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Electrooxidation of Phenazopyridine Hydrochloride and its Voltammetric and HPLC Determination in Human Urine and Tablet Dosage Form

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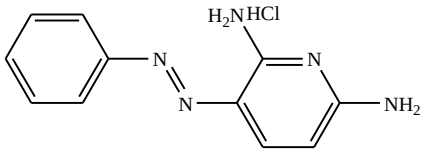
The electrochemical oxidation of phenazopyridine hydrochloride (PAP) was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques at ultra trace graphite electrode (UTGE). PAP exhibited a diffusion controlled process which is pH dependence. The dependence of the current and potential on pH, the concentration and nature of buffer, and scan rate was investigated to optimize the experimental conditions for the determination of PAP. It was found that the optimum buffer for the determination of PAP is 0.1 NaOH solutions with the pH 12.97. In the concentration range from $6.0x10^{-8}$ to $1.0x10^{-6}$ M, the current measured by DPV present a good linear property as a function of the concentration of PAP. Limit of detection (LOD) and limit of quantification (LOQ) were obtained as $8.1x10^{-10}$ and $2.7x10^{-9}$ M, respectively. PAP was determined in human urine and tablet dosage form. Precision and accuracy of the developed technique were checked by recovery studies in spiked urine and tablet dosage form. In addition, for the comparison, high performance liquid chromatography (HPLC) technique was applied for the determination of PAP in the same samples.

Keywords: Phenazopyridine hydrochloride; electrooxidation; differential pulse voltammetry; cyclic voltammetry; ultra trace graphite electrode

1. INTRODUCTION

Phenazopyridine hydrochloride [2,6-diamino-3-(phenylazo)pyridine hydrochloride, (PAP)] (Scheme 1), an azo dye and an analgesic drug, exerts an analgesic effect on the mucosa of the urinary

tract and provides symptomatic relief of pain, burning, urgency, frequency and other discomforts arising from irritation of the lower urinary tract caused by infection, trauma, surgery, endoscopic procedures, or the passage of sounds or catheters [1-3]. It is frequently used in combination with sulfonamides and antibiotics and it is given in conjunction with an antibacterial agent for the treatment of urinary-tract infections. Therefore, the determination of phenazopyridine hydrochloride in biological media, pharmacy and medicine is very important [3].



Scheme 1. The chemical structure of PAP

The United Stated Pharmacopeia recommends a direct spectrophotometric procedure for the assay of determination of PAP bulk drug and tablets [4,5]. The USP 23 gives a High Performance Liquid Chromatography (HPLC) method for PAP tablets assay [5]. PAP has been determined by a variety of analytical techniques [6,7].

The polarographic reduction of PAP has been investigated by Surmann and Aswakun [8] and Sabry [9] using a hanging mercury drop electrode (HMDE). In this work, we have reported the oxidation behavior of PAP by CV technique and the determination of PAP in tablet dosage form and spiked urine by DPV technique on the UTGE. The analytical parameters were established for the determination of PAP in real samples. DPV technique was proposed as an alternative technique to the HPLC technique in therapeutic drug monitoring. In the proposed DPV technique is sensitive, selective, simple, rapid and low cost electro-analytic technique for the determination of PAP in human urine and the pharmaceutical dosage form. At the same time, there is no time-consumed sample preparation step prior to drug assay.

In the first part of this study, the electro-analytical oxidation of PAP on UTCE was investigated by CV and DPV techniques. In the second part, PAP was determined in human urine and pharmaceutical tablet form on UTGE by DPV technique. The explanation of the electrode behavior can provide information on clinical activities and interaction of drug [3,10-12]. The determination of the trace amount of analytics especially in biological samples and in pharmaceuticals is a well-known problem. Therefore, in order to reduce the effect of interfering components and to enrich the analytics of interest, sample pretreatment is necessary in most cases [3].

2. EXPERIMENTAL

2.1. Apparatus

A Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) was employed for the voltammetric measurements, with a three-electrode system consisting of a UTGE (surface size φ = 7

mm, disk diameter R= 2 mm Metrohm) working electrode, a platinum wire auxiliary electrode and Ag/AgCl (KCl 3 M, Metrohm) reference electrode. UTGE was polished manually with polishing alumina (prepared from φ = 0.01μm aluminium oxide) on alumina polish pad then rinsed with ultra pure deionized water and ethanol respectively. All measurements were carried out after the deoxygenation of the solutions with argon gas for 5 min and for 60 s before each measurement.

