# Electrochemical Characterization and Voltammetric Anodic Stripping Methods for the Determination of Valsartan

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*In this study, electrochemical behaviour of valsartan (VSN), an agent used for the treatment of hypertension and VSN-serum protein binding affecting the activity, distribution, excretion, and toxicity of pharmaceutical agents in the body were investigated on glassy carbon electrode by using voltammetric methods. Furthermore, adsorptive stripping methods were developed for the direct determination of the same agent in pharmaceutical preparations and human urine samples. The results were also compared with those obtained by a standard method based on UV absorption and the discrepancies were found to be insignificant at 95 % confidence level.*

*Keywords: Valsartan, voltammetry, adsorptive anodic stripping voltammetry*

Cardiovascular diseases are complications involving any sort of disfunction of heart and blood vessels (arteries and veins). Valsartan (VSN), namely, N- valeryl- N[[2- (1H-tetrazol- 5- yl)biphenyl-4- yl] methyl] valine is an angiotensin II receptor, that relaxes blood vessels causing them to widen, which in turn lowers the blood pressure and improves the blood flow [1-3]. The chemical structure of valsartan is given in figure 1.

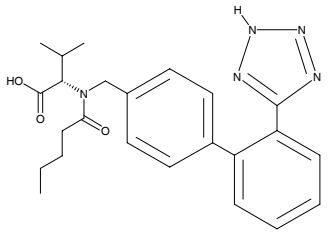

Fig. 1. Chemical structure of Valsartan

An accurate, precise and rapid analytical procedure for the determination of VSN in pharmaceutical preparations is of great significance as monitoring its concentration in the human body may turn out to be crucial. To this end, spectrophotometry and high performance liquid chromatography (HPLC) have been utilized [4-11], but due to complicated preconcentration procedure or a need for multi-solvent extraction appear to make these techniques fairly complex in the analysis of real samples, especially in cases where the concentration of the analyte is relatively low. Electrochemical adsorptive stripping methods, especially differential pulse voltammetry (DPV) and square-wave voltammetry (SWV), decrease the analysis time remarkably compared to the time spent by chromatographic methods. Also, these methods have the advantage of being relatively less expensive, highly sensitive and they have a low limit of detection. Moreover, they usually do not need any pretreatment, and they are expeditious which makes them promising as potentially routine methods for the analysis of biological samples and pharmaceutical formulations [12-14].

To the best of our knowledge, no attempt to investigate the electrochemical oxidation behaviour and co-rrespondingly no trial of determination by anodic stripping has appeared in the literature for VSN. Here, we aim to investigate electrochemical oxidation of VSN and to develop two voltammetric methods, differential pulse adsorptive stripping voltammetry and square wave adsorptive stripping voltammetry for its determination.

## Experimental part

*Equipment, material and methods*

CHI 660C electrochemical workstation was used for electrochemical measurements of VSN. The electrochemical measurements were related to cyclic voltammetry (CV), controlled potential coulometry (CPC), square-wave anodic adsorptive stripping voltammetry (SWAAdSV) and differential pulse anodic adsorptive stripping voltammetry (DPAAdSV). The electrochemical cell used has three electrodes: glassy carbon electrode (GCE) (MF-2012) as the working electrode, Ag/AgCl (CHI 111) as the reference and platin electrode as the auxiliary electrode.

All *p*H measurements were carried out with pH ion meter having a combined glass pH electrode (912600) calibrated *p*H 4.13 and *p*H 8.20 stock buffer solutions before measurements.

Double-distilled deionized water was supplied from a Ultra Pure Water System. All the data were obtained at ambient temperature.

*Reagents and solutions*

Analytically pure Valsartan was obtained from Nobel Pharm. Ind. and was used without further purification. The chemicals not mentioned here were all of analytical grade.

The stock solution of valsartan was prepared by dissolving a known amount of valsartan in methanol. The concentration of stock solution was $1.0 \cdot 10^{-3}$ mol/L. Working solutions of VSN were prepared by dilution of the stock solution with supporting electrolyte (Britton Robinson Buffer, BR).

Phosphoric acid, boric acid, acetic acid used in the preperation of BR buffer solution [15] and sodium hydroxide used to adjust the *p*H of the supporting electrolyte were of analytical reagent grade. Double-distilled deionized water was used in all the solutions.

All VSN solutions were protected from light and kept in refrigerator at +4 °C.

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*Cleaning and polishing electrode*

Glassy carbon electrode has a sensitive surface and the surface cleaned as instructed with a purpose–made cleaning pad and an alumina suspension provided by the instrument company. GCE was sonicated shortly in ethanol to clean the contaminations and remove the suspension residues.

