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A new method for determination of fenoprofen in urine and human plasma by differential pulse adsorptive stripping voltammetry

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ABSTRACT

A novel selective and sensitive electrochemical method is developed for determination of fenoprofen by adsorptive stripping voltammetry (DPAdSV). Fenoprofen gave well resolved diffusion controlled cathodic peak at -0.989 V vs. Ag/AgCl reference electrode in phosphate buffer. Optimal conditions were obtained at pH 4.4, accumulation potential 0.15 V, accumulation time of 40 s, and scan rate of 120 mV/s. Under the optimized conditions, linear calibration curves were established for the concentration of fenoprofen in the range of 0.418-52.26 μ g/ml, with detection limit of 0.081μg/ml. The relative standard deviation of the methodfor 10 runs at 0.418 and 4.18 μg/m lfenoprofen was 3.18%, 2.49%,, respectively. The method was applied to the determination of fenoprofen in various biological samples with satisfactory results.

KEYWORDS

Fenoprofen;
Adsorptive stripping voltammetry;
Biological samples.

INTRODUCTION

Fenoprofen is one of the nonsteroidal antiflammatory drugs (NSAID), which are used in the management of mild to moderate pain, fever and inflammation processes, whereas their antitumor potential has acquired limited attention to date[1-3]. Fenoprofen have rather short plasma half-lives, therefore, repeated doses must be given to maintain the therapeutic effect[4] and was approved by the Food and Drug Administration (FDA) in March 1976. Fenoprofen blocks the enzymes that make prostaglandins (cy- clooxygenases), resulting in lower concentrations of pro- staglandins. As a consequence, inflammation, swelling, pain and fever are reduced. It is a propionic acid derivative (Figure 1) which shows very low aqueous solubility and freely soluble in alcohols[5].

A number of chromatographic methods for determination of fenoprofen in plasma[6–11], serum[7] and urine[9, 10] appeared in the literature. Each of these methods requires a sample preparation based on simple acetonitrile deproteinization[6,7], liquid–liquid extraction[8–10] or on-line dialysis[11]. HPLC was also used to study binding of fenoprofen to human

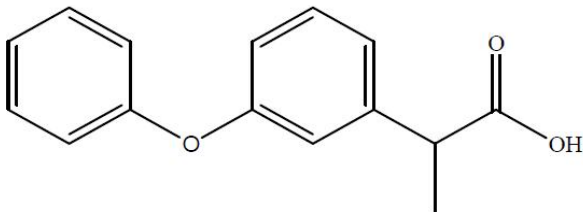

Figure 1 : Structure of fenoprofen

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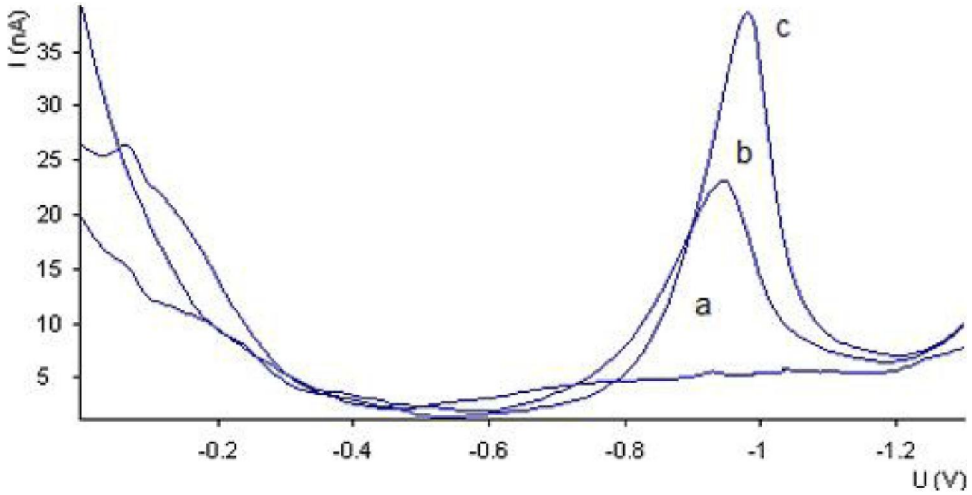

