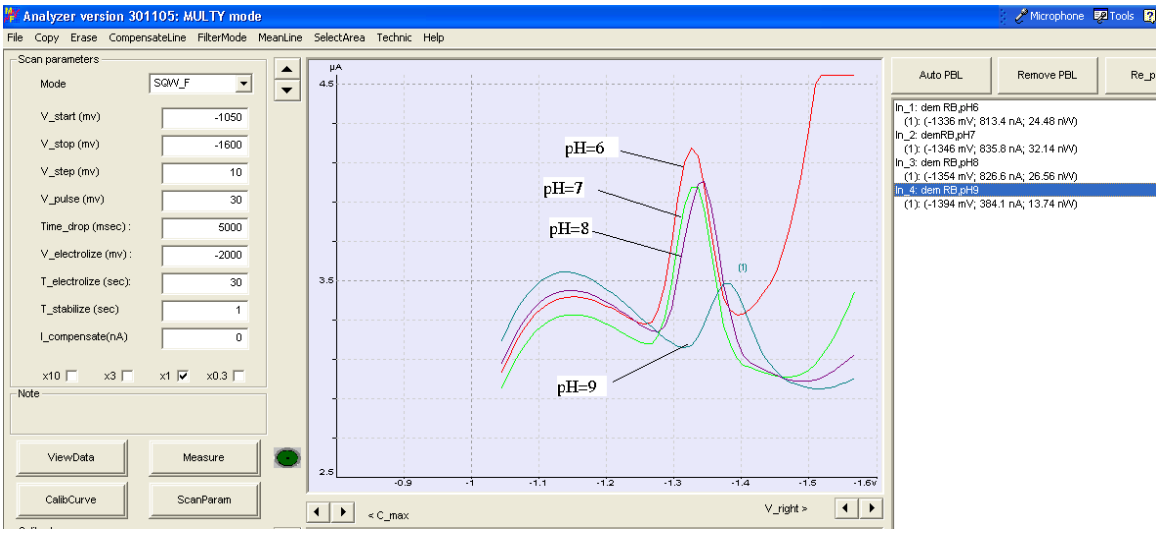

Fig 6: Peak of ciprofloxacin in Britton-Robinson at various pH values.

Buffer CH_3COONH_4 at pH 7.0 shows the highest intensity so we choose this buffer for further researchs

3.2.6. Effect of CH_3COONH_4 concentration

Table 6: Ciprofloxacin in CH_3COONH_4 at various concentration

[CH_3COONH_4] (M)	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
0.01	No peak signal				
0.05	-1346	833.2	825.6	839.4	832.7
0.10	-1326	859.0	851.3	846.4	852.2
0.15	-1316	836.1	838.2	839.3	837.9
0.20	-1316	823.5	828.9	828.2	826.9
0.25	-1316	822.7	818.3	819.2	820.1

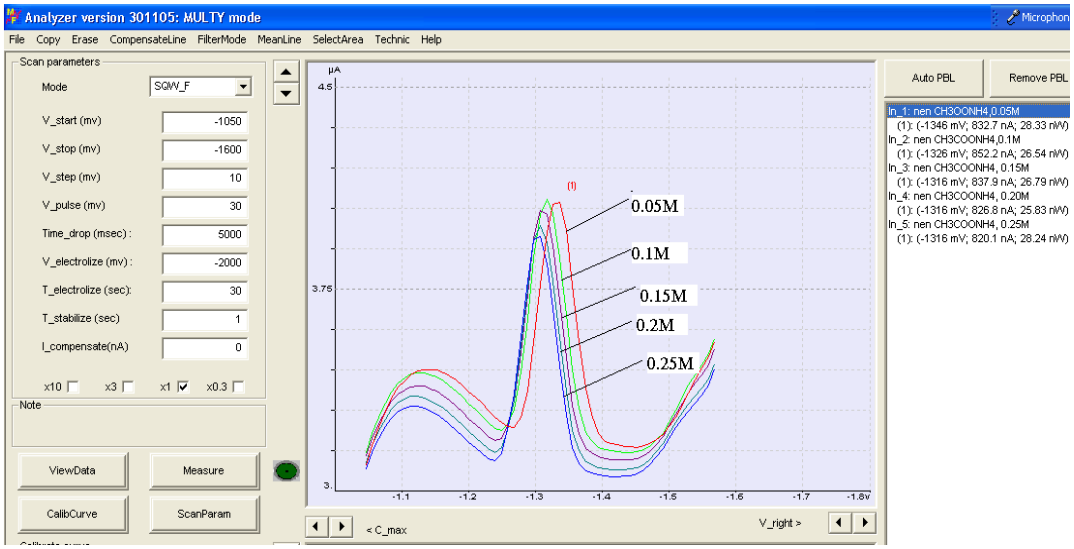

Fig 7: Peak of ciprofloxacin in CH_3COONH_4 at various concentrations.

