



## Determination of Meropenem by Spectrophotometric-Application to Pharmaceutical Preparations

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DOI: <http://dx.doi.org/10.25130/tjps.25.2020.012>

### ARTICLE INFO.

#### Article history:

-Received: 17 / 6 / 2019

-Accepted: 10 / 11 / 2019

-Available online: / / 2019

**Keywords:** Meropenem, spectrophotometry, charge transfer.

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### 1- Introduction

Meropenem (MER) Fig. (1) is abroad –spectrum antibacterial agent, with activity against the majority of gram positive, gram negative and anaerobic bacteria, MER is colorless to white crystals. springily soluble in water, very slightly soluble in alcohol. practically insoluble in acetone and in ether [1].MER ( $t_{1/2}$ , 1h) is similar to imipenem but it is stable to renal dihydropyridase[2].

MER was determined in pure and pharmaceutical formations by formation of dark yellow colored product between MER and 1,2-naphthoquinone-4-sulphonic acid measured at 449 nm in basic medium[3].The drug was determined by formation of a color red chromogen with brucine and sodium periodate in acidic medium, the maximum absorption was at 523 nm, Beer's law was obeyed in the range of concentration between (2-12 $\mu$ g/ml) of MER the apparent was 0.07 $\mu$ g.cm<sup>-2</sup>[4].

The stability of the drug has been studied at two temperatures 4.25 °C and 40°C in both acidic and basic medium using UV spectrophotometric technique at 280 nm. [5].

An ion pair between MER and bromothymol and bromocresol at (420 and 418) nm was studied respectively. The method was sensitive but need to extraction steps[6].

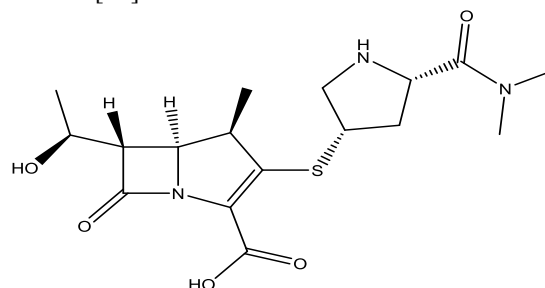
Other methods were used for determination of MER based on chromatographic procedures were also reported[7,8,9,10,11].

### ABSTRACT

A simple and sensitive spectrophotometric method was described for the determination of Meropenem in pure and in pharmaceutical formulations. 2,3 dichloro 5,6 dicyano 1,4 benzoquinone(DDQ) has been used for determination of meropenem by formation of charge transfer complex measured at 345 nm. Beer's law is obeyed in the concentration range of (0.625-12.5 $\mu$ g/ml) The molar absorptivity ( $2.3889 \times 10^4$ ) l.mol<sup>-1</sup>.cm<sup>-1</sup>, Sandell's sensitivity index is 0.0161 $\mu$ g.cm<sup>-2</sup>, The method is precise (relative standard deviation RSD% is better than  $\pm 3.32\%$ ) and accurate (relative error in the range of -0.97 to -0.60%) depending on the concentration level.

The method was applied successfully to the assay of Meropenem in pharmaceutical preparation in the form of injection.

2,3-dichloro-5,6-dicyano 1,4-benzoquinone has been used for determination of domperidone. The formed complex exhibits maximum absorption intensity at 458 nm in acetonitrile and 394 nm in chloroform[12]. The present study included determination of MER as a  $\pi$ -electron donor, with 2,3-dichloro 1,4-dicyanobenzoquinone as an acceptor, and applied the method to determine MER in pharmaceutical preparations. Chemically MER is 3-[5-(dimethylcarbamoyl) pyrrolidin-2-yl] sulfanyl-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid with the following structure [13].



**Fig.1: structure of Meropenem**  
 $C_{17}H_{25}N_3O_5S$  M.Wt = 383.46 g.mol<sup>-1</sup>

### 2- Experimental

#### Apparatus

The spectrophotometric measurements were carried out on UV – Visible spectrophotometer CARY 100

Conc. double beam, using 1 cm quartz cells, pH measurements have been done by senso direct pH200 (Lovi bond) and Denever Instrument balance has been used for weight measurement.