All pH measurements were made with a Metrohm Model 744 pH meter (Herisau, Switzerland. All measurements were carried out at ambient temperature of the laboratory (25-30 °C).

For the analytical application, the following parameters being employed: pulse amplitude 50 mV; pulse time 0.04 s, voltage step 0.009 V, voltage step time 0.04 , potential step 10 mV (DPV) ; the scan rate in the range 10-1000 mV/s (CV).

The HPLC system is Agilent 1100 series (Agilent Technologies, USA), including a quaternary solvent delivery pump, a thermostat column compartment with HP 1049 A - programmable electrochemical detector (ECD) and a manual sample injection valve with a 20 μL loop. Chromatographic separation was carried out using a Zorbax Eclipse XDB-C_8 (150 mm x 4.6 mm i.d., 5μm) column.

2.2. Reagents and Materials

PAP and its tablet dosage forms were kindly supplied by Faco Inc. (Istanbul, Turkey). A stock solution of 1.0 x 10^{-3} M was prepared by dissolving an accurate mass of the drug in an appropriate volume of ultrapure water and kept in the dark in a refrigerator. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution by ultrapure water. All solutions were protected from light and were used within 24 hours to avoid a possible decomposition. 0.1 M NaOH; pH 12.97 (Merck), 0.5 M sulphuric acid; pH 0.51 (Riedel, Germany, 95-97 % m/m), 0.067 M phosphate buffer (Na_2HPO_4, Riedel, Seelze, Germany and sodium dihydrogen phosphate; pH 4.48-7.25 (NaH_2PO_4, Riedel, Seelze, Germany,), 0.1 M acetate buffer; pH 3.29-5.24 (Acetic acid: Riedel,Seelze, Germany, 100 m/m % and sodium hydroxide: Riedel, Seelze, Germany) and 0.04 M Britton Robinson buffer; pH 2.30-12.13 (acetic acid: Riedel, Seelze, Germany, 100 m/m %; boric acid; Merck, Darmstadt, Germany, and phosphoric acid, Carlo Erba, Rodeno, France, 85 m/m %) were used for the supporting electrolytes. Ultra pure water obtained from Sartorius Arium model Ultra Pure Water Systems was used in order to prepare supporting electrolytes. Methanol and other chemicals were used at analytical-reagent grade (Merck). Azo-silin® tablets (Faco, Inc, Istanbul, Turkey) were labeled to contain 50 mg PAP per tablet.

Methanol (HPLC gradient, J.T. Baker 8402), o-phosphoric acid (Redel-de Haen 30417) and bi-distilled water were used for mobile phase. Distilled water was used after filtered with 0.22 μm pore diameter Millipore Express©- PVDF vacuum filter unit.

2.3. Calibration graph for voltammetric determination

The pure PAP was dissolved in ultra pure water to obtain 1.0x10^{-3} M stock solution. For optimum analysis conditions described in the experimental section, a linear calibration curve by DPV

was constructed in the PAP concentrations range $6.0x10^{-8}$ to $1.0x10^{-6}$ M. The repeatability, accuracy and precision were all checked.

2.4. Tablets assay procedure and recovery studies by voltammetry

Ten azo-silin® tablets (labeled to contain 50 mg PAP per tablet) were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration $1.0x10^{-3}$ M**,** was weighed and transferred into a 10 mL calibrated flask and completed to the volume with ultra pure deionized water. The contents of the flask were centrifuged for 15 min at 4000 rpm to dissolution and then diluted with the ultra pure deionized water. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with 0.1 M NaOH supporting electrolyte solution. Each solution was transferred into the voltammetric cell and deaerated oxygen with argon gas (analytically pure with 99.99 %) for 5 min in the supporting electrolyte solution and for 60 s before each measurement as for the pure PAP. The nominal content of the corresponding regression is equal to previously plotted calibration plots.

To study the accuracy and repeatability of the applied methods, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, known amounts of the pure PAP were added to the pre-analyzed azo-silin® tablets formulations of PAP and mixtures were analyzed by the proposed DPV technique. The recovery results were calculated using the related calibration equations after five repeated experiments.

