**INTERNATIONAL JOURNAL**
**OF**
**PHARMACEUTICAL SCIENCES**
**AND**
**RESEARCH**

# ASSAY OF ANTIBACTERIAL DRUG MEROPENEM IN PHARMACEUTICALS BY VOLTAMMETRIC TECHNIQUES

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**ABSTRACT:** In present study the electro-oxidative behaviour and determination of meropenem at a glassy carbon electrode were investigated using cyclic, differential pulse anodic adsorptive stripping voltammetry (DPAAdSV) and square wave anodic adsorptive stripping voltammetric (SWAAdSV) techniques. The dependence of the peak current and peak potentials on pH, concentration and scan rates were examined. The oxidation of meropenem gave a well-defined single irreversible peak at glassy carbon electrode vs Ag/AgCl (3M KCl) reference electrode. The oxidation process was diffusion controlled. A linear response was obtained between $1.0 \times 10^{-6}$ to $4.0 \times 10^{-5}$ M in aqueous media for differential pulse and square wave techniques. The recovery studies were also achieved to check sensitivity and selectivity of the proposed methods. The proposed methods were applied for the determination of meropenem from pharmaceutical dosage forms without any time-consuming extraction, separation, adsorption steps. The value of LOD was achieved $1.27 \times 10^{-7}$ M and $2.24 \times 10^{-7}$ M for DPAAdSV and SWAAdSV respectively.

**INTRODUCTION:** Meropenem **(Scheme)**, chemically (4R,5S,6S)-3- [[(3S,5S)-5-dimethylcarbamoyl pyrrolidin-3-yl]- thio]-6-[(1R)-1- hydroxyethyl]-4-methyl-7-oxo-1- azabicyclo [3,2,0] hept-2-ene-2-carboxylic acid, is a new parenteral carbapenem antibiotic with a very broad spectrum of antibacterial activity against the majority of gram-positive and gram-negative pathogens. It is more active in vitro than imipenem against Entero-bacteriaceae and Pseudomonas aeruginosa, but less active against gram positive cocci. Meropenem is more stable to ring opening by human renal dehydropeptidase I (DHP-I) than imipenem and consequently does not require concomitant administration of a DHP- 1 inhibitor.

This antibiotic has shown clinical efficacy in the treatment of a wide range of serious infections such as intra-abdominal infections, urinary tract infections and lower respiratory tract infections including patients with cystic fibrosis.[1-2]

A survey of literature has revealed several analytical methods for the determination of meropenem and its main metabolite (ICI-213689) in biological fluids, including high-performance liquid chromatography (HPLC), capillary zone electrophoresis and microbiological assay. The literature reports few methods for the quantification of meropenem in pharmaceutical dosage form. Most of reported methods require sample pre-treatment and extraction of the drug prior to the analysis [3-5].

To date, no electrochemical method is available in literature for determination of meropenem in pharmaceutical formulations. Electro-analytical methods have long been used for the determination

of a wide range of drug compounds due to their simplicity, low cost, and relatively short analysis time when compared to other techniques. Additional application of electrochemistry includes the determination of electrode mechanism.[6-12] Redox properties of organic molecules can give insights into their metabolic fate or their *in vivo* redox processes or pharmacological activity. Such use of electro-analytical measurements has found a vast of applications, including environmental monitoring, determination of a wide range of drug compounds[13-19] industrial quality control and biomedical analysis[20]. Glassy carbon electrodes are the most common carbon-based electrodes, since they have excellent mechanical and electrical properties, wide potential range, chemically inert nature, and impermeability to gases. They are easily mounted, polishable, and compatible with all common solvents. Their performances are relatively reproducible. Thus, they offer many applications in many different areas.
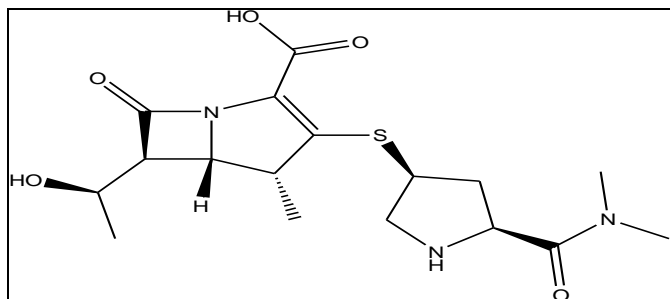


**SCHEME: CHEMICAL STRUCTURE OF MEROPENEM**

In present paper, a validated Square Wave Anodic Adsorptive Stripping Voltammetry (SWAAdSV) and Differential Pulse Anodic Adsorptive Stripping Voltammetry (DPAAdSV) methods are described for determination of meropenem in pharmaceuticals without the necessity for prior drug extraction.

