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Stability constants and voltammetric determination of ramipril in tablet and real urine samples

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ABSTRACT

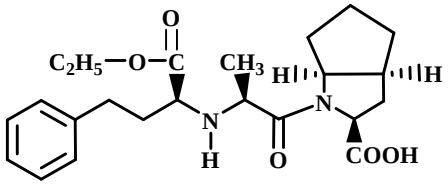
Five metal ions viz; Fe(III), Al(III), Cr(III), La(III), Y(III) were selected to elucidate the interaction of these metal ions with ramipril (RMP) using potentiometric method. The protonation constant of the ligand and stability constants of complexes formed have been tabulated at ionic strength I=0.05M $NaNO_3$ in aqueous solutions at $25^0C \pm 0.1$. Complexes of 1:1, 1:2 and/or 1:3 metal to ligand ratios are formed depending on the nature of the ligand or metal ions. The order of stability constants of the binary complexes was examined. Different parameters such as medium, pH, accumulation potential, scan rate, accumulation time and ionic strength were tested as optimal to optimize the conditions for the determination of RMP by square wave cathodic stripping voltammetry method. The adsorbed form is reduced irreversible using 0.6 M Britton-Robinson buffer (pH~9.0). Linear concentration ranges from 0.083 to 0.417ng/mL at accumulation times 15, 30 and 60 s, respectively, could be determined successfully. The standard addition method was used to determine RMP in pure solutions, tablets and in biological fluids with satisfactory results. The data obtained are compared with standard official method.

KEYWORDS

Ramipril;
Potentiometry;
Square wave cathodic stripping voltammetry;
Dosage forms;
Urine.

INTRODUCTION

Ramipil (RMP), 2[N-(S)-1-ethoxycabonyl-3-phenylpropyl]-L-alanyl]-(1S, 3S,1S)-2-azabicyclo [3,3,0]octane-3-carboxylic acid, is an orally active inhibitor of angiotesin. Converting enzyme (ACE) with antihypertensive activity[1].



Structure of RMP

It is used in the treatment of all forms of hypertension, heart failure and following myocardial infraction to improve survival in patients with clinical evidence of heart failure[2]. Despite the importance of RMP, little has been published concerning its determination, viz; GC[3,4], HPLC[5,6], enzymatic assay with GC or

HPLC[7], radioimmunoassy[8], derivative spectroscopy[9], ion selective electrode derivative potentiometry [10,11], and atomic absorption spectroscopy[12], most of these methods are laborious, time-consuming and require highly sophisticated instrumentation. The potentiometric method has been used extensively in many branches of solution chemistry. It is by for the most accurate and widely applicable technique currently available for the study of ionic equilibria[13]. Recently, much attention has been paid to the study of binary complexes of transition metals with molecules of biological and pharmaceutical interest[14-17]. Furthermore, it has been suggested that the presence of metal ions in biological fluids could have a significant effect on the therapeutic action of drugs[18].

Also, stripping voltammetry is a very sensitive method for the determination of many traces of organic compounds and metal ions achieving it is low level of detection by combining accumulation process with a voltage scanning measurements[19,20]. Carbon paste electrodes are convenient and often used as working electrodes for the voltammetric measurements because of their attractive properties. From analytical point of view, these electrodes exhibit rather low background currents over a wide range of potentials when compared with other solid electrodes, and after are new ability of their surface as well as a high versatility and simplicity of modification[21,22]. The present work is a continuation of our studies in field of drug analysis using mercury and modified carbon paste electrodes[23-26].

The stability of RMP in aqueous buffer solutions has been studied as a function of pH. The rate of RMP; loss and the mode of degradation are dependent upon the pH of solution. In addition, RMP is characterized by its very low ability to absorb light in the UV region with barely discernible maxima at about 257nm (molar absorptivity in methanol at 257nm is about 290L $mol^{-1}cm^{-1}$). However, little information is available on complexes containing RMP drug[27,28].

In this manuscript, we report for the first time, on metal complexes of RMP of the type M^{+n}-RMP such as, Fe(III), Al(III), Cr(III), La(III), Y(III), nitrates ions. The dissociation constants of the drug and stability constants of their complexes were determined. Also, the aim was to investigate the square wave cathodic stripping voltammetric determination of RMP in dosage forms (tablets) and in biological fluids(spiked and real urine sample) at a paraffin oil bare carbon paste electrode (CPE).