*Preparation and analysis of samples*

Valsartan tablets each with nominal Valsartan content of 10 mg were used as pharmaceutical samples. Ten tablets were weighed, finely powdered and mixed so as to obtain homogeneous powder. Then the average mass per tablet was determined. A sample equivalent to one tablet was weighed and transferred into a calibrated flask of 100.0 mL volume, then methanol was added. The contents of the flask were sonicated for about 15 min to achieve complete dissolution of VSN. Following dissolution step, content of flask was centrifuged 15 min at 1500 rpm to remove the insolubles. The clear solution was kept in a refrigerator at +4 °C and was labeled as the stock tablet solution. Aliquots corresponding to pharmaceutical samples of nominally known concentrations were taken from this stock solution and transferred to the electrochemical cell and diluted to the volume with BR and the *p*H was adjusted to the desired value. Then the concentration of VSN was determined by using direct calibration against standard VSN solutions.

Similarly, spiked human urine and serum samples were analyzed. Urine and Serum samples, obtained from healthy individuals were stored as frozen until their use. After a gentle thawing, 1.0 mL aliquot volumes of serum (or urine) was added to the electrochemical cell containing 9.0 mL of BR buffer and then sufficient volumes from the stock tablet solution were transferred to this cell. After deareation with nitrogen, measurements were performed to determine VSN content of the cell by using direct calibration methods.

## Results and discussions

*Electrochemical behaviour of VSN*

Cyclic Voltammetry (CV) was used to investigate the electrochemical properities of VSN. In CV voltammogram, a well-defined oxidation peak were observed at a potential of about 1.1 V at *p*H 3.0 (60 $mVs^{-1}$ *vs* Ag/AgCl electrode) but there is no cathodic peak. This result shows that oxidation of VSN on GCE is irreversible. BR solution scanned alone gives rise to no peak at all. Peak current was found to increase linearly with increasing concentration of VSN. These results indicate that anodic oxidation peak is due to the oxidation of VSN molecules.

To investigate the effect of scan rate on anodic peak potential and anodic peak current of VSN at glassy carbon electrode, scan rate was changed in the range 0.005-1.0 $Vs^{-1}$ (for 3.0 . $10^{-4}$ $molL^{-1}$ VSN) by using cyclic voltammetric technique. The peak potential shifts to more anodic values with increasing scan rate (fig 2). This behaviour indicates that the oxidation process is of irreversible or quasi-reversible nature. At lower scan rate the oxidation of VSN was found to be controlled by adsorption. When the scan rate was changed from 0.005 to 1.0 $Vs^{-1}$ in 3.0 . $10^{-4}$ $molL^{-1}$ VSN solution, a linear dependence of anodic peak current $i_{p,c}$ (μA) upon the scan rate ($Vs^{-1}$) was found as given by the equation $i_{p,a}$ (μA) = 9.5815×ν – 0.3758 with $R^2$ = 0.9924. This confirms an adsorption behaviour. Also, a plot of logarithm of peak current (A) versus logarithm of scan rate ($Vs^{-1}$) gave a straight line with a slope of 0.67 for VSN. In addition to this, the plot of peak current versus square root of scan rate was constructed and the graph shows that, it is not linear when scan rate is extremely low but linear when the scan rate higher than 0.125 $Vs^{-1}$.

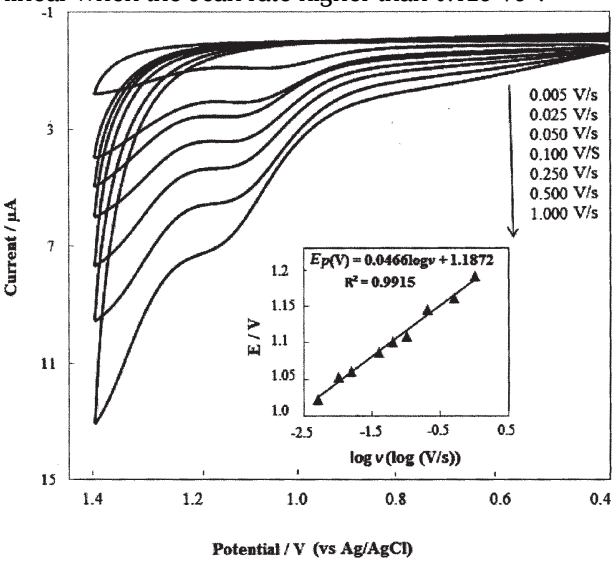



Fig. 2. Cyclic voltammograms of 3.0 . $10^{-4}$ $molL^{-1}$ VSN in BR with different scan rates at *p*H 3.0. The plot of peak potential versus logarithm of scan rate ($Vs^{-1}$) is inset in figure 2

Furthermore, to investigate the electrode process for VSN, CV voltammograms with 5 cycles are recorded. The oxidation peak current of VSN indicated a spectacular decrease during the successive cyclic voltammetric sweeps. The peak current disappears after the first scan and then the other scans remain virtually unchanged.