3.3 Effect of different technical machine operation

3.3.1 Effect of V_{pulse}

Table 7: Ciprofloxacin at various Vpulse values

V_{pulse} (mV)	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
10	-1346	658.5	663.7	659.1	660.4
20	-1336	812.2	814.5	812.2	813.0
30	-1326	856.8	859.1	853.7	856.5
40	-1316	816.5	813.5	811.0	813.7

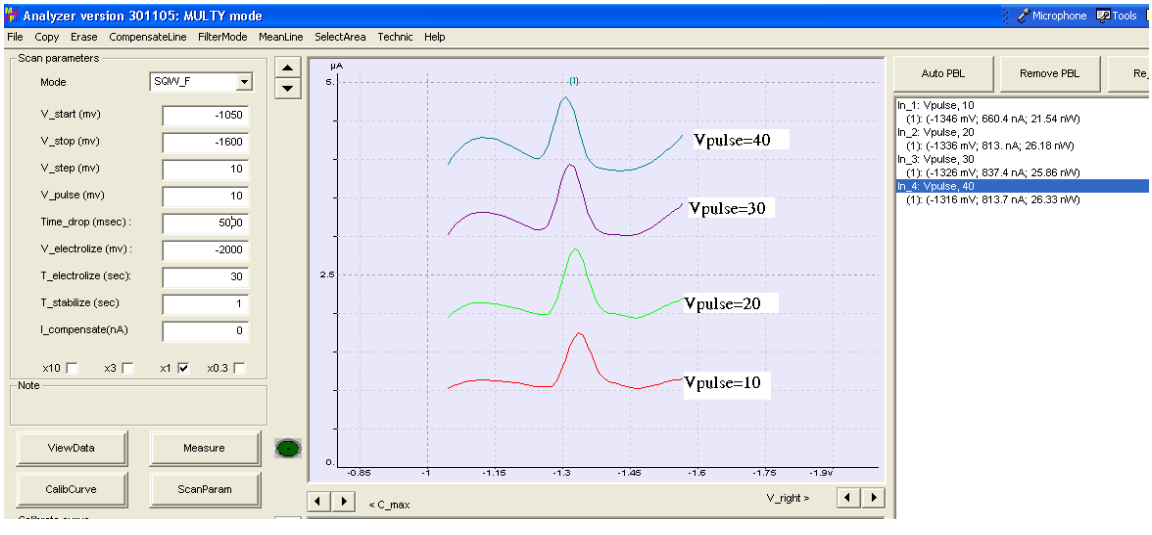

Fig 8: Peak of ciprofloxacin by various V_{pulse} values

3.3.2 Effect of V_{step}.

Table 8: Ciprofloxacin at various V_{step} values

V_{step} (mV)	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
2	-1294	146.1	145.8	145.8	145.9
4	-1308	318.1	315.0	317.2	316.8
6	-1318	478.4	480.1	484.4	481.0
8	-1324	644.0	637.7	641.5	641.1
10	-1326	853.7	859.1	857.8	856.9

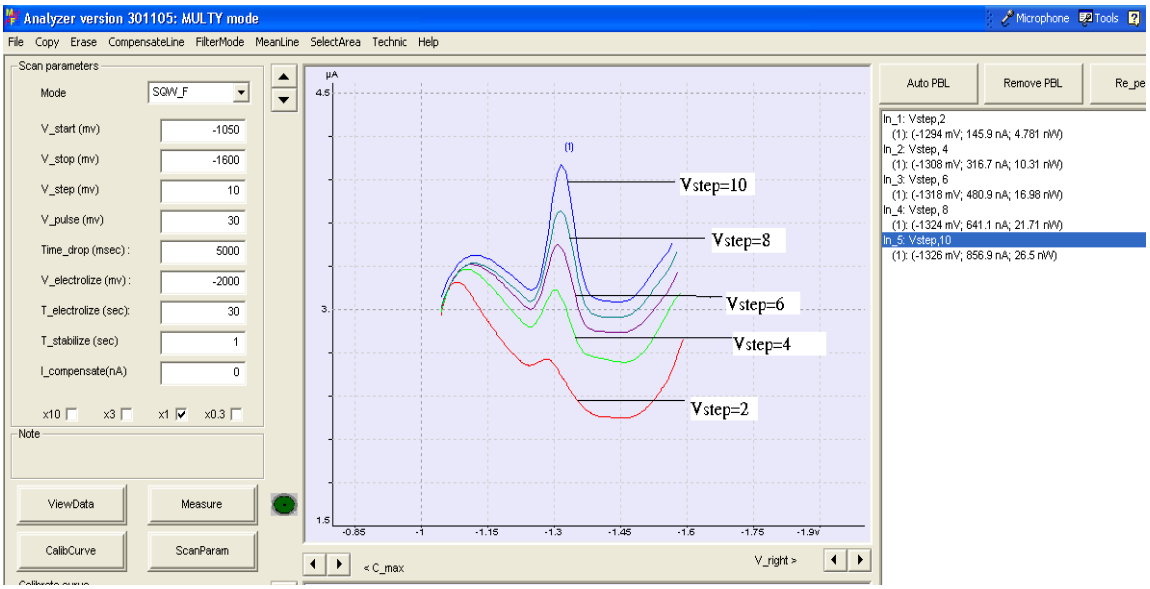

Fig 9: Peak of ciprofloxacin by various V_{step} values

3.3.3 Effect of T_{drop}

Table 9: Ciprofloxacin at various T_{drop} values

T_{drop} (ms)	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
1000	-1326	301.0	302.7	300.6	301.4
2000	-1326	470.2	463.8	469.1	467.7
3000	-1326	621.9	618.1	623.2	621.1
4000	-1326	727.4	728.1	731.6	729.1
5000	-1326	856.8	853.5	859.5	856.6