EXPERIMENTAL

Apparatus

Calvin-Bjerrum technique as adopted by Irving and Rossoti[29] or Kather and Munshi[30] were used to determine the dissociation constants of the ligand (RMP) and the formation constants of their metal complexes with RMP at $25^0C \pm 0.1$ in aqueous solutions. All pH measurements were made with VWR scientific products Model 2000, USA.

All voltammetric experiments were performed with EG&G Princeton Applied Research (PAR Princeton, NJ, USA) Model 273 A potentiostat, controlled by the model 270/250 electrochemical software version 4.30. A three-electrode cell was employed incorporating a hand-make working carbon paste electrode that prepared as previously mentioned[21], an Ag/AgCl (saturated KCl) reference electrode and platinum wire was used as a counter electrode. Mass transport was achieved with a Teflon-coated bar at approximately 400 rpm using a magnetic stirrer (KIKA Labortechinik, Germany).

Reagents and materials

The solutions of Fe(III), Al(III), Cr(III), La(III) and Y(III) ions (Merck and BDH) as nitrates were prepared and titrated complexometrically by EDTA[31]. In potentiometric method sodium hydroxide and RMP solutions (Merck) were prepared in bidistilled water as fresh solution.

In voltammetric method, RMP (Merck) stock standard methanol solution was prepared at 25^0C and kept in brown volumetric flask. The working standard solutions were prepared daily by serial dilution of stock standard solution.

Pharmaceutical formulations

Tritace® protect tablet (Aventis Pharmas. A. E, Aventis Pharma-Germany) labeled to contain 10 mg RMP per tablet.Real urine sample was taken from healthy volunteers previously taken the drug.

General analytical procedure

The following solutions were prepared and titrated potentiometrically with 0.2M standard free sodium hydroxide solution standardized against standard potas-

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sium hydrogen phthalate, a=0.001M HNO_3, b =a+ 0.001 M RMP and c = b+0.001 M metal nitrate solution. The total volume was adjusted to 50 cm^3 by adding doubly-distilled water in each case. The titration's were performed at 25 C°±0.1 and ionic strength of I = 0.05M $NaNO_3$.

The preconcentration step was performed by immersing the carbon paste electrode in stirring 15ml sample solution for a given period of time at potential range from -0.7 to -1.6V. Then stop the stirring and a delay period of 10 s to settle the solution and decrease the background current, square wave voltammogram was recorded in the negative potential direction. A renewed carbon paste surface was used for each measurement.

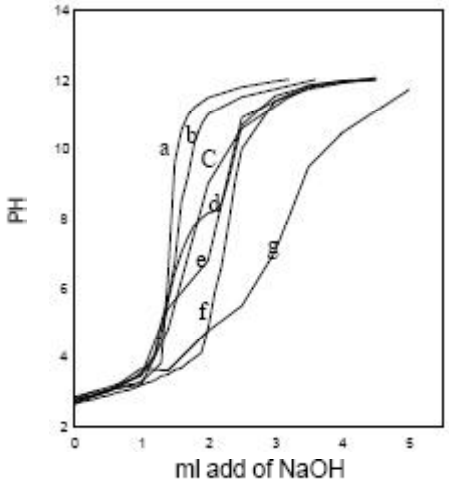


Figure 1 : Titration curves of RMP-metal complexes at I = 0.05 M : (a) acid, (b) RMP, (c) La(III), (d) Y(III), (e) Cr(III), (f) Fe(III), (g) Al(III)

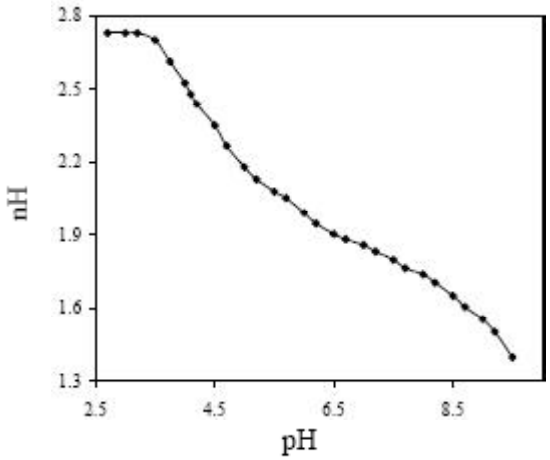


Figure 2 : Protonation constant curve of RMP at ionic strength I = 0.05M.