### Reagents and solution

All chemicals are used of an analytical grade. MER was purchased from the state company for drug industries and medical applications (Astrazeneca UK limited).

**MER solution  $100 \mu\text{g}.\text{ml}^{-1}$ :** was prepared by dissolving 0.01g of MER in 100 ml distilled water in a volumetric flask.

**MER solution  $25 \mu\text{g}.\text{ml}^{-1}$ :** A 12.5 ml of  $100 \mu\text{g}.\text{ml}^{-1}$  is diluted with distilled water to the mark in 50 ml volumetric flask.

### Coupling reagent ( $1.1 \times 10^{-4}$ )M :

2,3 Dichloro-5,6-dicyano-1,4-benzochinon solution (DDQ) was prepared by dissolving 0.0012 g of DDQ (Fluka Agbuchs.SG) in 5ml ethanol then the volume was completed to 50 ml with distilled water in volumetric flask.

### Buffer Solution pH7

Borate buffer solution was prepared by dissolving 0.05 M Disodium tetra borate with 10% Boric acid [14].

## Results and Discussion

### Optimization of Reaction

#### Selection of coupling reagent

Effect of different coupling agents on the absorption intensity and color contrast has been investigated for better analytical results. The reagents tested are: 2,3-Dichloro 1,4-naphthochinon, Sulphonimid, 7,7,8,8-tetracyanoquinon dimethan. Only 2,3-Dichloro-5,6-dicyano-1, 4benzochinon gives maximum absorption intensity with a good color contrast ( $\Delta\lambda = 40\text{nm}$ ), therefore it is selected for subsequent investigations.

### Study of reaction medium

#### Effect of Buffer solution:

The effect of buffer solution on the absorbance of formed colored products was studied by using 1ml of various types of buffer (phosphate, Borate, Carbonate). The obtained results in table (1) showed that the maximum absorbance was attained using 1 ml of borate buffer.

**Table 1: Effect of Buffer**

| 1ml of buffer solution added | Absorbance | pH   |
|------------------------------|------------|------|
| Borate Buffer                | 0.422      | 7.0  |
| Phosphate Buffer             | 0.367      | 6.9  |
| Carbonate                    | 0.342      | 6.95 |

#### Effect of borate buffer volume :

The effect of different volume of borate buffer was studied, the highest absorbance for complex is shown using 1 ml of borate buffer. Table(2)

**Table 2: Effect of borate buffer volume**

| ml of borate buffer | Absorbance |
|---------------------|------------|
| 0.25                | 0.356      |
| 0.5                 | 0.392      |
| 1                   | 0.423      |
| 1.5                 | 0.385      |
| 2                   | 0.364      |
| 2.5                 | 0.350      |

#### Effect of coupling agent amount:

Effect of (0.5-4) ml of  $1.1 \times 10^{-4}$  M DDQ reagent has been added to increasing concentration of drug and keeping the concentration of buffer constant, then diluted to the mark by distilled water and wait for (45minute) at room temperature, then measure the absorbance against blank solution at 345 nm. Table(3)

**Table 3: Effect of coupling agent amount**

| ml of $1.1 \times 10^{-4}$ M of DDQ | Absorbance / $\mu\text{g}$ of MER |       |       |       |       | Determination coefficient $R^2$ |
|-------------------------------------|-----------------------------------|-------|-------|-------|-------|---------------------------------|
|                                     | 5                                 | 10    | 25    | 50    | 75    |                                 |
| 0.5                                 | 0.061                             | 0.075 | 0.093 | 0.142 | 0.198 | 0.9928                          |
| 1                                   | 0.091                             | 0.122 | 0.165 | 0.229 | 0.301 | 0.9934                          |
| 1.5                                 | 0.163                             | 0.199 | 0.233 | 0.321 | 0.388 | 0.9921                          |
| 2                                   | 0.243                             | 0.267 | 0.323 | 0.432 | 0.498 | 0.9914                          |
| 2.5                                 | 0.306                             | 0.342 | 0.423 | 0.522 | 0.655 | 0.9959                          |
| 3                                   | 0.322                             | 0.377 | 0.461 | 0.573 | 0.713 | 0.9930                          |
| 3.5                                 | 0.335                             | 0.399 | 0.495 | 0.631 | 0.765 | 0.9910                          |
| 4                                   | 0.431                             | 0.453 | 0.534 | 0.633 | 0.789 | 0.9934                          |