Figure 2 : Differential pulse voltammograms of phosphate buffer (pH 5.5) containing: a): 0.00 µg/ml, b): 5.22 µg/ml and c): 15.67 µg/ml of fenoprofen. Conditions: accumulation potential: +0.1 V, accumulation time: 50 s and scan rate: 60 mV/s

serum albumin[1,12]. Although some reported methods have their respective advantages, they also have some deficiencies in the sensitivity, selectivity, simplicity, cost and unsuitability for automatic or continuous analysis. So, it is necessary to develop a simple, sensitive and selective method for determination fenoprofen. Differential Pulse Adsorptive Stripping Voltammetry is proper method for determination of drug[13-21]. At this time there is no report on the direct determination of fenoprofen with any electrochemical method. Only inone papers the interaction of fenoprofen with bovine serum albumin (BSA) onto the proposed electrochemical sensor was studied[5].

In this paper we report a Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV) procedure for determination of fenoprofen. The method is applied to the determination of fenoprofen in various biological samples with satisfactory results.

EXPERIMENTAL

Apparatus

DPAdsv measurement were made using a 746 VA-Trace Analyzer, (Metrohm, Switzerland) connected to an electrode stand, 747 VA-Stand,(Metrohm, Switzerland).

The three-electrode configuration was used comprising a Metrohm multimode electrode(MME) in hanging mercury drop electrode (HMDE) state as working electrode, a double junction Ag/AgCl (3M KCl, saturated AgCl, and 3M KCl in the bridge) reference electrode and a Pt wire auxiliary electrode. All potential quoted are relative to the Ag/AgCl reference electrode. A rotating Teflon rod stirred solutions in the voltammetric cell. The mercury was triple-distilled quality, and medium drop size of the HMDE was selected. All experiments were done at the room temperature. pH measurement were made with a Metrohm pH meter model 827 (Switzerland). Eppendorf reference variable micropipettes (10-100 and 100-100 µl) were used to pipette micro liter volume of solution. All glassware and storage bottle were soaked in 10 % nitric acid overnight and thoroughly rinsed with deionized water prior to use.

Reagents and solutions

All chemical reagents were of analytical grade and were purchased from Merck (Germany). All solutions were prepared with doubly distilled water. The stock solutions of 1.0×10^{-3} M fenoprofen (fenoprofen calcium salt hydrate, Merck) was prepared with The 0.013g of purefeno profen accurately weighed and transferred to a 25mLvolume tricflask and thendis solved in 4ml of ethanol, and we havethe volumewith doubly distilled water. Solution under these conditions was stable for a week. The stock solutions should be kept in the refrigerator.

Recommended procedures

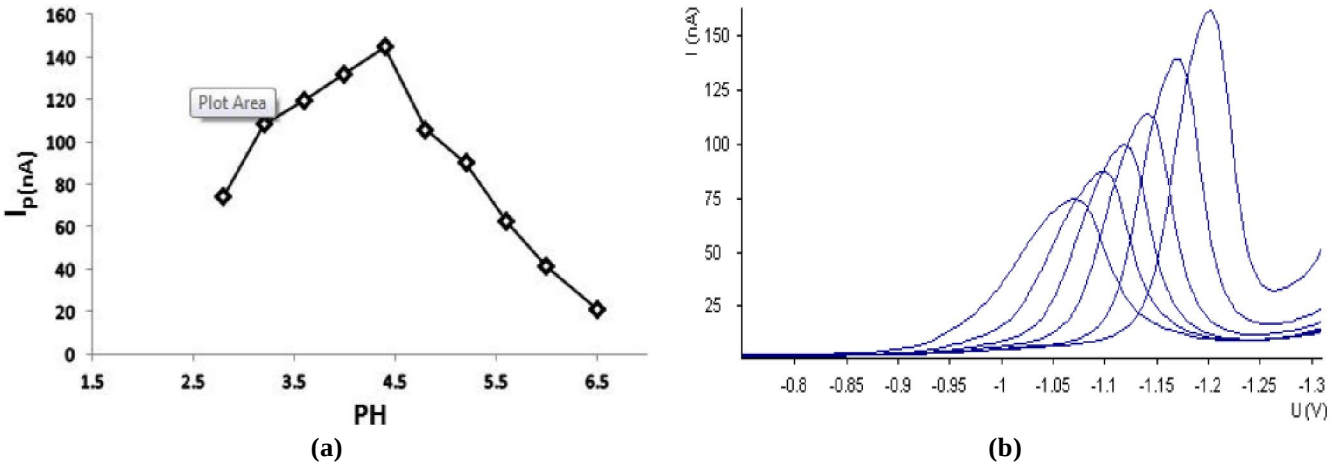

Figure 3 : Effect of pH on the peak current of 31.35 µg/ml of fenoprofen (a) and corresponding voltammograms (b). Conditions: accumulation potential: +0.1 V, accumulation time: 50 s and scan rate: 60 mV/s