For the determination of RMP in biological fluids (spiked and real urine samples), 1mL aliquot of urine (blank or containing drug) was transferred to 250mL separating funnel containing 5mL of diethyl ether (Merck). The mixture was thoroughly shaken for 15min, then, the organic layer was transferred to a glass tube, and the solvent was evaporated in water bath to dryness. The residue was reconstituted in methanol. Then, 30 µL urine sample (containing 0.833ng/mL of drug in case of spiked urine and unknown amount of excreted drug in real urine samples), was added to 15ml 0.6M Britton-Robinson buffer pH~9.0. The solution was stirred at 400 rmp at open circuit conditions and the square wave voltammogram was recorded.

Also, in case of dosage forms, ten tablets of the drug were weighed into a small dish, powdered and mixed well. A suitable portion was weighed and dissolved in 100mL of methanol, shaken well and filtered, then transferred into a calibrated flask and it was completed to volume with bidistelled water. 30µL of each solution was then added to the measurement cell.

In all measurements the square wave voltammogram was recorded in negative potential direction.

RESULTS AND DISCUSSION

Potentiometric studies

The titration curves are shown in figure 1. The average number of proton attached per ligand $\bar{n}H$, was calculated[29]

$$\bar{n}H = Y + \frac{(V_1 - V_2)(N^0 + E^0)}{(V_0 - V_1)(Tcl^0)} \quad (1)$$

Where Y = 2 (number of dissociable protons in the ligand), V_o is the initial volume, V_1 and V_2 are the volume of alkali required to reach the same pH in mineral acid (HNO_3) and (HNO_3+RMP), respectively. Tcl^o is the total concentration of ligand, N^o is the normality of the alkali and E^o is the initial concentration of free acid.

Calculation of proton ligand dissociation constants were carried out by plotting $\bar{n}H$ against pH at the three ionic strengths are shown in figure 2. The values of log K_1^H and log K_2^H (the first and second proton dissociation constants of RMP) are the pH values corresponding to 0.5 and 1.5, respectively. The SUPERQUAD computer program[15,32] was used to refine the overall protonation or formation constants by a least squares fit. The data are collected in TABLE 1.

TABLE 1 : Protonation constants of RMP and stability constants of metal ions at ionic strength=0.05M

Metal ions	$LogK_1$	$LogK_2$	$LogK_3$
H^+	9.2	4.0	--
Fe(III)	9.704	7.42	7.13
Al(III)	-	9.585	8.374
Cr(III)	9.504	7.822	-
Y(III)	9.585	8.733	-
La(III)	9.316	8.302	-

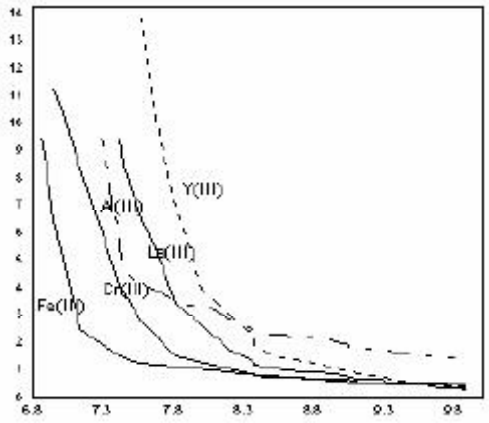


Figure 3 : Formation curves of binary metal ion complexes with RMP at I = 0.05M

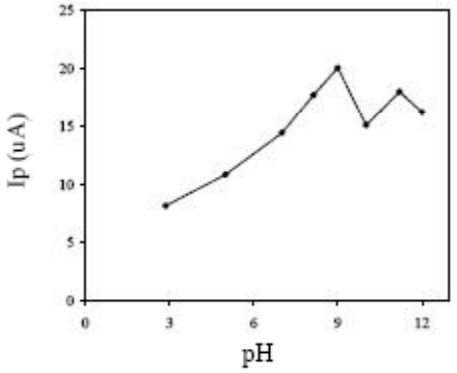


Figure 4 : Plot of Ip versus pH using Britton-Robinson buffer solution in presence of 833 ng/mL of RMP

It is worth mentioning that the ligand do not hydrolysis under the optimum experimental conditions. This is indicated by the rapid attainment of equilibrium during the titration time. The titration curves of the metal-ligand solutions (c) are well separated from that of the ligand solution (b) (Figure 1). Thus, replacement of H^+ ion is due to complexation. $\bar{n}$ (average number of ligand molecules attached per metal ion) and pL (free ligand exponent) values were calculated using Irving and Rossoti equation[29].