**MATERIALS AND METHODS:**
Meropenem was purchased from local pharmacy under the trade name Merocrit® (Cipla) and was used without further purification. A stock standard solution of bulk Meropenem ($1 \times 10^{-3}$ M) was prepared in water and stored at 4°C until assay. The solution was next filtered through a 0.45 μm-pored Wattmann filter paper. The Working solutions ($1 \times 10^{-5}$ to $1 \times 10^{-4}$ M) were prepared daily by appropriate dilution of the standard solution of bulk

Meropenem with water just before use. A series of BR buffer of pH values 2.8 to 11 was prepared and used as a supporting electrolyte.

**Apparatus:**
Model 1230A (SR 400) electrochemical analyser (CHI Instrument, USA) was employed for electrochemical techniques, with a totally automated attached to a PC with proper CHI 100W version 2.3 software for total control of the experiments and data acquisition and treatment. Athree electrode cell system was used with activated glassy carbon electrode (ø = 3 mm, CHI) as working electrode, Ag/AgCl (3 M KCl) as the reference electrode and a platinum wire as the auxiliary electrode. A magnetic stirrer (CAT.NO-1250-2 LAB-LINE INSTRUMENT, INC. USA) and a stirring bar provided the convective transport during the pre-concentration step. A digital pH-meter (CHINO- DB-1011) was used for measuring the pH values of the investigated solutions

**Pre-treatment of the Glassy carbon Electrode:**
The glassy carbon electrode was polished with 0.5 μm alumina powder on a polishing cloth prior to each electrochemical measurement. Then it was thoroughly rinsed with methanol and double distilled water and gently dried with a tissue paper.

**General Analytical Procedure:**
10 mL of the total solution containing BR buffer of pH 3.4 and the appropriate concentration of the Meropenem was transferred into the electrochemical cell, through which a pure deoxygenated nitrogen stream was passed for 10 min to remove the oxygen gas before measurements. Electrochemical pre-treatment was always performed in the same solution in which the measurement was subsequently carried out. The accumulation of Meropenem at the working electrode was carried out for a selected time while the solution was stirred at 2000 rpm.

The stirring was then stopped and after a rest period cyclic voltammetry was initiated in the anodic direction over the range 0.6 to 1.6 V vs Ag/AgCl/KCl reference electrode at room temperature. Differential pulse anodic adsorptive stripping and square wave anodic adsorptive stripping measurements were carried out under

similar conditions. Quantification of Meropenem was performed by means of both calibration curve and standard addition methods. The irreversibility of the oxidation process was investigated by using Cyclic Voltammetry.

## RESULTS AND DISCUSSION:

The electrochemical behaviour of Meropenem was studied by cyclic voltammetric, square wave anodic adsorptive stripping voltammetric and differential pulse anodic adsorptive stripping voltammetric techniques on glassy carbon electrode. In all electrochemical methods Meropenem gave one well defined oxidation peak in BR buffer at pH 3.4.

### Cyclic Voltammetric Behaviour

Cyclic voltammograms of Meropenem were recorded within a wide range of the potential (0.6 to 1.6V) at different pH values, scan rate and concentration. Meropenem gave one well defined oxidation peak, no peakwere observed in the cathodic direction of the reverse scans, suggesting the irreversible nature of the electrode process. **Fig. 1** depicts cyclic voltammograms of 1.0 x $10^{-4}$ M meropenem in BR buffer (0.4M pH 3.4) on glassy carbon electrode at different scan rates. The initial parameter for cyclic voltammetry are as follows: initial potential ($E_i$) = 0.6 V, high potential ($E_h$) = 1.6 V, low potential ($E_L$) = 0 V, scan rates = 50-250 $mVs^{-1}$, quit time 5 sec and sensitivity 10 $\mu AV^{-1}$.
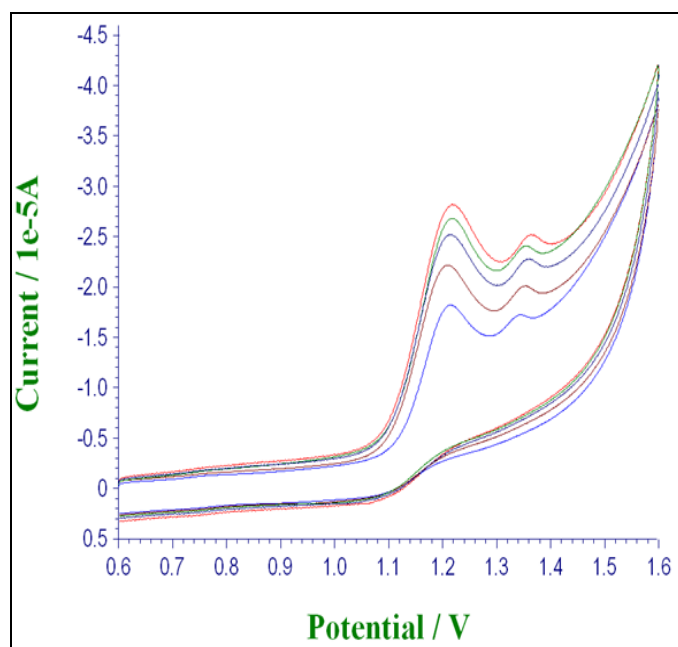