Table (3) shows that an increasing intensity of the colored product with increasing the volume of DDQ reagent but 2.5 ml gives the best value of determination coefficient therefore, it is selected.

#### Effect of temperature:

The effect of temperature on the intensity of product complex was studied at temperatures (R.T. 5, 35 and,  $50^\circ\text{C}$ ). The obtained results, showed that maximum

absorbance occurs at R.T. Table (4) shows that the reaction need 45 min to reach completion.

**Table 4: Effect of temperature with time**

| Time | Absorbance of 25 µg / ml of MER |       |       |       |
|------|---------------------------------|-------|-------|-------|
|      | R.T                             | 5°C   | 35°C  | 50°C  |
| 5    | 0.188                           | 0.047 | 0.061 | 0.072 |
| 10   | 0.225                           | 0.074 | 0.080 | 0.081 |
| 15   | 0.246                           | 0.081 | 0.092 | 0.099 |
| 20   | 0.262                           | 0.094 | 0.101 | 0.112 |
| 25   | 0.291                           | 0.110 | 0.120 | 0.121 |
| 30   | 0.314                           | 0.117 | 0.128 | 0.129 |
| 35   | 0.344                           | 0.131 | 0.130 | 0.138 |
| 40   | 0.380                           | 0.142 | 0.140 | 0.147 |
| 45   | 0.423                           | 0.155 | 0.152 | 0.151 |
| 50   | 0.421                           | 0.141 | 0.143 | 0.139 |
| 55   | 0.420                           | 0.133 | 0.132 | 0.129 |
| 60   | 0.420                           | 0.120 | 0.121 | 0.119 |

**Effect of surfactants:**

In order to study the effect of surfactants on absorption intensity, 3 ml of anionic sodium dodecyl sulphate (SDS), cationic (cetylpyridinium chloride (CPC), and neutral (cetyltrimethyl ammonium bromide) (CTAB) surfactants were used at different order of additions. As shown in Table(5)

**Table (5): Effect of surfactant**

| Order                            | CTAB  | CPC   | SDS   |
|----------------------------------|-------|-------|-------|
| MERO + DDQ + buffer + surfactant | 0.237 | ----- | 0.213 |
| MERO + DDQ + surfactant + buffer | 0.249 | ----- | 0.205 |
| MERO + surfactant + DDQ + buffer | 0.203 | ----- | 0.222 |
| MERO + buffer + surfactant + DDQ | 0.299 | ----- | 0.218 |

(\*) reading without surfactant is equal 0.422 (order: MER+DDQ+buffer)

The addition of CPC 4 order produced turbid solution and the addition of SDS decreases the absorption intensity while the addition of CTAB doesn't exhibit any change on absorption intensity, therefore the use of surfactant was excluded.

**Effect of order addition of reactants :**

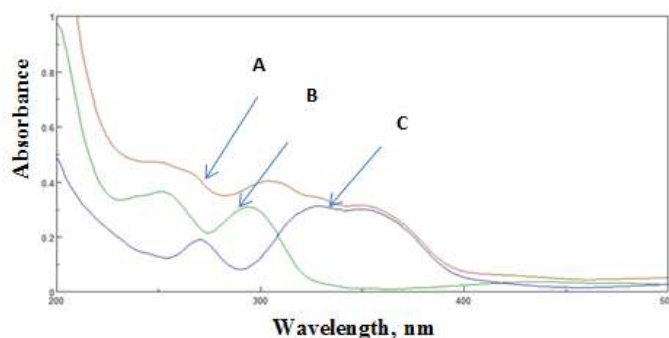
Few trials were performed to check the influence of order of addition of reactants on the color development and the results are presented in table (6). The order of addition of serial number (III) is recommended.