$$\bar{n} = \frac{(V_3 - V_2)(N^0 + E^0)}{(V_0 + V_2)\bar{n}HTcM^o} \quad (2)$$

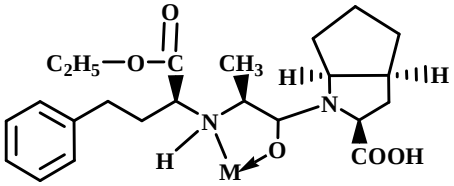
$$pL = Log\left[\frac{(1 + K_1^H[H^+] + K_2^H[H^+]^2 + K_3^H[H^+]^3 + - - -)}{(Tcl^0 - \bar{n}TcM^0)} \times \frac{V_0 + V_3}{V_0}\right] \quad (3)$$

Where V_1, V_2 and V_3 are the amounts of alkali reach the same pH in the free acid, free acid + ligand and free acid + ligand + metal, respectively. T_cM^o denotes the total concentration of metal present in the solution.

The $\bar{n}$ values were plotted against the corresponding pL values to get the formation curves of the metal complexation equilibria. The formation curves are shown in figure 3. From these formation curves, the values of stability constants at the ionic strength I = 0.05 M (TABLE 1) were determined using the half-integral method[30].

RMP has two sites, the first one is protonation of imino group and the second site is the dissociation of H^+ ion from carboxylic group[COOH] these sites are shown as follow:

The order of stability constants of the different binary complexes formed between RMP and transition metal ions investigated in this study is in the expected



Metal ion-RMP complex

Irving-Williams order[33] for (1:1) metal to ligand at I = 0.05 M $NaNO_3$:

Fe(III) > Y(III) > Cr(III) > La(III)

Square wave voltammetric methods

1. Effect of buffer type, pH, and ionic strength

The effect of type of buffer used as electrolyte (Acetate buffer, citrate buffer, phosphate buffer, acetate buffer and Britton-Robinson (universal buffer) on the analytical signal was tested. Both the peak height and peak shape were taking into consideration when choosing type of buffer. A study of the influence of the ionic strength of the selected medium on the definition of the voltammeteric peak revealed that minimal background current, the best curve and the highest peak were obtained in 0.6 M of Britton-Robinson buffer.

The effect of pH on the reduction peak of 0.833

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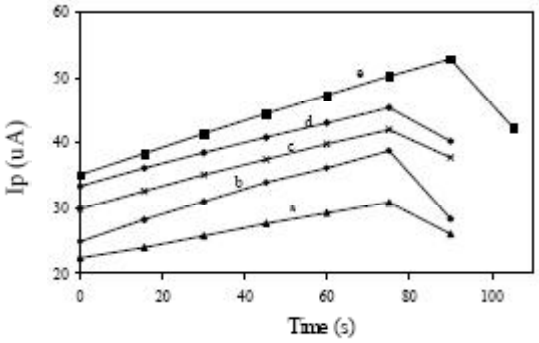

Figure 5 : Effect of accumulation time on different concentration of RMP: (a) 0.0833, (b) 0.833, (c) 8.33 and (d) 83.3 ng/mL of RMP

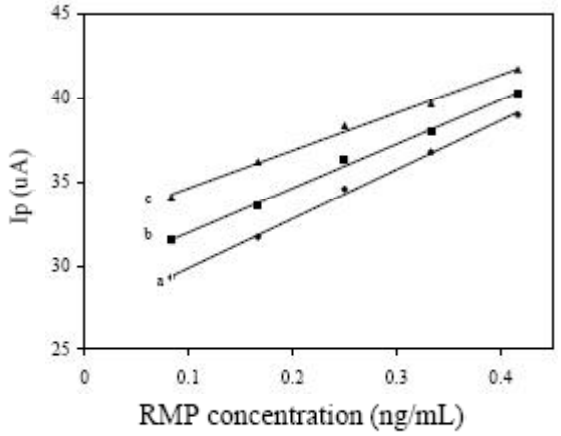

Figure 6 : Plot of Ip versus concentrations of RMP using 0.6 M of Britton-Robinson buffer (pH~9.0) at different accumulation times: (a) 15s, (b) 30s and (c) 60s

µg/mL RMP at CPE was studied over the pH range 2.0-12 by square wave voltammetry as shown in figure 4. A small current was observed at pH~2, which increased gradually up to pH~9.0 which used in all measurements. The cathodic potential of RMP is shifted linearly towards less negative values with increasing the pH over than 9.0.