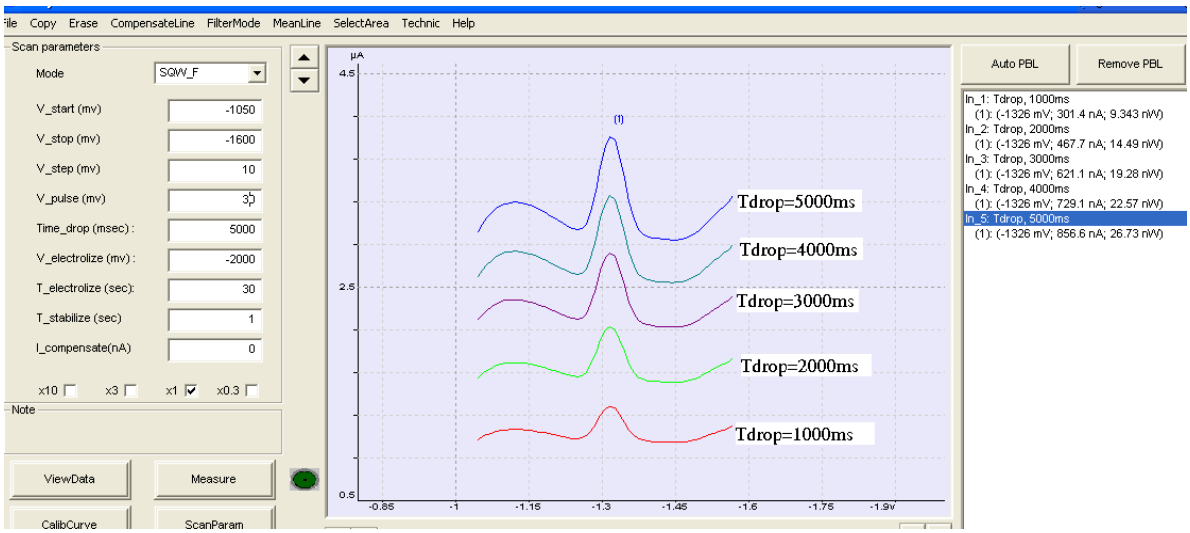

Fig 10: Peak of ciprofloxacin by various T_{drop} values

Remarks: optimal conditions for ciprofloxacin determination by square wave voltammetry as folows CH_3COONH_4, 0.10M; pH = 7.0; V_{pulse} = 30mV; V_{step} = 10 mV; T_{drop} = 5000 ms.

3.4 The accuracy of analyzing data

Table 10: Accuracy of analyzing results

[cipro] (mg/L)	E_{peak} (mV)	Intensity of current, I (nA)						
		I_1	I_2	I_3	I_4	I_5	Tb	s_i^2
0.814	1354	54.05	52.56	53.97	50.51	52.3	51.88	s_1^2=2.102
2.036	1346	196.5	194.9	194.3	198.5	196.4	196.1	s_2^2=2.672
4.072	1336	430.0	429.3	429.7	430.6	430.6	430.0	s_3^2=0.323

Demonstration by Bartlet:

$$s_{th}^2 = \frac{(5-1)(2.102+2.672+0.302)}{3(5-1)} = 1.692;\ f_{th}\log s_{th}^2 = 2.740;\ \sum f_i \log s_i^2 = 1.035$$

$$B = 2.303\ (2.740 - 1.035) = 3.927;\ C = 1+\frac{1}{3k-3}\left(\sum_{i=1}^{n}\frac{1}{f_i}-\frac{1}{f_{th}}\right) = 1.194;$$

$$\chi^2_{TN} = B:C = 3.927:1.194 = \underline{3.289}\ ;\ \chi^2_{LT} = \chi^2_{0.95,2} = \underline{5.991}$$