These results confirm that the electrode process is controlled by absorption under diffusion conditions. *p*H is one of the most important parameter which changes the potential and current size of electrochemical signal. For a close investigation on the effect of *p*H, BR buffers with a wide *p*H working range (2.0-12.0) was used. *p*H values of 3.0 . $10^{-4}$ $molL^{-1}$ VSN solutions were adjusted to in the range

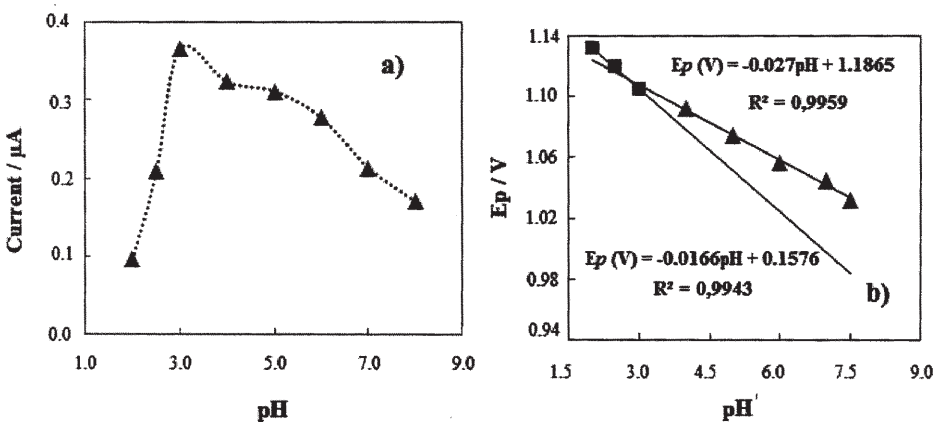



Fig. 3. Influence of pH on **a)** peak currents **b)** peak potential on SWV voltammograms of VSN sample

2.0-12.0 by using BR buffer and square wave voltamograms were recorded (fig.3a). At the end of the assay, it was seen that when *p*H values were adjusted in the range of *p*H 2.0-3.0, the peak current increases. After *p*H 3.0, the peak current was observed to decrease. In contrast, peak potential shifts to more cathodic potentials (fig.3b). As a result, the most suitable peak shape, symmetry and the highest peak current was observed at *p*H 3.0, therefore optimum *p*H was chosen to be 3.0.

The number of protons accompanying electrochemical oxidation reaction of VSN was calculated from the equation (1) [16]

$$E_p = E^0 + \frac{RT}{nF}\ln\frac{[Ox]}{[\mathrm{Re}\ d]} - \frac{\partial RT}{nF}\ln[H^+] \quad (1)$$

In this equation, $\partial$ is the proton number which is accompanied to the oxidation mechanism of VSN. By using the slope of peak potential versus *p*H graph (fig.5b) and equation (1), $\partial$/n ratio was calculated and found to be 0.46 at *p*H 3.0, which means that, 2 electrons were transferred and that $H^+$ was added to oxidation mechanism.

To calculate the number of electrons in the oxidation reaction of VSN, bulk electrolysis was carried out. At the end of the electrolysis, by using the equation Q= nFN, electron number of oxidation mechanism was calculated. The value of electron number was found to be 1.76 ± 0.18, therefore the number of electrons transferred was assumed to be 2.

The number of electrons transferred was also calculated by using CV studies at different scan rates. In this procedure, equation (2) and equation (3) was used [17].

$$i_p = \frac{n^2F^2\Gamma A\upsilon}{4RT} \quad (2)$$

$$Q = nFA\Gamma \quad (3)$$

To calculate the number of electrons, the value of $\Gamma$ in equation (3) was inserted in equation (2) and equation (4) was established.

$$n = \frac{4i_pRT}{FQ\upsilon} \quad (4)$$

In this equation; $i_p$ is current (A); F, Faraday constant (C/mol $e^-$); R, ideal gas constant (J/mol K); T, absolute temperature (K); $\Gamma$ is the surface overlap factor (mol/cm$^2$) and $\upsilon$ is the scan rate (V/s). On the basis of this equation, the number of electrons transffered was found to be 1.72 ± 0.16 at the scan rates of 0.005-1.0 V/s.