The supporting electrolyte solution (1ml of 0.1 M NaOH/H_3PO_4 buffer solution, pH 4.4) containing 6×10^{-5} M fenoprofen was transferred into the electrochemical cell and purged with nitrogen for at least 100 s. the accumulation potential (0.15 V vs Ag/AgCl) was applied to a fresh mercury drop while the solution was stirred for a period of 40 s. after 40 s of accumulation time, the stirring was stopped and voltammograms were recorded from -0.750 V to -1.310 V with a potential scan rate of 120 mV/s and pulse amplitude of mV. All data were obtained at room temperature.

Sample preparation and determination

In order to determination the application of the reported method in practical analysis, the procedure was employed to detect fenoprofen in human plasma and urine samples that were prepared as follows:

Determination of fenoprofen in human plasma

To prepare plasma samples, plasma samples were mixed with different peopleand15 mLacetonitrilewereaddedto10 mL of plasma. Then the mixture was centrifuged at a speed of 4500rpmfor 15 min. than 1.0 ml aliquot of the supernatant fluid was taken into a 100 ml calibrated flask for determination fenoprofen. The concentration of fenoprofen in the working solution was determined under the optimum conditions by DPAdSV method. The results for the determination of plasma are listed in TABLE 1.

Determination of fenoprofen in urine

The fresh urine sample was taken. Deproteinization of the sample was achieved by adding 2 ml of 10% trichloroacetic acid and centrifuged the mixture at 4500 rmp for 20 min. Then 5.0 ml aliquot of the supernatant fluid was taken into a 100 ml calibrated flask for determination fenoprofen. The accuracy was tested by standard addition method. The results for the determination of urine are listed in TABLE 1.

RESULTS AND DISCUSSION

Fenoprofen electrochemical behavior was studied. The results of the initial testing of the drug in phosphate buffer solution at pH =5.5 indicatedfenoprofencharacteristics is absorbed surface hanging mercury drop electrode. The sample solution containing the fenoprofen show a peak at -0.989 V in pH 5.5 (Figure. 2). This peak current increased with increasing concentration of fenoprofen.

Effects of variables

To obtain the best sensitivity in the determination of fenoprofen the influence of different parameters such as pH, accumulation potential, and accumulation time and scan rate were investigated.

Influence of supporting electrolyte and pH

Preliminary experiments were carried out with different types of buffers such as acetate, phosphate, citrate, borate, Britton-Robinson and ammonia-am-

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TABLE 1 : Determination of fenoprofen in human plasma and urine samples

	Added	Found	Recovery
Samples	(μg/ml)	(μg/ml)	(%)
Plasma	0.0	N.D	–
	5.22	5.388±0.21	103.1
	52.26	51.583±0.28	98.71
Urine	0.0	N.D	–
	5.22	5.136±0.18	98.39
	52.26	51.583±0.31	97.54

TABLE 2 : Interference study for fenoprofen determination

Species	Tolerance limit ($S_{pecies}/W_{fenoprofen}$)
glucose,saccharose	1000
Ca^{+2},Na^{+}, Cl^{-}	300
K^{+},Mg^{+2},Al^{+3}	200
Co^{+2} Cu^{+2}, Mn^{+2}	100
Ni^{+2},Fe^{+3},Zn^{+2},CN^{-}	2

monium. The result showed that the peak shape for fenoprofen was improved in the presence of phosphate buffer solution. Therefore,phosphate buffer was used for optimization of pH. The influence of pH on the cathodic stripping peak currents of fenoprofen was studied in the pH range of 2.5-6.5 of phosphate buffer (t_{acc}=50 s and E_{acc}= 0.1 V). The results are shown in Figure 3. The results show that the peak currents of fenoprofen increasing the pH to about 4.4. Considering that the pKa of fenoprofen equal to 4.5 and optimum pH of the fenoprofen is 4.4, it can be concluded that the species as a molecular absorbed on the surface of the drop. Because, the fenoprofen has been protonated in lower pH than 4.4 and deprotonated in higher pH, respectively. Thus, pH 4.4 was adopted for further studies.