**Table 6: Effect of order of Addition of reactants on color development.**

| Drug | Order of addition      | Absorbance | Recommended Order of addition |
|------|------------------------|------------|-------------------------------|
| MER  | (I) D + DDQ + Borate   | 0.418      | III                           |
|      | (II) DDQ + D + Borate  | 0.411      |                               |
|      | (III) Borate + D + DDQ | 0.426      |                               |
|      | (IV) Borate + DDQ + D  | 0.420      |                               |

**Absorption spectrum:**

Under the optimum reaction conditions studied as above, the absorption spectrum of the product against blank (fig 2) shows that the wavelength of the maximum absorption intensity is 345 nm. This wavelength has been used in subsequent investigations.

**Fig . 2: Absorption spectrum of Meropenem**

A : sample against blank

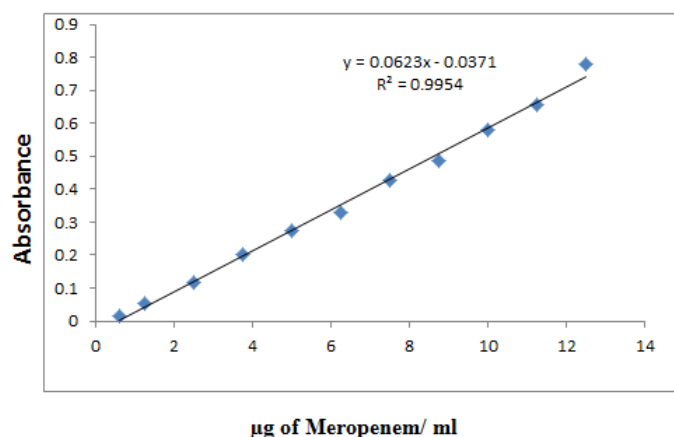
B : sample against distilled water

C : Blank against distilled water

**Procedure and Calibration curve**

To increasing volumes (0.25-5) ml of 25 µg.ml<sup>-1</sup> of standard (MER) solution following reagents has been added in the following order: 2.5 ml DDQ 25 µg.ml<sup>-1</sup>

, 1ml of borate buffer pH7, The volume completed to 10 ml. In volumetric flask with distilled water, the absorbance has been measured at 345nm against blank.



**Fig. 3: calibration curve of spectrophotometric method**

A linear calibration curve is obtained over the range (6.25-125 μg) of MER in 10 ml ( $0.625 - 12.5 \mu\text{g ml}^{-1}$ ) with Molar absorptivity ( $2.3889 \times 10^4$ )  $\text{l.mol}^{-1}.\text{cm}^{-2}$  and sandell's sensitivity index ( $0.0161$ )  $\mu\text{g.cm}^{-2}$

#### Accuracy and precision :

To check the accuracy and precision of the calibration curve. MER was determined at three different concentrations and the determinations were replicate five times, the results are shown in table (7), which indicates good accuracy and precision.