The following equation which expresses adsorption phenomena validated by Garrido [18] was used to calculate the diffusion coefficient of VSN:

$$i_p = 1.06\times10^6 n^2 AC\upsilon D^{1/2}t_p^{1/2} \quad (5)$$

In this equation, $t_p$ is the puls time; D is the diffusion coefficient of the electroactive substant, cm$^2$/s; A is the surface area of working electrode, cm$^2$, (A = $\pi$ . 0.15$^2$ = 0.071 cm$^2$), C is the analytical concentration of electroactive substance, mol/cm$^3$.

Diffusion coefficient was calculated at relatively lower scan rates, as the adsorption is the effective process during slow scans. Within the range of 0.005 – 1.000 V/s scan rate, average diffusion coefficient was found to be 3.76 ± 2.18 . $10^{-8}$ cm$^{-2}$s$^{-1}$.

In the literature, diffusion coefficent for diffusion – controlled electrochemical reactions are reported to lie within the range $10^{-5}$ – $10^{-6}$ cm$^2$ s$^{-1}$. The coefficient we found for VSN is well outside this range and we tentatively suggest that, at the surface of the glassy carbon electrode, the oxidation of VSN is controlled by adsorption under diffusion conditions.

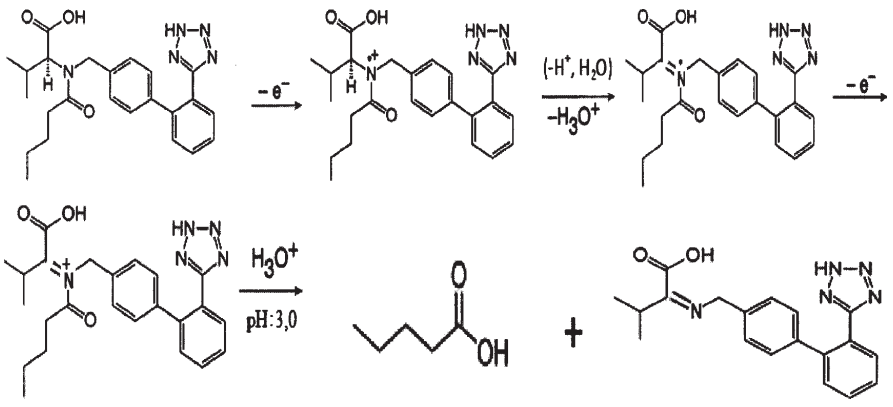
Surface overlap factor of VSN was calculated from the equation (3) as (6.45 ± 0.82) . $10^{-10}$ mol/cm$^2$).

*Proposed mechanism*

Evaluation of the data we obtained leads to the conclusion that in methanol-water medium at *p*H 3.0, the oxidation of VSN at GCE is irreversible and adsorption controlled. The electrochemical procces involves two electrons and one proton. We could not obtain appropriate data to comment on which part of the molecule was involved in the oxidation but the tertiary amine group might be the active site that undergoes the change through the mechanism given in scheme 1

*Electroanalytical determination of VSN*

Electrochemical behaviour of VSN was examined and optimum parameters were determined. Using the optimum parameters found, we attempted to develop a new, reproducible, and accurate method for the analysis of VSN in biologicial fluids and pharmaceutial preperations. The Voltammetric signals were optimized by changing the instrumental parameters and variables like accumulation time, the accumulation potential and *p*H. The voltammetric signals of methods are changed with the signal parameters and software of voltammetric device so, for VSN, optimum instrumental parameters are determined. After optimizing instrumental parameters, the most suitable accumulation potential and accumulation time were also found for SWAAdSV and DPAAdSV. To determine accumulation potential ($E_{ac}$), *p*H was fixed at as 3.0 and the concentration of the sample adjusted to 0.03 mM. Ten different potentials in the range of 0.0 – 1.0 V were tried to accumulate for VSN on GCE and the results were recorded by using SWAAdSV and DPAAdSV techniques. The results show that, peak