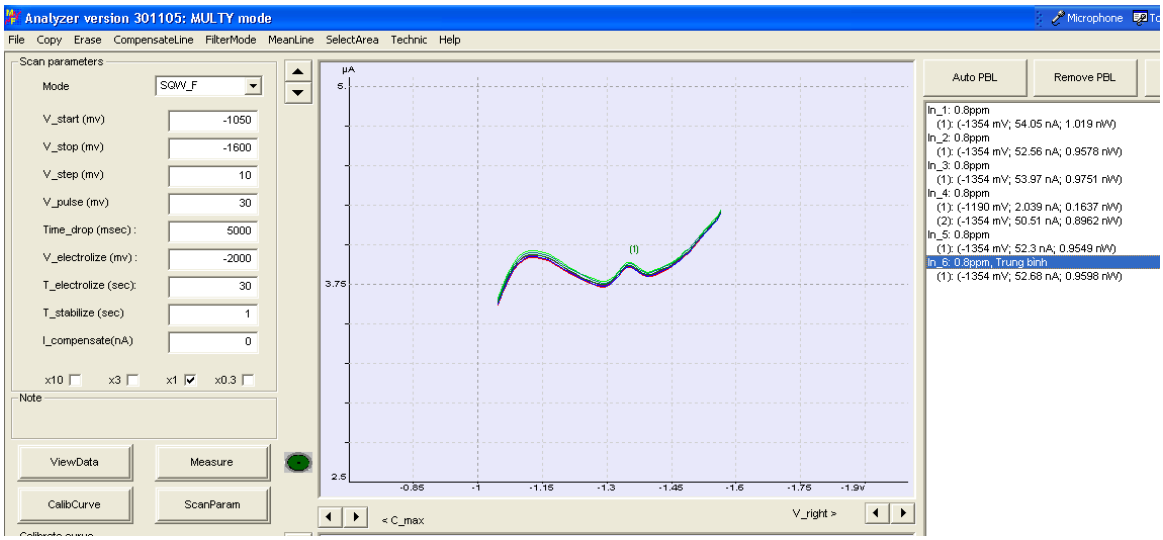

Fig 11: Peak of ciprofloxacin at 0.814mg/L

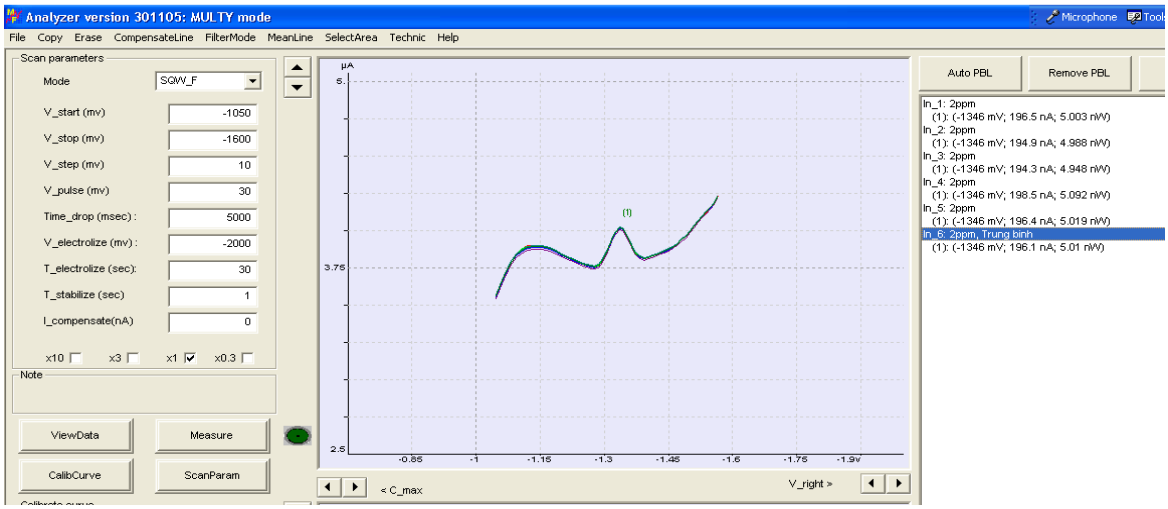

Fig 12: Peak of ciprofloxacin at 2.036mg/L

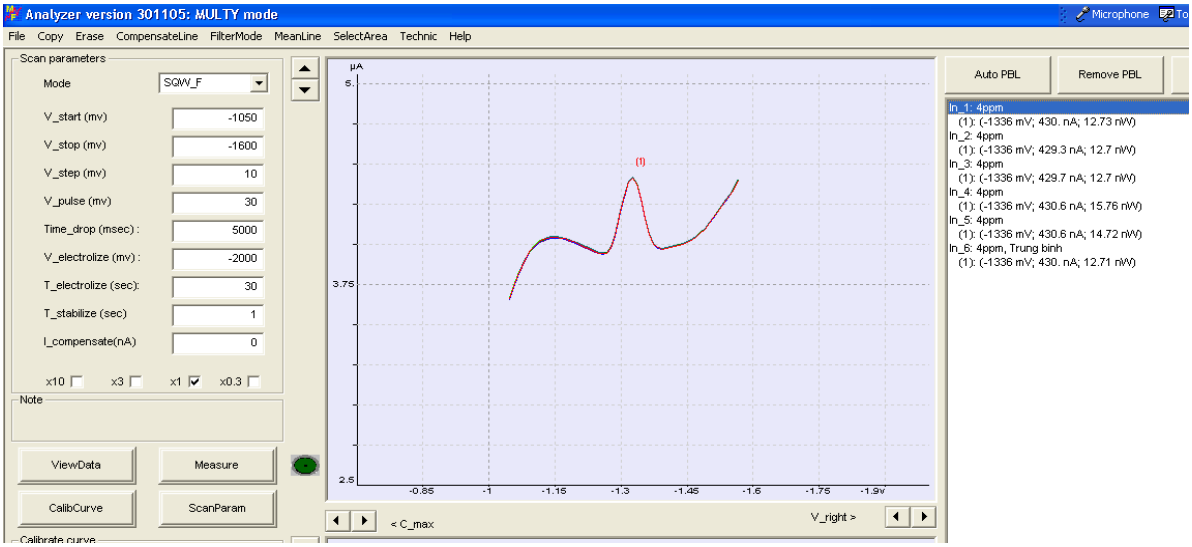

Fig 13: Peak of ciprofloxacin at 4.072mg/L

3.5 Linear range of ciprofloxacin analysis, limit of detection LOD, limit of quantification LOQ

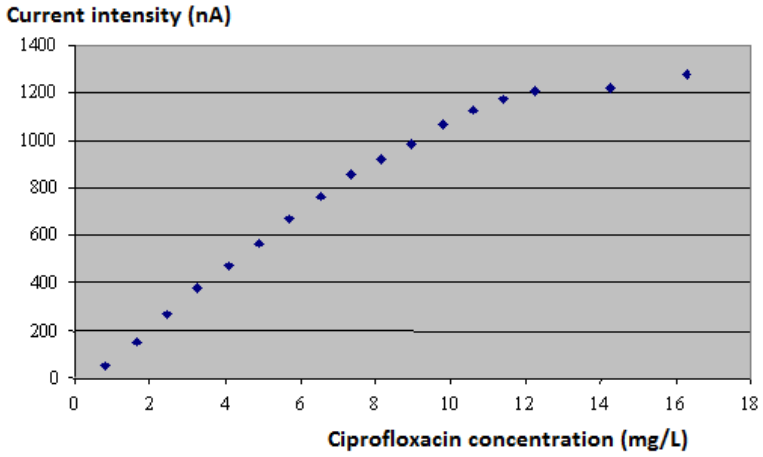

Fig 14: Linear range of ciprofloxacin analysis

Table 11: Calibration curve of ciprofloxacin by mode SQW-F

Ciprofloxacin concentration (mg/L)	Intensity of current, I (nA)				
	Measure # 1	Measure #2	Measure # 3	Mean	s_i^2