2.5. Voltammetric analysis procedure of spiked human urine

Spiked human urine sample obtained daily from a volunteer was diluted 1: 9 with ultrapure water. Firstly, the voltammetric measurement of 0.5 M NaOH as supporting electrolyte was done. No peak was observed. Then 600 µL of the diluted urine solution was added to 0.5 M NaOH solution and its voltammogram was also recorded as urine and supporting electrolyte. After that, 40µL urine sample (1mL urine + 8 mL methanol + $1.0x10^{-3}$ M PAP solution) was put into this cell and its voltammogram was recorded. Then 20 µL of $1.0x10^{-4}$ M PAP solution was added four times successively and their voltammograms were recorded individually after each addition .Calibration curve was plotted using obtained results [3,13].

3. RESULTS AND DISCUSSION

3.1. Electrochemical Oxidation of PAP

The electrochemical oxidation behavior of PAP was investigated in various supporting electrolyte (pH 0.51-12.97) on the UTGE by cyclic and DPV techniques. In strong basic media, only one oxidative peak of PAP was observed.

The voltammetric response was strongly pH dependent. The DPV peak potential of the oxidation shifted with increasing pH Fig. (1a). The effect of pH on the peak current was shown at Figure. (1b). The maximum current was observed at the 0.1 M NaOH (pH 12.97). Therefore, this pH value and supporting electrolyte were chosen to carry out the electroanalytical determination of PAP.

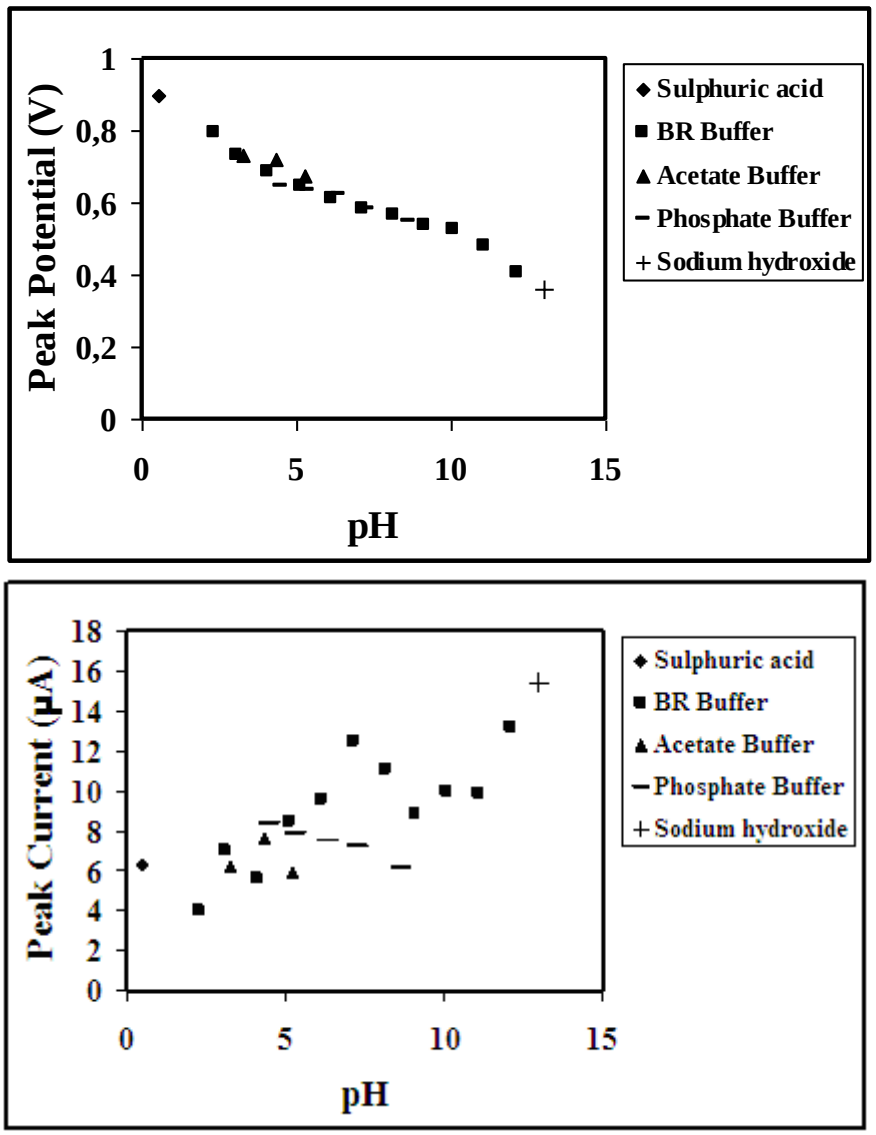


Figure 1. The changing of pH on the peak potential (a) and peak current (b) of $5x10^{-5}$ M PAP in various supporting electrolyte by DPV voltammograms.