Scheme 1
Suggested reaction mechanism of VSN

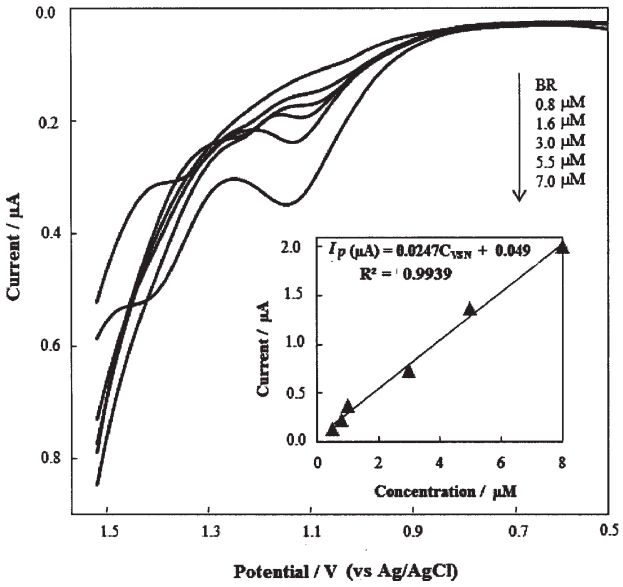



Fig. 4. Voltamograms of VSN at different concentrations in DPAAdSV. Calibration curve. (pH:3.0; $E_{acc}$:800 mV, $t_{acc}$: 60s) is inset in figure 4

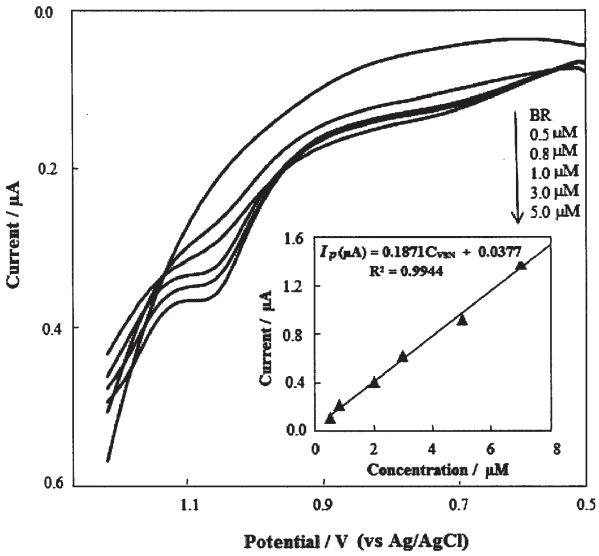



Fig. 5. Voltamograms of VSN at different concentrations in SWAAdSV. Calibration curve. (pH:3.0; $E_{acc}$:200 mV, $t_{acc}$: 45s) is inset in figure 5

current increases in the potential range of 0.0-0.8 V and then decreases after 0.8 V. Considering peak shape and peak current, most suitable accumulation potential was chosen as 0.8 V.

SWAAdSV trials indicate that the peak current increases in the potential range 0.0-0.2 V and then decreases after 0.2 V. Consequently, optimum accumulation potential was selected 0.2 V.

To determine the appropriate accumulation time ($t_{ac}$) at *p*H 3.0 for SWAAdSV and DPAAdSV, the concentration of working sample was kept constant at 0.03 mM and eight different accumulation times were chosen in the range of 0.0-120 s. Optimum accumulation times were found to be 45 s and 60 s for SWAAdSV and DPAAdSV, respectively.

Using the best instrumental and working parameters described above calibration studies were performed to prepare the calibration curves. The linear concentration range of VSN was investigated by using different standard solutions with concentrations in the range $1.0 \cdot 10^{-7}$ molL$^{-1}$ - $1.0 \cdot 10^{-5}$ molL$^{-1}$. For each concentration five reproducible measurements were taken and results of measurements were used to plot the calibration curve. Result of concentration studies indicated that an average peak current of oxidation peak changes linearly with VSN concentration, in the range from $8.0 \cdot 10^{-7}$ molL$^{-1}$ to $7.0 \cdot 10^{-6}$ molL$^{-1}$ for DPAAdSV and $5.0 \cdot 10^{-7}$ molL$^{-1}$ to $5.0 \cdot 10^{-6}$ molL$^{-1}$ for SWAAdSV. The calibration curves can be seen inset in figure 4 and figure 5.