1.222	105.0	104.1	103.8	104.3	0.390
1.629	150.1	150.1	150.3	150.17	0.013
2.036	196.5	200.0	198.5	198.33	3.083
2.444	242.3	248.4	244.0	244.9	9.910
2.851	285.9	285.9	286.3	286.03	0.053
3.258	333.0	337.3	331.2	333.83	9.823
3.666	380.7	381.1	383.1	381.6	1.653

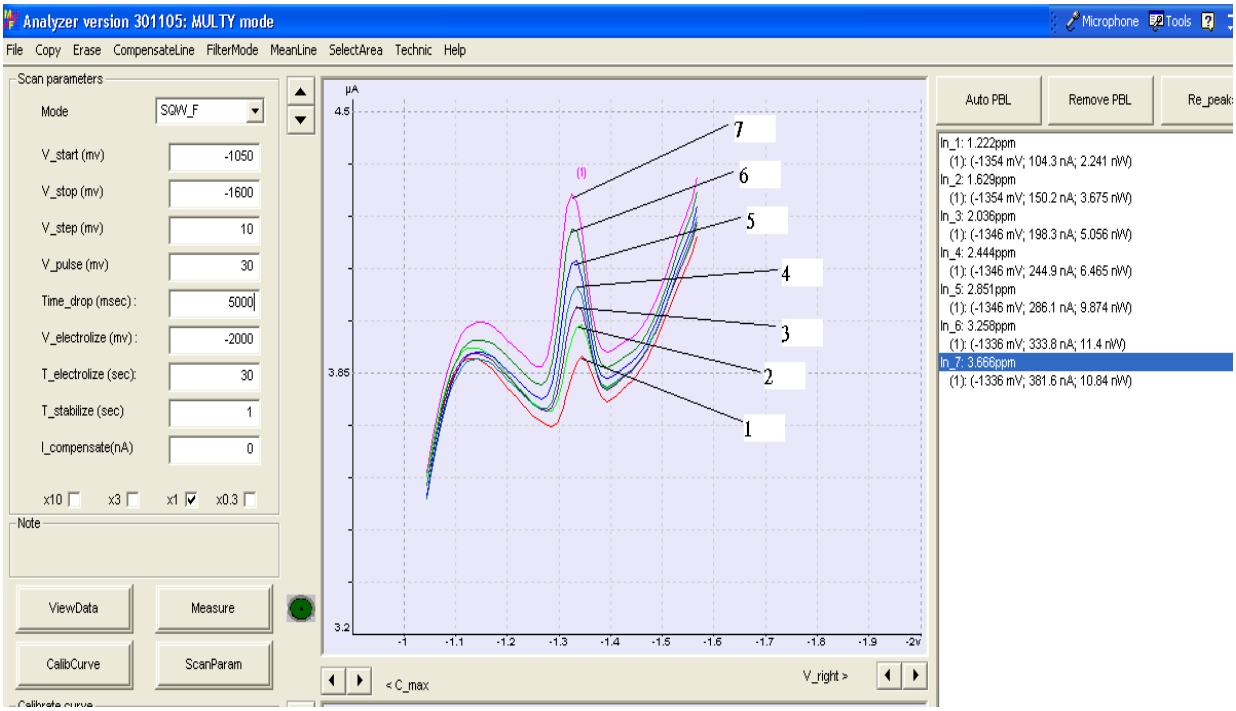

Fig 15: Peak of ciprofloxacin on calibration curve by mode SQW-F.

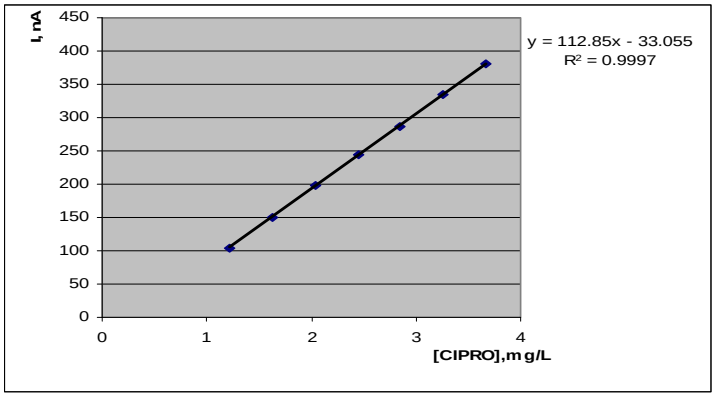

Fig 16; Calibration curve of ciprofloxacin by SQW-F

Regression equation y = 112.85x – 33.055. Correlation ratio $R^2 = 0.9997$. $S_{residue} = 1.426$, m = 5, f = 7+5-2=10, $t_{0.95,10} = 2.23$.