2. Effect of accumulation time and reproducibility

The dependence of the peak current on accumulation time was studied for five levels of concentration named as (0.0833, 0.833, 8.33, 83.3 and 833ng/mL of RMP. The stripping signal increased linearly with increase accumulation time up to 75s for all concentrations (Figure 5). Repeating three experiments on 8.33µg/mL at 60s accumulation time showed the reproducibility of the adsorption process with correlation coefficient (0.9984) and standard deviation ±0.01.

3. Effect of concentration and detection limit

The square wave cathodic stripping peak for RMP yields a well-defined concentration dependence using SWAdSV method. Calibration plots over the RMP concentration range, following different preconcentration times were investigated. A well defined peak was observed over the concentration range 0.083-2.196ng/mL RMP and over the range from 3.748-41.650ng/mL RMP, at 15, 30 and 60s (Figure 6), respectively, with the stirring at -0.7V. The results showed positive deviation from linearity at concentrations higher than 0.0.417ng/mL at 15, 30 and 60s, and over the concentrations 33.320 ng/mL at 15s and over the concentration 24.990 ng/mL of RMP at 30 and 60s, respectively. However, TABLE 2 illustrates the linearity ranges.

The detection limits were estimated as 3σ/b where b is the slope and =standard deviation (SD) of the intercept[20,24,25]. Also, a quantitative limit was computed as 10σ/b. The results obtained from the proposed method show that RMP can be detected from 2×10^{-10}M (0.0833ng/mL); with relative standard deviation 0.02%, correlation coefficient r = 0.9962(n = 5) at accumulation time 60s.

4. Effect of interference

Different concentrations of DL- valine and DL- alanine and Urea ranged from 1×10^{-6} to 1×10^{-5}mol dm^{-3} were added on 1×10^{-6}mol dm^{-3} of RMP, then the voltammograms were recorded . The results showed that no significant interference. The addition of 1×10^{-6} and 1×10^{-5} mol dm^{-3} from Glycine on RMP showed increase in the current peak by about 3.97% and 18.38% respectively. Also, Ascorbic acid was showed no significant interference on peak response of RMP.

Furthermore, the effect of some metal ions such as Fe (III), Cu (II) and Cd (II) on the peak response of 1×10^{-6} mol dm^{-3} of RMP was studied. Different concentrations of Fe (III), Cu (II) and Cd (II) ranged from 1×10^{-6} to 1×10^{-4} mol dm^{-3} were added.

In the presence of Fe (III), the current decrease by 4.19% at 1×10^{-6} , 5.00 % at 1×10^{-5} M. But in the case of Cu (II) and Cd(II) ions , no interferences were observed

Analytical applications

The proposed method was successfully applied to determine RMP in pharmaceutical preparations, spiked and real urine samples.

1. Pharmaceutical preparations

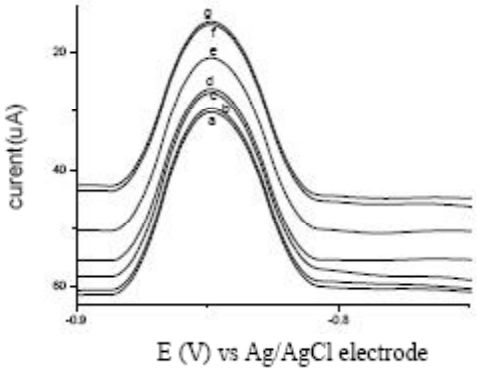

Figure 7 : Typical SWAdS voltammograms of Tritace® tablet in presence of 0.6 M Britton-Robinson buffer (pH~9.0) at 60s

The square wave voltammogram of the tablet sample was recorded after preconcentration time for 15, 30 and 60s in 0.6 M Britton-Robinson buffer (pH~9.0). The content of the tablet in the cell was determined by standard addition method[26]. One peak was observed on addition of pure drug to sample at (-0.85V as shown in figure 7 on increasing the RMP concentration, the peak current was increased linearly from 3.749 to 29.572ng/mL at 15s, from 3.749 to 21.245ng/mL at 30 and 60s, which fitted equation Y=0.326X+29.597 with correlation coefficient 0.9973, Y=0.298X+31.834 with correlation coefficient 0.9958 and Y=0.323X+34.145 with correlation coefficient 0.9937, respectively. The obtained values were validated statistically by studied t-test for accuracy and F-test for precision with the official method[21,23].