*Application of method to dosage form and biological samples*

VSN was analyzed in biological fluids in urine and serum as well as pharmaceutical samples, by using DPAAdSV and SWAAdSV methods. Tablets produced by NOVARTIS were used as pharmaceutical sample. Ten tablets were weighed together and the average weight of one tablet was determined. The weighed tables were ground in a mortar. From the ground batch, a sample with a mass equivalent to the average weight of one VSN tablet was taken and transferred into a volumetric flask of 100 mL volume. 50 mL of ethanol was added and then the flask was kept in an ultrasonic bath for 30 min to dissolve all the active ingredient VSN. Then the total volume was completed to 100 mL with ethanol and the flask was kept in a fridge at +4°C for the settling of the insoluble components. Following the settling process, known volumes of sample were taken from the clear part of this standard solution of VSN and it was diluted by using BR before assay. The voltammograms were recorded by using DPAAdSV and SWAAdSV methods.

Before analyzing the VSN content of biological fluids, serum and urine samples were taken from healthy individuals and stored at -25°C. For each assay, a known volume of standard VSN or tablet solution was added into 1 mL serum sample and this solution was divided into two parts. Upon the first part was added some acetonitrile to settle the serum proteins. After this procedure, the clear supernatant was taken and diluted with BR and then used for electrochemical measurement. The second part of the solution was analyzed as such without separating the proteins. As seen in figure 6 and 7, when the serum sample with protein was added in VSN solution, the oxidation peak that is observable in the BR buffer solution was lost in DPAAdSV and SWAAdSV methods. These results indicate that VSN does interact with the serum proteins, so the removal of the proteins is essential.

For each assay, standard VSN or standard tablet solution was added in 1.0 mL urine sample and before the voltammetric recording, the total volume was diluted to 10 mL with BR. After this process, voltammograms were recorded by using proposed methods (fig 8). It is seen that, there is no peak at the oxidation potential region of VSN in the urine sample without VSN. But, there is a peak at the

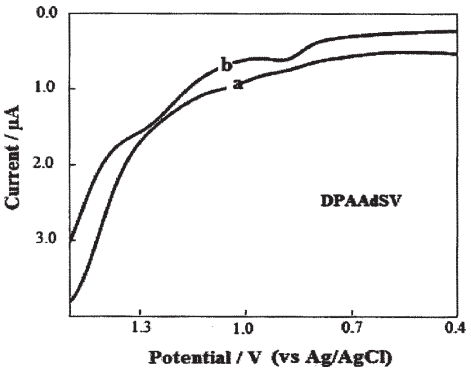



Fig. 6. Voltammograms of **a)** serum sample with protein and VSN **b)** serum sample without protein and VSN in DPAAdSV. ($C_{VSN}$: $3.0 \cdot 10^{-6}$ M; pH: 3.0; GCE; $E_{acc}$: 800 mV; $t_{acc}$: 60 s)

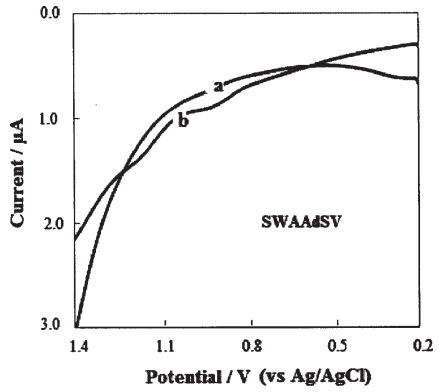



Fig. 7. Voltammograms of **a)** serum sample with protein and VSN **b)** serum sample without protein and VSN in SWAAdSV. ($C_{VSN}$: 3.0 . $10^{-6}$ M; *p*H: 3.0; GCE; $E_{acc}$: 200 mV; $t_{acc}$: 45 s)

potential of 0.6 V which belongs to urine. As the VSN concentration increases, the peak current of VSN also increases but the peak potential at 0.6 V remains unchanged.

Recovery studies were carried out in biological and pharmaceutical samples to demonstrate the applicability of the proposed methods. The results were given in table 1.

*Method validation*

The imported parameters required for the validation of an analytical method are the linearity range, limits of detection and quantification, accuracy, repeatability, stability, selectivity and robustness [19].

Two different calibration curves were constructed by plotting the variable VSN concentrations versus average peak currents. For the calibration curve of DPAAdSV, the working range in $molL^{-1}$ VSN was found to be 8.0 . $10^{-7}$ – 7.0 . $10^{-6}$. For SWAAdSV, the range 5.0 . $10^{-7}$ – 5.0 . $10^{-6}$ mol $L^{-1}$ appears to be applicable.

The values obtained from calibration studies are evaluated by using the method of least squares and the parameters obtained are shown in table 2.