LOD = 0.0165mg/L = 16.5g/L; LOQ = $\frac{10}{3}$ LOD = 0.055mg/l = 55g/L.

3.6 Scanning on mode PSA-F (Stripping square wave voltammetry)

3.6.1 Effect of $V_{electrolize}$

Table 12: Ciprofloxacin at various $V_{electrolize}$ values

$V_{electrolize}$ (mV)	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
0	-1326	278.7	271.0	283.1	277.6
-200	-1326	287.8	291.1	290.8	289.9
-400	-1326	306.6	307.8	311.7	308.7
-600	-1326	327.8	325.1	321.5	324.8
-800	-1326	340.4	343.0	340.9	341.5
-1000	-1326	368.3	369.0	366.2	367.8
-1200	-1326	383.9	383.0	382.7	383.2
-1400	-1354	80.93	79.85	80.96	80.58
-1600	-1354	74.68	75.45	73.94	74.69
-1800	-1354	68.38	67.70	69.86	68.65
-2000		No peak signal			

3.6.2 Effect of $T_{electrolize}$

Table 13: Ciprofloxacin at various $T_{electrolize}$ values

$T_{electrolize}$ (s)	E_{peak} (mV)	Intensity of current, I (nA)			
		Measure # 1	Measure #2	Measure # 3	Mean
1	-1346	141.8	148.2	149.0	146.3
2	-1336	213.4	213.8	216.5	214.6
3	-1336	254.7	257.7	257.9	256.8
4	-1336	295.5	295.7	293.1	294.4
5	-1326	340.1	340.9	341.6	340.9
6	-1326	383.9	383.0	382.7	383.2
7	-1326	393.9	397.5	394.1	395.2
8	-1326	425.2	425.2	420.9	423.8
9	-1326	466.4	467.8	467.2	467.1
10		Dropped mercury			

3.6.3 Effect of stiring

Stiring has strongly affected to peak intensity

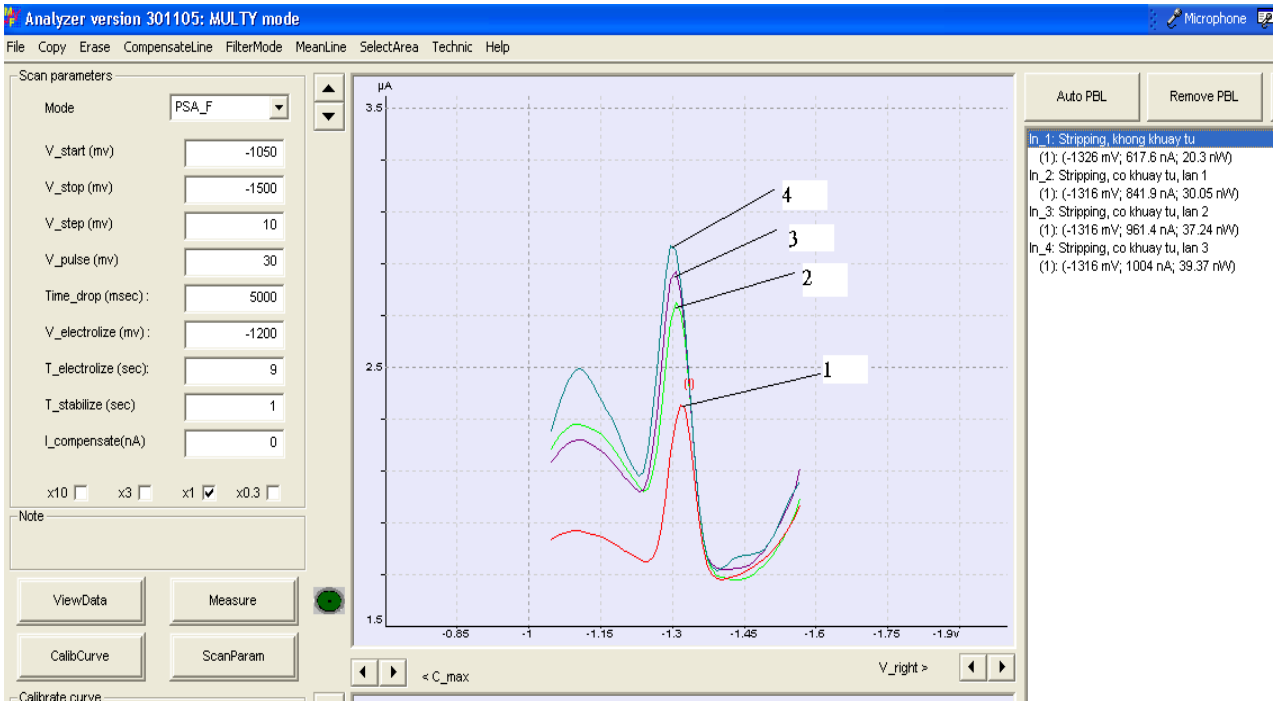

Fig 17: Effect of stiring to ciprofloxacin peak intensity

3.6.4 Linear range, limit of detection LOD, limit of quantification LOQ

Table 14: Calibration data of ciprofloxacin by mode PSA-F

Concentration (mg/L)	Intensity of current, I (nA)				
	Measure # 1	Measure #2	Measure # 3	Mean	s_i^2
0.203	41.36	38.4	39.37	39.71	2.277
0.407	92.93	91.76	92.86	92.52	0.431
0.611	143.8	146.3	145.6	145.23	1.663
0.814	190.2	194.1	194.5	192.9	5.643
1.018	241.8	242.4	243.3	242.5	0.570
1.222	291.5	291.4	290.7	291.2	0.190
1.426	339.6	336.9	340.2	338.9	3.090