2. Real urine samples

The proposed method was also applied to the determination of RMP in human urine samples from healthy volunteers who received as single oral dose of 10mg of Tritace® tablet. The samples of individual were collected up to 24 h after administration of tablet and urinary volumes were recorded as well. RMP was well separated from organic components and excipients did not interfered[34]. The result obtained summarized in TABLE 3, shown that a small amount of an administered dose are excreted in the urine. The results showed a high correlation coefficient r > 0.9992. Also, the obtained result from the proposed method for voltammetric method[27,28], in which about 65 % of an oral dose is excreted in human urine in the first 24 hours.

3. Accuracy and repeatability

Applying the proposed method for the analysis of RMP in dosage forms, spiked and real urine samples exhibited the correlation coefficient of 0.9937,0.9962, 0.9985 at 60s respectively, the standard deviation of both slopes ±0.33, ±0.28, ±0.10% at 60 s respec-

TABLE 2 : Characteristic of linear regression of calibration curves for RMP in 0.6M Britton-Robinson buffer (pH~9.0) using SWAdSV at different deposition times

Deposition time (s)	Linearity range (ng/mL)	Correlation coefficient	Slope (μA/ngmL^{-1})± SD	Intercept (μA)±SD
15	0.083 - 0.417	0.9987	29.341±0.845	29.934±0.234
	3.748 - 33.320	0.9990	0.303±0.008	29.016±0.159
30	0.083 - 0.417	0.9983	26.405±0.877	29.334±0.242
	3.748 - 24.990	0.9975	0.276±0.008	31.177±0.170
60	0.083 - 0.417	0.9970	22.503±1.007	32.361±0.278
	3.748 - 24.990	0.9933	0.221±0.010	34.322±0.205

TABLE 3 : Analysis of RMP in tablet, spiked and real urine sample

Sample	Accumulation time (s)	Detection limits (ng/mL)	Linearity range (ng/mL)	Slope (μA/ ng mL^{-1})±SD	Intercept (ng/mL)±SD	Correlation coefficient
Tritace® tablet[a]	15	2.227	3.749 - 29.572	0.326±0.014	29.597±0.242	0.9973
	30	2.487	3.749 - 21.245	0.298±0.019	31.834±0.244	0.9958
	60	3.046	3.749 - 21.245	0.323±0.026	34.145±0.329	0.9937
Spiked urine sample	15	0.036	0.083 - 0.417	27.388±0.329	27.388±0.329	0.9972
	30	0.027	0.083 - 0.333	25.182±0.828	29.686±0.229	0.9984
	60	0.041	0.083 - 0.333	20.204±1.011	33.127±0.279	0.9962
Real urine sample after 12h	15	0.311	2.307 - 33.320	0.434±0.003	31.139±0.045	0.9999
	30	0.743	2.307 - 24.99	0.408±0.008	30.837±0.101	0.9993
	60	0.814	2.307 - 16.66	0.387± 0.012	35.296±0.105	0.9985
Real urine sample after 24h	15	0.641	0.833 - 20.825	0.697±0.016	32.147±0.149	0.9987
	30	0.455	0.833 - 12.495	0.778±0.021	33.358±0.118	0.9985
	60	0.427	0.833 - 4.165	1.741±0.089	33.510±0.247	0.9961

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tively and the intercept of 34.15, 33.13, 35.30 at 60s respectively indicating adequate precision and accuracy of the proposed method.

CONCLUSION

The SWAdSV method with carbon paste electrode for the quantitative determination of RMP was found to be simple and highly sensitive in dosage forms and biological fluids. A detection limit of 2×10^{-10}M (0.0833ng/mL) at 60 s accumulation time, the standard deviation 0.02 % was obtained in pure solution. The method can be used successfully to assay the drug in dosage form as well as in spiked and real urine samples. It has some distinct advantages over existing methods regarding sensitivity; time saving and minimum detect ability. Moreover, it can be directly applied to the determination of RMP in urine without prior extraction, and this is an advantages over HPLC which necessitates a clean-up procedure before application. The method is sensitive enough to monitor the drug level after therapeutic doses.

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