The accuracy of the proposed methods was studied by using analytical applications of VSN. The applications include urine and tablet samples. The accuracy of the

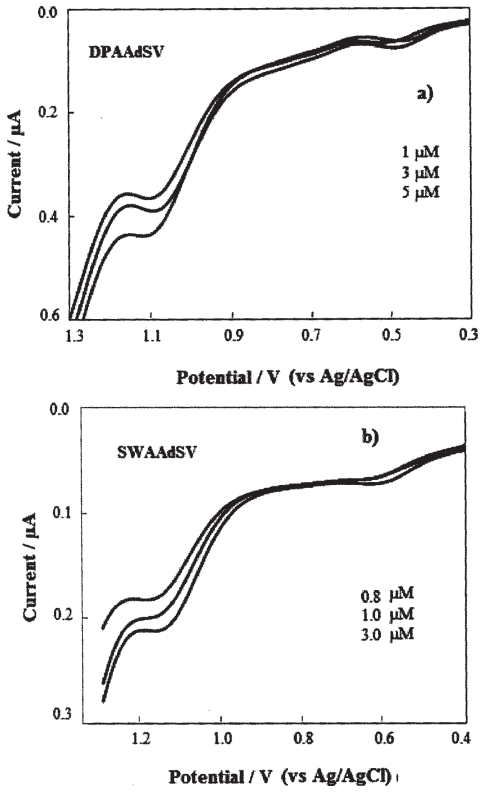



Fig. 8. Voltammograms of VSN in urine sample in a) DPAAdSV and b) SWAAdSV. (pH: 3.0; GCE; For DPAAdSV, $E_{acc}$:800 mV, $t_{acc}$: 60s; for SWAAdSV, $E_{acc}$:200 mV, $t_{acc}$: 45s)

proposed methods appears to be excellent, since the relative standard deviation of recovery values ranges between 1.11 and 4.25 %. Also, the recovery values of SWAAdSV in tablet samples are about 100.6 % and for urine samples, it is in the range 99.08 - 101.9 %. Similarly, for DPAAdSV, recovery values for tablet samples are found about 100.6 % and for urine samples it is in the range 97.63-100.5% .

To indicate the reproducibility of proposed methods, the stability of peak currents and peak potentials was investigated. For this purpose, at optimum conditions when VSN concentration was 1.0 . $10^{-6}$ $molL^{-1}$, the

**Table 1**
RESULTS OF DETERMINATION OF VSN IN URINE SAMPLE BY USING DPAAdSV

| Sample | Added VSN,µg | Found VSN, µg | %Recovery * | RSD** % |
|---|---|---|---|---|
| VSN in Urine | 7.62 | 7.00;7.60;7.50;7.50;7.60 | 97.63±3.85 | 3.36 |
| | 13.06 | 13.34;12.56;13.04;13.40;13.10 | 100.2±2.91 | 2.52 |
| | 21.77 | 22.04; 21.50; 21.45; 21.56;21.50 | 99.26±1.94 | 1.12 |
| Tablet in Urine | 7.62 | 7.45;7.50;7.74;7.80;7.75 | 100.3 ±2.36 | 2.09 |
| | 13.06 | 13.56;12.61;13.04;13.48;13.00 | 100.5 ± 3.10 | 2.96 |
| | 21.77 | 21.76;21.56;21.72;21.85;21.94 | 99.98 ± 1.10 | 0.65 |

* Results of recovery values are given as mean ± $ts/\sqrt{n}$ (at 95 % confidence level)

** RSD is relative standard deviation

| Calibration parameters | SWAAdSV | DPAAdSV |
|---|---|---|
| Linearity Range, μM | 0.5 - 5.0 | 0.8 - 7.0 |
| Potential, V | 1.104 | 1.056 |
| Slope of Calibration Curve ($m$), $\mu A \mu M^{-1}$ | 0.1871 | 0.0247 |
| Intercept ($b$), μA | 0.0377 | 0.049 |
| SD of Slope ($s_m$), $\mu A\ \mu M^{-1}$ | 0.0067 | 0.0015 |
| SD of Intercept ($s_b$), μA | 0.0026 | 0.0024 |
| Regression Coefficient ($R^2$) | 0.9939 | 0.9944 |
| Limit of Detection (LOD), μM | 0.2932 | 0.1824 |
| Limit of Quantification (LOQ), μM | 0.9676 | 0.6021 |
| Repeatability of peak current in day (RSD %) | 0.717 | 0.225 |
| Repeatability of peak current in different days (RSD %) | 4.151 | 5.917 |
| Repeatability of peak potential in day (RSD %) | 0.452 | 0.093 |
| Repeatability of peak potential in different days (RSD %) | 2.063 | 0.833 |

**Table 2**
REGRESSION DATA OF THE CALIBRATION CURVE FOR ASSAY OF VSN BY SWAAdSV AND DPAAdSV

voltammograms were recorded by using SWAAdSV and DPAAdSV and this process was repeated 5 times. Relative standard deviation values of peak currents and peak potentials obtained from stripping voltammograms were calculated throughout a whole day and found to be within 0.225 and 0.093 %, respectively for DPAAdSV, 0.717% and 0.452% for SWAAdSV. This results show that, within a day, the reproducibility of peak potential and peak current values are excellent.