The effects of the potential scan rate between 10 and 1000 mV/s on the peak potential and the PAP peak current were evaluated. The cyclic voltammograms of PAP in 0.1 M NaOH was given at Figure. (2).

The linear relationship existing between peak current and square root of the scan rate between 10-1000 mV/s ($I_p(\mu A) = 0.5168v^{1/2} - 0.6815$, correlation coefficient 0.994) were observed and this indicated that the oxidation process is predominantly diffusion-controlled. In addition, a plot of

logarithm of peak current versus logarithm of scan rate gave a straight line (correlation coefficient 0.978) with a slope of 0.62 (very close to 0.5), which is the expected value for an ideal reaction of solution species [3,12-15].

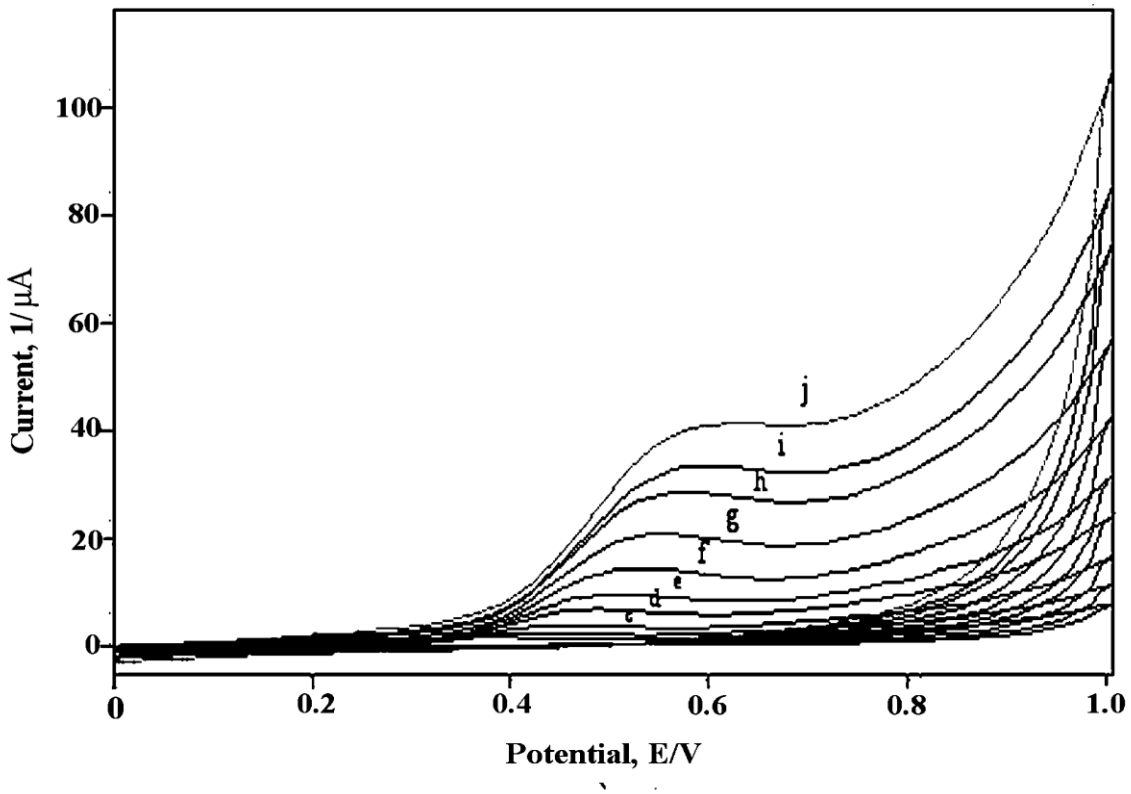


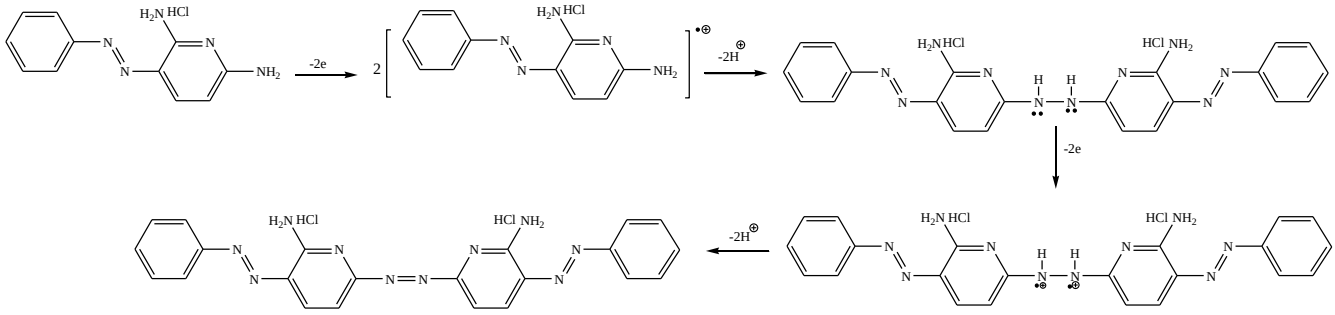
Figure 2. The cyclic voltammograms of 1 x 10^{-4} M PAP in 0.1 M NaOH (pH 12.97) on the UTGE. Scan rates: 10, 25, 50, 100, 150, 250, 400, 600, 750, 1000 mV/s.

The cylic voltammogram of PAP exhibited only one anodic peak, with no peak on the reverse scan, indicating the totally irreversible nature of the electrode reaction. In addition, for an irreversible oxidation process, the peak potential Ep shifts to less negative values with the increasing of scan rate. Therefore, the oxidation process of PAP was proved to be irreversible [3].