**FIG.1: CYCLIC VOLTAMOGRAMS OF 1.0 X $10^{-4}$ M MEROPENEM AT DIFFERENT SCAN RATES 50, 100, 150, 200, AND 250 mV/s ON BR BUFFER OF pH 3.4.**

### Effect of pH:

The influence of the pH on the oxidation process was studied and an evolution of voltammograms was observed. Only one voltammetric peak was observed in the whole pH range studied. An increase in pH produced a shift in the oxidation peak to more positive potential. The peak current was also dependent on the pH, implying the involvement of the protons in the current –limiting electrode process. The maximum peak current value for the voltammetric peak was obtained at the pH range 3.4.

### Effect of scan rate:

The cyclic voltamograms of 1.0 x $10^{-4}$ M meropenem was recorded at different scan rates at $25^oC$. Both peak potential and peak current were affected by scan rate. The whole procedure for cyclic voltammetry was repeated for Meropenem with different scan rates ranging from 50 to 250$mVs^{-1}$ while other parameter being kept constant. The applied scan rates were 50, 100, 150, 200 and 250$mVs^{-1}$. Graphs of $E_p$ (V) vs log ($\upsilon$ /$mVs^{-1}$) and $i_p$($10^{-5}$ A) versus ($\upsilon^{1/2}$ /$mVs^{-1}$) were plotted.

For a totally irreversible process the relationship between the peak potential $E_p$ (V) and scan rate $\upsilon$ ($mVs^{-1}$) is expressed as[21]:

$$E_p = E^0 + \frac{RT}{\alpha nF} \ln \frac{RTk}{\alpha nF} + \frac{RT}{\alpha nF} ln\upsilon \qquad (i)$$

Where $E^0$ is the formal potential, $T$ the temperature, $\alpha$ is the transfer coefficient, $n$ is number of electrons involved in the rate determining step, $k$ is electrochemical rate constant and $F$ is the Faraday constant. A straight line was observed when $E_p$(V) was plotted against log ($\upsilon$ /$mVs^{-1}$) at a particular concentration and pH value of 3.4. (**Fig. 2**):

$$E_p(V)=0.0563\log(\upsilon/mVs^{-1}) + 1.1067 \quad R^2 = 0.9937 \quad (ii)$$

The slope of these plots indicates the electrochemical oxidation of Meropenem in our experimental condition is totally irreversible. The value $\alpha n_\alpha$, as calculated from the slope is 1.049.Generally, $\alpha$ is assumed to be 0.5 in a totally irreversible electrode process. Thus two electrons were involved in the electro oxidation of meropenem. For a totally irreversible process, the peak current is given by the equation (ii) [22-23]

$$i_p = 2.99 \times 10^5 \, n \, [\alpha n_\alpha]^{1/2} \, ACD^{1/2} \upsilon^{1/2} \quad (iii)$$

where $n_\alpha$ is the number of electrons taking part in the rate determining step of electrode process, $\alpha$ is electron transfer coefficient, A is apparent surface area of the electrode ($cm^2$), C is the concentration of the electro active species (mM), D is the Diffusion Coefficient of the electro-active species ($cm^2 \, s^{-1}$) and $\upsilon$ is the scan rate ($mVs^{-1}$).
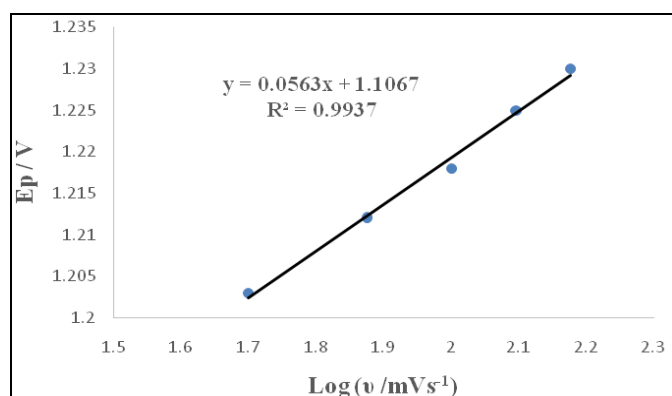