Influence of accumulation potential

The effect of the accumulation potential on the peak of fenoprofen was studied in the range of 0.3 to -0.3 (t_{acc}=50 s). As shown in Figure.4, the accumulation potential does not significant effect on the intensity of the peak current. It can be concluded that the species as a molecular absorbed on the surface of the drop.

Influence of accumulation time

The effect of the accumulation time on the stripping peak currents of fenoprofen was studied in the range of 10-70 (E_{acc}=0.15 V). As shown in Figure.5, the peak currents increased initially with increasing pre-concentration time, indicating that before adsorptive equilibrium is reached, the longer accumula-

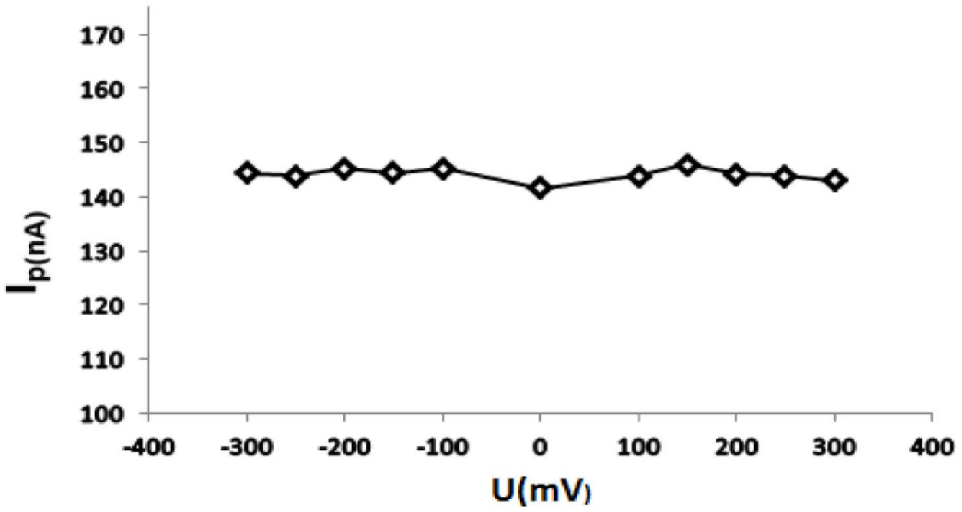

Figure 4 : Effect of accumulation potential on the peak currents of fenoprofen. Conditions: phosphate buffer (pH 4.5), accumulation time: 50 s and scan rate: 60 mV/s

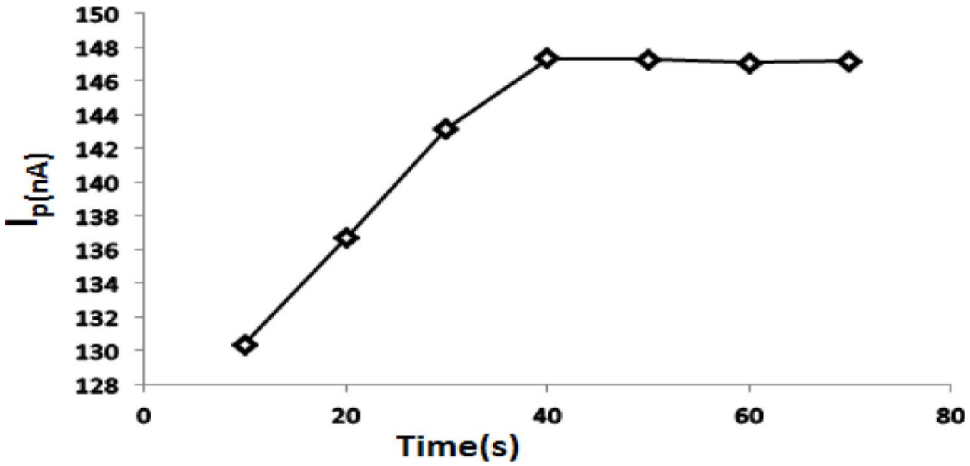

Figure 5 : Effect of accumulation time on the peak currents of fenoprofen. Conditions: phosphate buffer (pH 4.5), accumulation potential, +0.15 V and scan rate: 60 mV/s