On the basis of our electrochemical experiments and the theoretical calculations described, we propose that the direct oxidation of PAP proceeds according to the mechanistic steps shown in Schemes 2.

The mechanism of the electrode reaction may be given in three steps:

In the first step, PAP molecule gives two e^- and two H^+. In the second step, formed compound gives two e^- and two H^+. During these steps, two :N: atoms occur. In the last step, dimerazition reaction may be occurred between these two :N: atoms. All this mechanistic steps may be given as follows:



Scheme 2. Mechanistic picture for the direct electrochemical oxidation of PAP

3.2. Validation of the proposed voltammetric technique

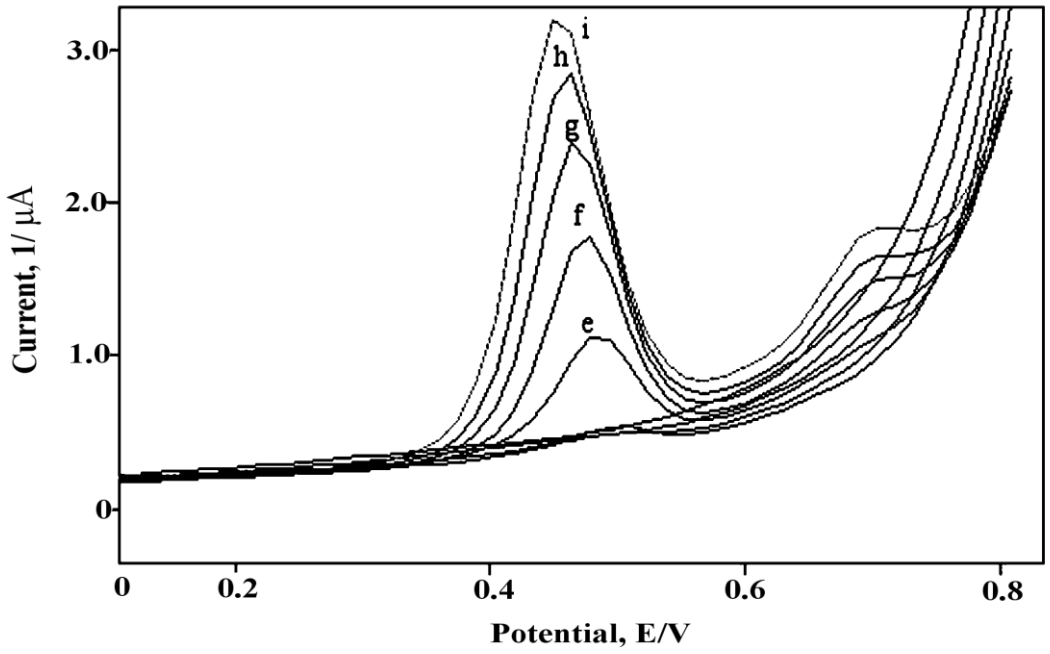


Figure 3. Variation of oxidation peak current on DPV voltammograms with concentration of PAP in 0.1 M NaOH (pH 12.97) conditions: a) supporting electrolyte b) $6x10^{-8}$; c)$8 x10^{-8}$ d)$1x10^{-7}$; e) $3x10^{-7}$; f) $5x10^{-7}$; g) $7.0x10^{-7}$ h) $9x10^{-7}$; i) $1.0x10^{-6}$ M PAP.