**FIG.2: ANODIC PEAK POTENTIAL ($E_p$/ V) AS A FUNCTION OF LOG $\upsilon$ ($mVs^{-1}$)**

The effect of potential scan rates on the oxidation peak current of meropenem was studied and the oxidation peak current was proportional to the square root of scan rate $\upsilon^{1/2}$ ($mVs^{-1}$) in the given pH range, as prescribed for a diffusion controlled process. Plot of the peak current ($i_p/10^{-5}A$) versus square root of the scan rates $\upsilon^{1/2}$ ($mVs^{-1}$) as shown in the **Fig. 3** is described by the following equation:

$$i_p \, (\mu A) = 0.1842 \, \upsilon^{1/2}((mVs^{-1})) - 0.1765 \quad R^2 = 0.9971 \quad (iv)$$

As the scan rate was increased from 50 $mVs^{-1}$ to 250 $mVs^{-1}$ at a fixed concentration of meropenem, (i) the peak potential shifted anodically, (ii) the peak current increased steadily and (iii) the peak current function($i_p/AC \, \upsilon^{1/2}$), exhibited nearly constancy[24].
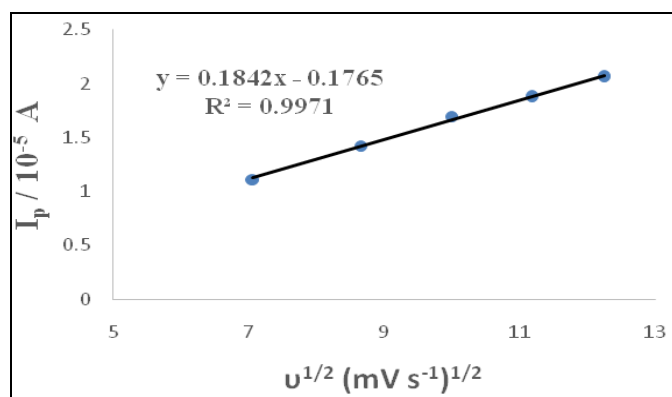


**FIG.3: PLOT OF ANODIC PEAK CURRENT ($i_p$/ $\mu A$) AS A FUNCTION OF $\upsilon^{1/2}$($mVs^{-1}$).**

The linear relationship existing between peak current and square root of scan rate (with slope 0.1842) showed that the oxidation process is predominantly diffusion controlled in the whole scan rate range studied.

**Validation of the Analytical Procedure:**
Quantitative evaluation is based on the linear correlation between the peak current and concentration. For analytical purposes, best response was obtained in the aqueous media for glassy carbon electrode. The best single peak shape, peak current sensitivity, and reproducibility of these techniques were obtained in aqueous solution. The graph of meropenem concentration vs the peak current gave linear calibration curves using both techniques, indicating a diffusion-controlled process. The linearity of the calibration curve was obtained in the range of $1.0 \times 10^{-6}$ to $4.0 \times 10^{-5}$ M for both DPAAdSV and SWAAdSV techniques. Above these concentration ranges, the loss of linearity was probably due to the adsorption of meropenem on the electrode surface. The precision of the method was investigated by repeatedly (n = 5) measuring peak potential and peak current of meropenem within a day and over three consecutive days for both techniques.

The value of limit of detection (LOD) and limit of quantification (LOQ) were calculated as (3s/m) and (10s/m), respectively where s is standard deviation of response (three runs) and m is the slope of the calibration curve[24]. LOD and LOQ values confirmed the sensitivity of the proposed methods, were shown in **Table 1.** These results demonstrated good precision, accuracy and reproducibility. The solutions did not show any appreciable change in assay values even after two week period. However, all solutions used for the validation experiments were freshly prepared to ensure the stability of analyte in the solutions.