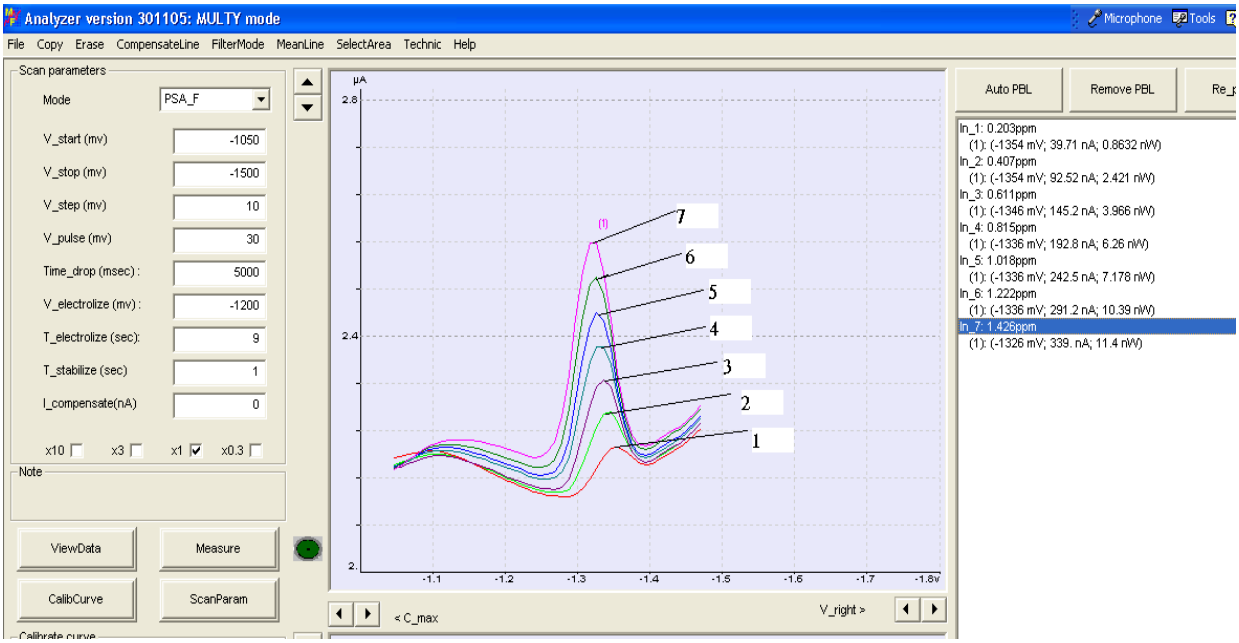

Fig 18: Peak of ciprofloxacin on calibration curve by mode PSA-F

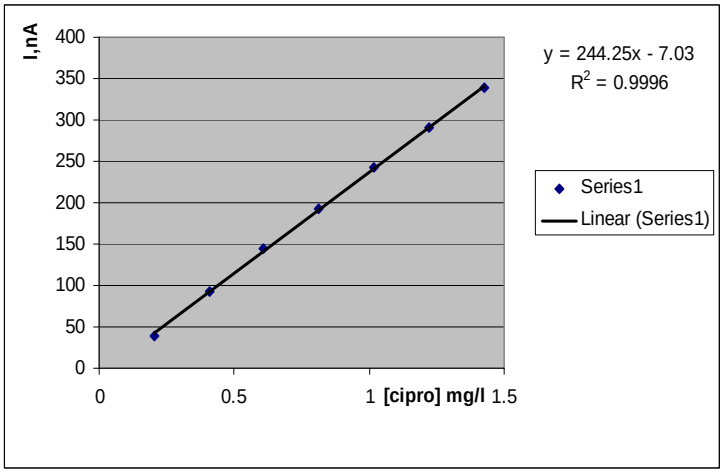

Fig 19: Calibration curve of ciprofloxacin by mode PSA-F

Regression equation: y = 244.25x – 7.03. Correlation ratio: $R^2 = 0.9996$; m = 5, f = n+m-2 = 10, $t_{0.95,\ f=10} = 2.23$. $S_{residue} = 1.99$.

LOD = 0.0106mg/L =10.6g/L. LOQ = $\frac{10}{3}$ LOD = 0.035mg/L = 35g/L.

3.7 Compare the analyzing results by two different modes: SQW-F and PSA- F

Table 15: Comparision of the analyzing results by mode SQW-F and PSA-F

Mode	E_{peak} (mV)	Intensity of current, I (nA)				
		Measure # 1	Measure #2	Measure # 3	Mean	s_i^2
SQW-F	-1354	52.56	55.36	50.42	52.78	6.137
PSA-F	-1336	190.2	194.1	194.5	192.9	5.643