DPV technique was used to develop a voltammetric methodology for determination of the drug in spiked pharmaceutical and urine. Under the optimized experimental conditions, a linear relationship between the oxidation peak current of PAP at UTGE and concentration can be established in the range of 2.5×10^{-8} to 2.5×10^{-6} M (Figure.3).

The equation of the linear regression plots was $Ip(\mu A)= 3x10^{6}C(M)–0.166$; correlation coefficient, r=0.995; n=5 repeat measurements. Standard deviations for intercept and slope of the calibration curve are given in Table 1.

Table 1. Electroanalytical determination parameters were obtained from the calibration plots of PAP in 0.1 M NaOH solution (pH 12.97) at UTGE by DPV technique

Parameters	Results
Measured potential (mV)	359
Linear concentration range (M)	$6x10^{-8}$-$1x10^{-6}$
Slope (μA M^{-1})	$3x10^{6}$
±S.D. of slope	0.01
Intercept (μA)	0.166
±S.D. of intercept	0.03
Correlation coefficient, r	0.995
Number of measurements (n)	5
LOD (M)	$8.1x10^{-10}$
LOQ (M)	2.7×10^{-9}
R.S.D % of repeatability of peak current	1.46*
R.S.D % of reproducibility of peak current	2.30*
R.S.D % of repeatability of peak current	2.92*
R.S.D % of reproducibility of peak current	1.81*

*Concentration of PAP : $3x10^{-7}$ M

Validation of the procedure for the quantitative determination of the PAP was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility, recovery, accuracy and precision (Table 1)

LOD and LOQ were calculated on the oxidation peak current using the following equations: LOD = 3 s / m, LOQ = 10 s / m (s is the standard deviation of the peak currents (six runs, m is the slope of the calibration curve) [3,15]. The LOD and LOQ were calculated as 8.1×10^{-10} and 2.7×10^{-9} M respectively. A good repeatability and reproducibility of the peak current and potential were calculated from five independent measurements (Table 1).

3.3. Determination of PAP in azo-silin® tablets by voltammery and HPLC techniques

The developed voltammetric procedure was applied with a great success for the quantitative determination of PAP and its commercially available tablets (*azo-silin®*) containing 50 mg of PAP. There was no need for any precipitation, evaporation or extraction step priors to drug assay. The accuracy of the applied technique was evaluated by its recovery during spiked experiments (the addition of known amounts of pure drug to pre-analyzed formulations of PAP). In order to detect interactions of excipients in this method, the standard addition technique was applied to the same preparations, which were analyzed by the calibration straight line. These results indicate the validity

of developed method for the quantitative determination of PAP in commercial tablets. The average recovery of five independent measurements was calculated to be 98.30 % when 2.50 mg of PAP was added in to the sample. This average percentage recovery showed no significant excipients interference, so the procedure was able to determine PAP in the presence of excipients and thus the applied method can be considered sensitive and reliable (Table 2).

Table 2. Application of the DPV and HPLC techniques to the assay of PAP in Azo-silin ® tablet dosage form

Applied Techniques	Voltammetry	HPLC
Linear concentration range (M)	6.0×10^{-8}-1.0×10^{-6}	5.7×10^{-8} -5.7×10^{-6}
LOD (M)	8.1×10^{-10}	2.53×10^{-8} M
LOQ (M)	2.70×10^{-9}	5.49×10^{-8}
Labelled claim of PAP in Azo-silin ® tablet (mg)	50.00	50.00
Amount found of PAP (mg)[a]	48.90	48.06
R.S.D. %	1.04	3.20
Bias (%)	2.20	3.80
Added PAP (mg)	2.50	2.50
Found PAP (mg)[a]	2.45	2.40
Recovery (%)	98.30	96.30
R.S.D. % of recovery	1.59	1.62
Bias (%)	1.70	1.73

[a] Each value is the mean of ten experiments.