**Application to Analysis of Pharmaceuticals:**
The validity of the proposed DPAAdSV and SWAAdSV methods at the glassy carbon electrode for the determination of meropenem in pharmaceutical dosage forms was investigated by assaying injection dosage form. (**Fig.4, 5**) illustrate the response of rise over successive concentration of meropenem in injection dosage form by

DPAAdSV and SWAAdSV. Without any sample extraction or filtration step, but just through dissolution and an adequate dilution of the analyte present in the solution of Merocrit injection. Both methods can be used for the direct determination of meropenem using the related calibration straight line. Recovery studies were carried out after addition of known amounts of pure drug to various pre-analysed formulations of meropenem.
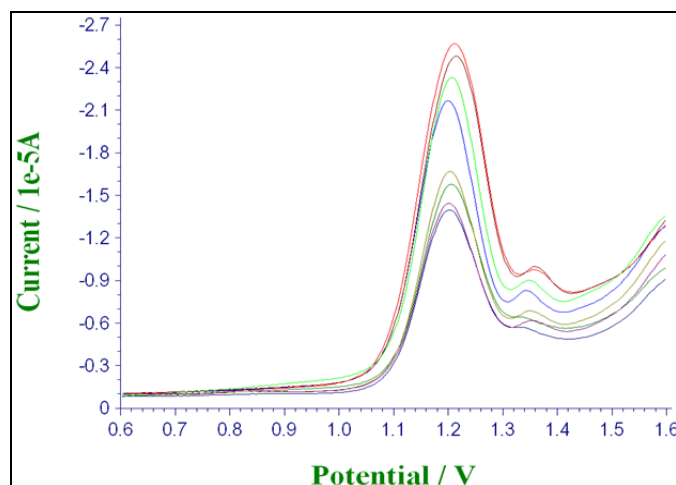
In order to detect interactions of the excipients, the standard addition technique was applied to the same preparations, which were analysed by calibration curve. The results showed that the proposed methods were successfully applied for the assay of meropenem in its injection dosage form (**Table 2**). The results showed that the proposed methods could be applied with great success for meropenen assay in pharmaceuticals without any interference.

**TABLE 1: REGRESSION DATA OF THE CALIBRATION LINES FOR QUANTITATIVE DETERMINATION OF MEROPENEM BY DPAAdSV AND SWAAdSV TECHNIQUES.**

| Analytical Parameter | DPAAdSV | SWAAdSV |
|---|---|---|
| Medium | Aqueous | Aqueous |
| Measured potential (V) | 1.21 | 1.15 |
| Linearity range | $1.0 \times 10^{-6}$ - $4.0 \times 10^{-4}$ M | $1.0 \times 10^{-6}$ - $4.0 \times 10^{-4}$ M |
| Slope of the calibration curve ($AM^{-1}$) | 0.1635 | 0.1447 |
| Intercept ($\mu A$) | 1.418 | 3.8555 |
| Correlation coefficient | 0.9975 | 0.997 |
| LOD (M) | $1.27 \times 10^{-7}$ | $2.24 \times 10^{-7}$ |
| LOQ (M) | $4.25 \times 10^{-7}$ | $7.47 \times 10^{-8}$ |
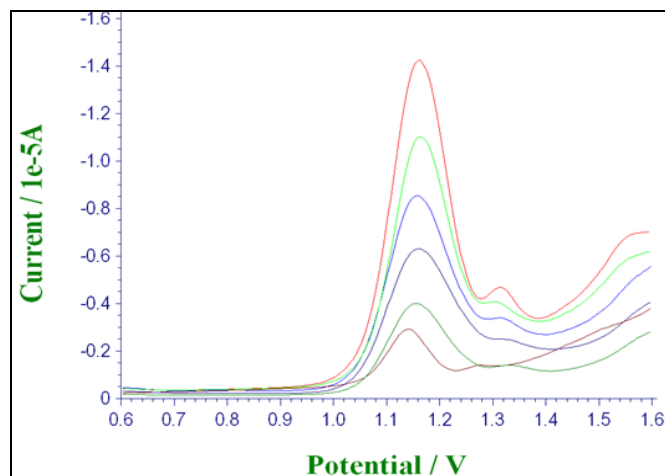