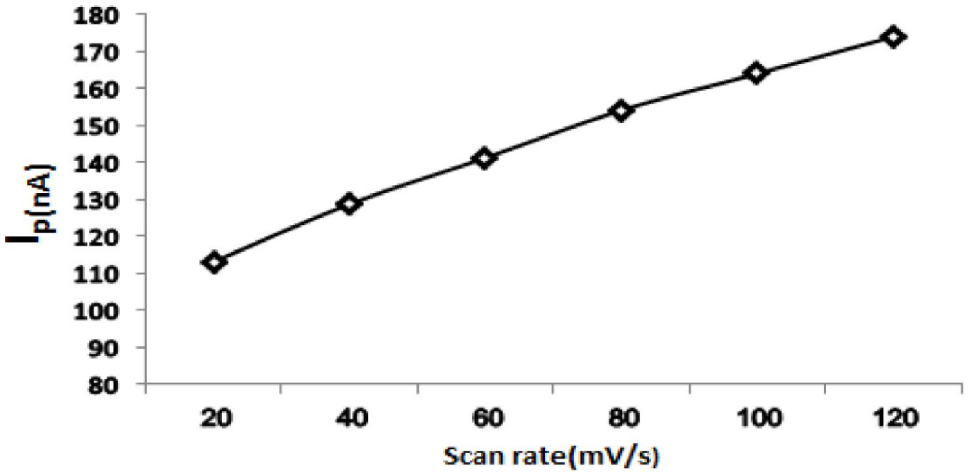

Figure 6 : Effect of scan rate on the peak currents of fenoprofen. Conditions: phosphate buffer (pH 4.5), accumulation potential, +0.15 V and accumulation time, 40 s

tion time, the more fenoprofen was adsorbed and thus the peak currents become larger. However, after a specific period of accumulation time, the peak currents tend to level off slowly as the equilibrium surface concentration of the adsorbed fenoprofen was approached. Therefore, an accumulation time 40 s was selected for further investigations.

Influence of scan rate

Figure 6 depicts the effect of scan rate on the stripping peaks of fenoprofen in the optimal conditions described above. The results show that the peak for fenoprofen increase nearly from 20 to 120 mV/s. therefore, the scan rate 120 mV/s was selected.

Linear range, detection limit and precision

To verify the linear relationship between peak currents and fenoprofen concentrations, a calibration graph was plotted under optimum condition (pH 4.4, accumulation potential 0.15 V, accumulation time 40 s and scan rate 120 mV/s) is shown in Figure.7. The calibration equation, obtained by least-squares method (Figure.8), is I= 4.9195C (µg/ml) + 10.47 (r^2 = 0.9979), where I is the peak current (nA). The stripping peak current of fenoprofen was found to be directly proportional to the fenoprofen concentration in the range of 0.418-52.26 µg/ml. The relative standard deviation for 10replicate analyses of solution containing 0.418 and 4.18µg/ml fenoprofen was 3.18% and 2.49%, respectively. A detection limit of 0.081 µg/ml of fenoprofen was estimated from 10 replicate determinations of blank solution under optimum conditions.

Interference study

Possible interference of other species in the

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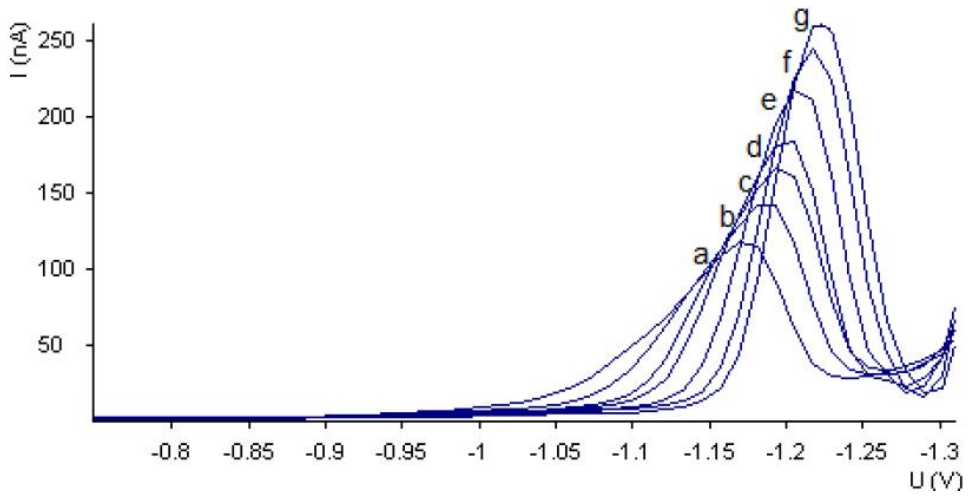