For the comparison, HPLC technique was applied for the determination of PAP in the only its tablet form (Table 2). The results indicated that there was no significant difference between the proposed voltammetric and HPLC techniques [3].

3.4. Determination of PAP in spiked urine by voltammery

Table 3. Quantitative determination of PAP in spiked human urine by voltammetry

Medium	Human urine
Added PAP (M)	9.90×10^{-7}
Found PAP(M)	1.01×10^{-6}
Number of measurements (n)	10
Average recovery (%)	102.00
R.S.D. (%)	4.16
Bias (%)	2.02

PAP was also determined in spiked human urine by only DPV technique. The applicability of DPV technique was also tested successfully by the standard additions of PAP (Table 3). The voltammograms of PAP in spiked human urine are shown in Figure. (4).

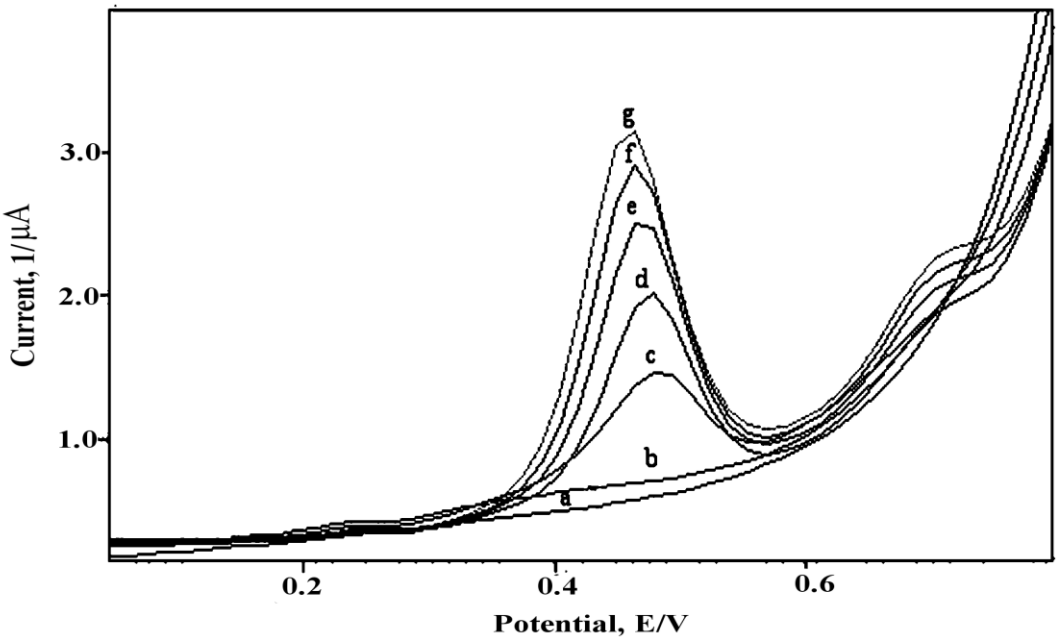


Figure 4. The voltammograms of PAP in spiked human urine

The recovery results of PAP in human urine samples were calculated from the related linear regression equations.

4. CONCLUSIONS

A simple, sensitive, selective DPV technique for the quantitative determination of PAP based on the electrochemical oxidation at UTGE was established. From the CV and DPV measurements, it is understood that electrode reaction process is irreversible and pH dependent. PAP was successfully determined in 0.1 M NaOH in tablets dosage and spiked human serum by DPV technique. The analysis was performed with good recoveries without any interference from the excipients in tablets.

The principal advantage of the DPV technique over the other techniques is that it may be applied directly to the analysis of pharmaceutical dosage forms and to the biological samples without the need for extensive sample preparation, since there was no interference from the excipients and endogenous substances. Another advantage is that the developed DPV technique is rapid, requiring about 5 min to run any sample and involves no sample preparing other than dissolving, diluting, precipitating, centrifuging and transferring an aliquot to the supporting electrolyte. Compared with HPLC, the proposed technique did not require time-consuming extraction or pre-treatment steps prior

to the drug assays. The proposed voltammetric technique is a good alternative to the HPLC, UV and the other drug analysis techniques. The possibility of monitoring of PAP in urine and tablets dosage form also makes the voltammetric technique useful for the pharmacokinetic, pharmacodynamic purposes as well as for the clinical and quality control laboratories [9,11].

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