**FIG.4: DPAADS VOLTAMOGRAMS OF MEROPENEM AT DIFFERENT CONCENTRATION ($2 \times 10^{-6}$ TO $1.2 \times 10^{-5}$M)**
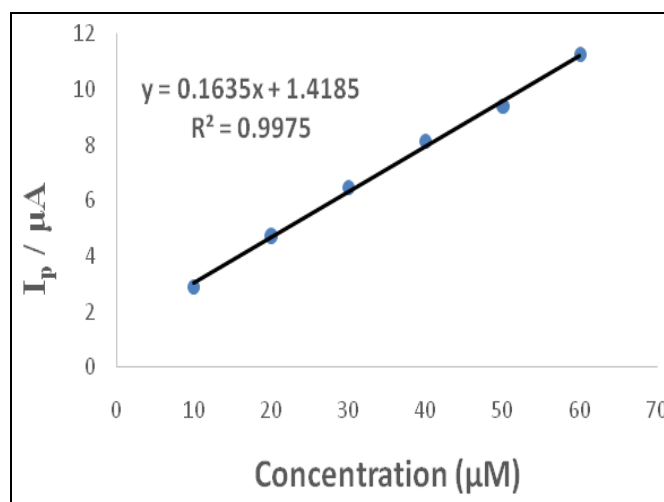
Under the optimized condition a linear correlation between DPAAdSV and SWAAdSV peak intensity and the drug concentration was obtained over the range $1.0 \times 10^{-6}$ - $4.0 \times 10^{-5}$ M for both DPAAdSV and SWAAdSV. The calibration equation can be written in the form (**Fig. 6, 7**)

$$I_p(\mu M) = 0.1635\ C\ (\mu M) + 1.4185 R^2 = 0.9975$$
(For DPAAdSV) (v)

$$I_p\ (\mu A) = 0.1447\ C\ (\mu M) + 3.8555 R^2 = 0.997$$
(For SWAAdSV) (vi)



**FIG.6: ANODIC PEAK CURRENT ($i_p / \mu A$) AS A FUNCTION OF CONCENTRATION ($\mu M$) OF MEROPENEM FOR DPAAdS VOLTAMMOGRAMS**



**FIG.5: SWAADS VOLTAMOGRAMS OF MEROPENEM AT DIFFERENT CONCENTRATION ($2 \times 10^{-6}$ TO $1.6 \times 10^{-5}$M)**
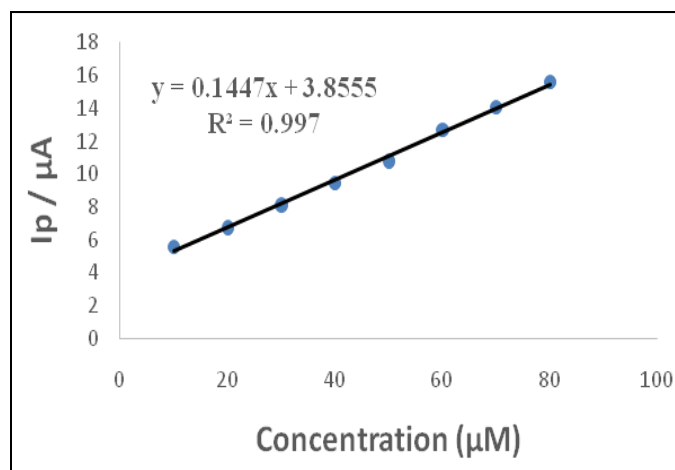
**FIG.7: ANODIC PEAK CURRENT (i$_p$/ µA) AS A FUNCTION OF CONCENTRATION (µM) OF MEROPENEM FOR SWAAdS VOLTAMMOGRAMS**

**TABLE 2: THE RESULTS FOR THE DETERMINATION OF MEROPENEM (MEROCRIT) FROM INJECTION DOSAGE FORM AND ACHIEVED RECOVERY OF DPAAdSV AND SWAAdSV EXPERIMENTS.**

| Parameter | DPAAdSV | SWAAdSV |
|---|---|---|
| Medium | Aqueous | Aqueous |
| Labelled claimed(mg) | 500.00 | 500.00 |
| Amount found (mg) | 499.65 | 499.69 |
| Average recovered % | 99.93 | 99.94 |
| RSD of recovery | 0.43 | 0.96 |

**CONCLUSIONS:** The voltammetric oxidation step of meropenem in different buffer solutions between pH 2.8 to 7.0 has been elucidated. The meropenem is irreversibly oxidized at high positive potentials. The proposed DPAAdSV and SWAAdSV technique for the determination of meropenem in pharmaceutical dosage form was found to be as simple and rapid as the reported method and even more selective, sensitive and validated than the reported method. The proposed voltammetric methods can be applied directly to the analysis of pharmaceutical dosage forms without the need of separation or complex sample preparation, since there was no interference from the excipients and endogenous substances. The proposed method is fully validated.

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