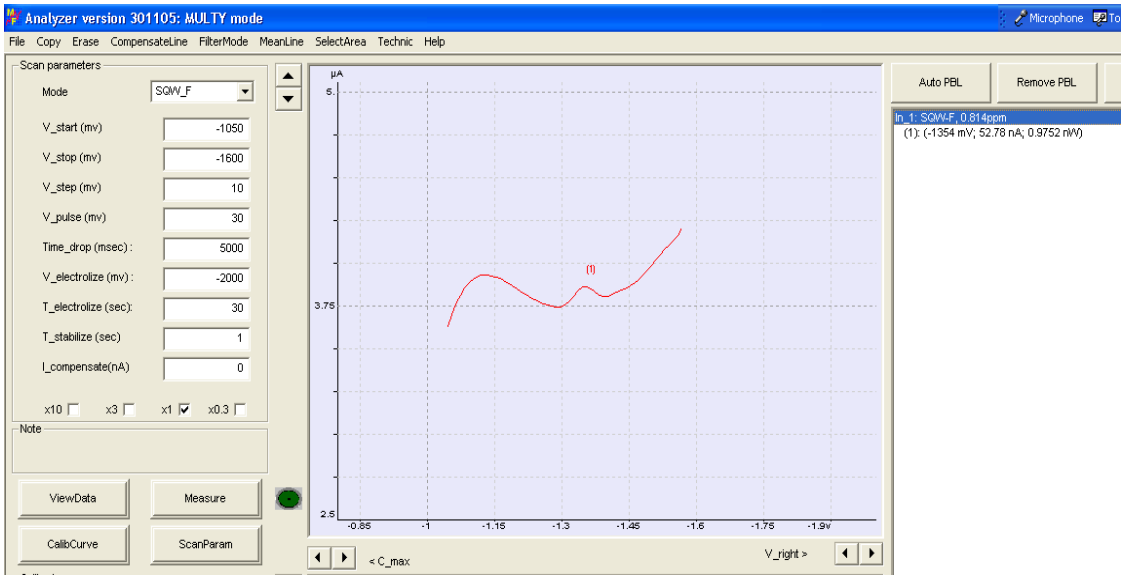

Fig 20: Peak of ciprofloxacin by mode SQW_F

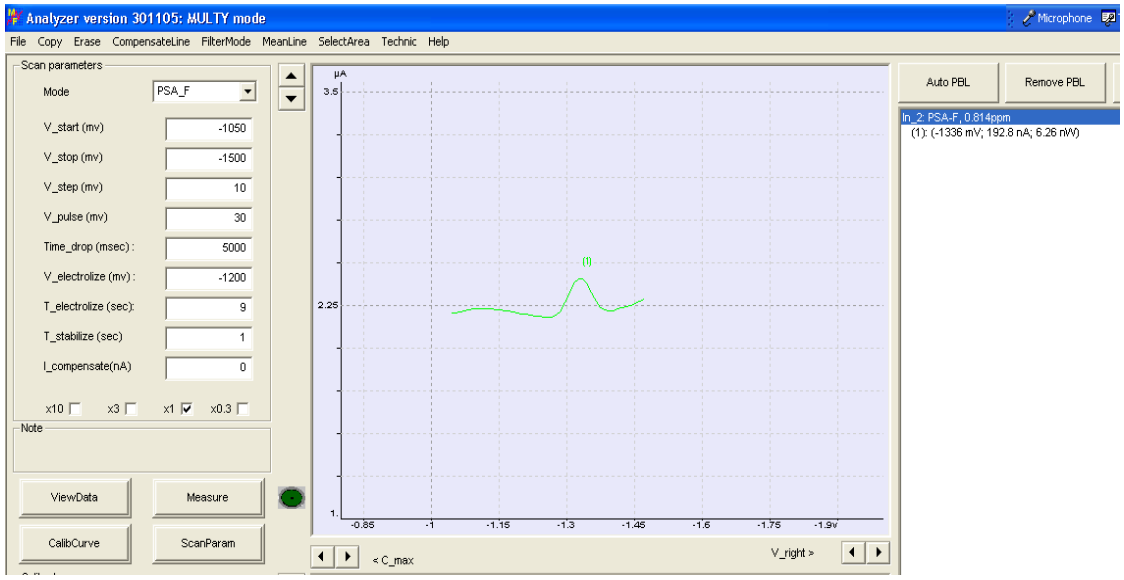

Fig 21: Peak of ciprofloxacin by mode PSA-F.

4. Conclusion

A rapid, sensitive and reliable method for the quantitation of veterinary drugs in food matrices was developed using SQW-F and PSA-F. The overall processing time for analysis was significantly shortened compared to traditional sample analyzing method. Accurate monitoring of chemical residue levels in food and agriculture products is essential to assure the safety of the food supply and manage global health risks. The analysis of chemical residues requires techniques sensitive enough to detect and quantify contaminants at or below the maximum residue limit of the compound in a given sample matrix. In addition, because of increased food safety regulations and the growing numbers of samples to be analyzed, it is critical that the analytical techniques provide high sample throughput.

5. References

1. Ministry of health – UNICEF, manual of essential drugs for the treatment of some diseases in the community, Ha Noi 1996.
2. Huyen NV. The facility either by your background, Science University National University, Ha Noi 1999.
3. Phuong Loan LS. Quantitative study of Norfloxacin in preparations by polarography scan square wave fast, thesis pharmacists College, the University of medicine and pharmacy, Ho Chi Minh City 2005.
4. Qui HV. Analyze storage, Educational Publishing House, Hanoi, Vietnam 2001.
5. Harris C. Quantitative Chemical Analysis. Fifth Edition, W.H.Freeman, 2000.
6. World Health Organization. The International Pharmacopoeia, Geneva, Spain, 200.