To investigate the reproducibility of peak potential and peak current in different days, five VSN samples were prepared in different days and then the voltammograms were recorded by using proposed DPAAdSV and SWAAdSV methods. According to these voltammograms, relative standard deviation values of peak currents and peak potentials were calculated and found to be 5.917 and 0.833% respectively for DPAAdSV and also for SWAAdSV, the corresponding values for the peak currents and peak potentials are 4.151 and 2.063% respectively. According to these results, in different days, the reproducibility of peak potential and peak current values also appear to be excellent.

The accuracy of proposed methods was evaluated by using the obtained relative standard deviation values of recovery studies in analytical applications. In SWAAdSV, the RSD value of recovery studies in tablet samples is about 3.47% and for urine samples, it is in the range of 1.1%-4.25%. For DPAAdSV, the RSD values are calculated and found to be about 1.94% for tablet samples and in the range of 0.65%-3.36% for urine samples. The relative standard deviations of recovery values are lower than 5%, so it can be said that the accuracies of the proposed methods are also acceptable.

Limit of detection (LOD) and limit of quantification (LOQ) values were calculated using the relations: LOD = $3s/m$ and LOQ = $10s/m$ [20]. Where S is the standard deviation of intercept and m is the slope of the calibration curve. LOD values of DPAADSV and SWAAdSV are $1.82 \cdot 10^{-7}$ molL$^{-1}$ and $2.93 \cdot 10^{-7}$ mol L$^{-1}$ respectively. Limit of quantification (LOQ) values were also calculated by using the equation LOQ = $10s/m$, and were found to be $6.02 \cdot 10^{-7}$ molL$^{-1}$ and $9.68 \cdot 10^{-7}$ molL$^{-1}$ respectively.

During the application of proposed method to biological samples and tablets, before adding a standard solution of VSN, voltammetric base line of biological medium was measured by applying the same procedures as carried out in the calibration studies. In such applications, no extra voltammetric signal in studying potential window indicates that there is no significant interferences of various inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological media. These results indicate that oxidation peak is specific to VSN and this peak can be used selectively to determine the VSN in biological fluids.

The sensitivity of the proposed methods to VSN was compared by using the slope of calibration curves. The sensitivity of SWAAdSV is higher by a factor of 7.5 than DPAAdSV and it can be said that, for VSN, proposed SWAAdSV method is more sensitive.

To compare the accuracy and precision values of the proposed DPAAdSV and SWAAdSV methods, *t*-test and *F*-test were applied. To investigate whether there is any difference between the precision of the two methods, F-test was applied. When the results were interpreted at the confidence level of 95%, it is seen that there is no difference in precision between proposed two new methods. After this process, *t*-test was also applied to proposed methods for comparing accuracy of the methods. At the confidence level of 95% there is no difference in accuracy between proposed new methods.

On the other hand, the two methods were compared with the one based on UV spectroscopy [21] to see if there is any difference in the precisions and accuracies or not. For this purpose, *F*-test and *t*-test were applied. At the confidence level of 95%, there is no difference in precision and accuracy between the proposed methods and UV spectroscopy method.

## Conclusions

In this study, the electrochemical oxidation process of Valsartan (VSN) was investigated by using voltammetric methods. Based on the findings, two new methods (DPAAdSV and SWAAdSV) were proposed for VSN assay in tablet samples and biological fluids. It is observed that, the sensitivity of proposed SWAAdSV method is higher than proposed DPAAdSV method.

The methods developed provide a sensitive, fast, cost-effective, high-throughput and simple approach to the determination of VSN in tablet dosage forms and spiked human urine samples. As applied to urine samples, the proposed method offers the advantage that no prior extraction procedure is required. Moreover, the proposed methods have distinct advantages over other existing methods regarding sensitivity, time-better detectability and no excipients as interfering with the analysis, not to mention the advantage of avoiding a separation step. The proposed methods appear to have a potential of

becoming alternatives to the chromatographic techniques.

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