Figure 7 : Typical voltammograms for determination of Fenoprofen under optimum conditions:a)10.45 µg/ml, b)20.90 µg/ml, c) 26.13 µg/ml, d) 31.35 µg/ml, e) 41.80 µg/ml f) 47.03 µg/ml and g)52.26 µg/ml of Fenoprofen

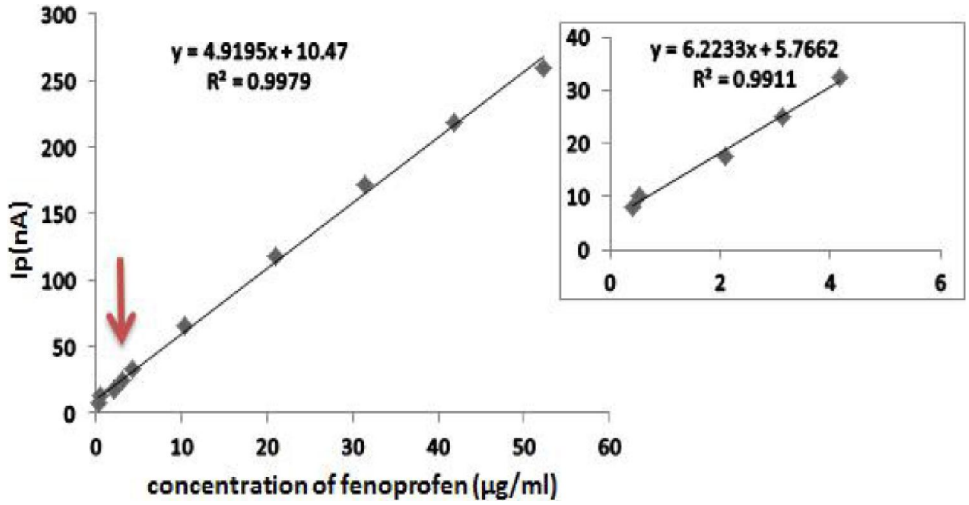

Figure 8 : Calibration graph obtained using the optimized conditions

TABLE 3 : Some critical points in present work compared with some previous works applied for determination of fenoprofen

Method	Linear range (µg/ml)	LOD (µg/ml)	Reference
HPLC	0.00-20.00	0.20	10
HPLC	10.00-60.00	0.50	11
HPLC	-	0.25	22
GLC	3.00-8.00	0.25	23
capillary isotachophoresis	10.45-209.04	10.45	24
DPAdSV	0.42-52.26	0.08	This work

adsorptive stripping voltammetric determination of fenoprofen was studied by addition of the interfering species to a solution containing 31.35 µg/ml of fenoprofen using the optimized conditions. The maximum tolerable concentrations of foreign species are shown in TABLE 2, where the tolerance limit was definedas the concentration of foreign species that produces a change in height of peak current of less than 5%. According to the results, the method is highly selective and therefore, has been successfully applied to trace determinations of fenoprofen in various biological samples without any prior separation or preconcentration steps.

Real samples analysis

To investigate the applicability of the proposed method for the determination of fenoprofen, the method was applied to the determination of fenoprofen in biological samples (human plasma and urine) by standard addition method. The data obtained for samples piked with known amounts of fenoprofen showed good recoveries. The results are given in TABLE 2.

CONCLUSION

A novel method is developed for determination of trace amount of fenoprofen by DPAdSV. The proposed method is sensitive, precise, selective and simple for determination of fenoprofen. TABLE 3 show some critical properties of present work compared with previous studies. Comparison of the present work with the results in this TABLE shows a good detection limit or linear calibration range compared to other studies.

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