**Table 7: Accuracy and Precision of the calibration curve**

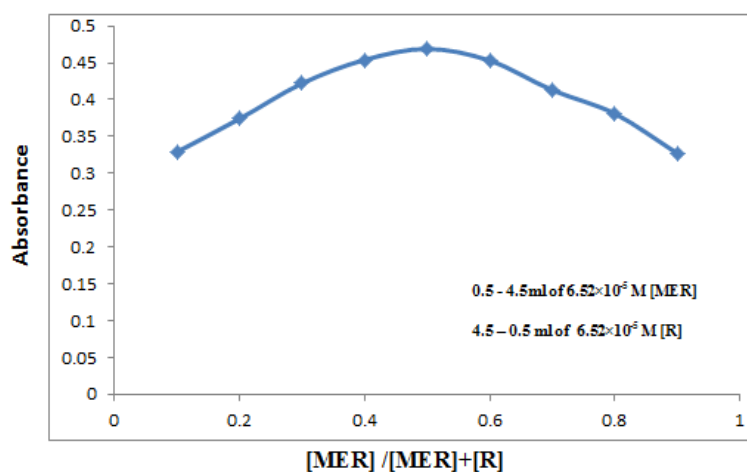
| Amount of MER taken $\mu\text{g}/10 \text{ ml}$ | Found of MER $\mu\text{g}/10 \text{ ml}$ | Relative error, % * | Relative standard deviation, % * |
|-------------------------------------------------|------------------------------------------|---------------------|----------------------------------|
| 10                                              | 9.903                                    | -0.97               | $\pm 3.32$                       |
| 50                                              | 49.73                                    | -0.54               | $\pm 1.23$                       |
| 100                                             | 99.40                                    | -0.60               | $\pm 1.39$                       |

(\*):Average of five determinations .

#### Stoichiometry of the Dye:

The ratio of the drug to the reagent has been studied using both mole ratio method and Job method [15] ,

Fig (4) and Fig (4) show that the ratio Drug : reagent is 1: 1.



**Fig .4: Job's method**

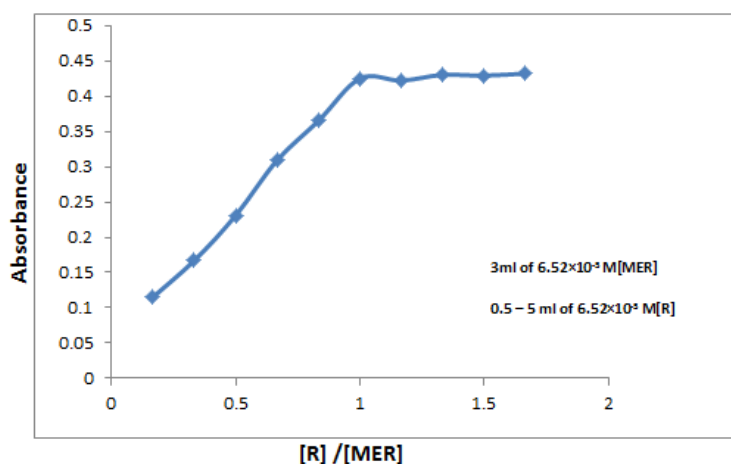


Fig.5: Mole-ratio method

**Effect of organic solvents:** The spectrophotometric characteristics of the colored product are more detectable using water.

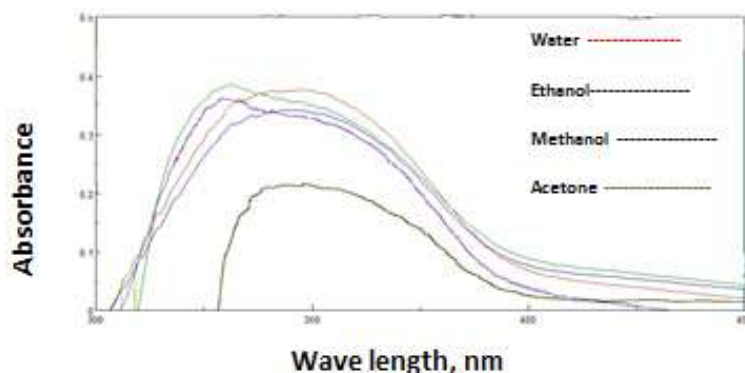


Fig.6: Effect of organic solvent

**Study of Interferences :** In order to realize the analytical application of this method, effect of some excipients has been studied by carrying out the determination of 25 µg of MER in 10 ml final volume in the presence of different excipients (glucose, starch, gum Arabic and lactose), the results exhibits no any interfering effect caused by supposed additives. As presented in Table(8).

Table 8: Effect of excipients on assay of MER

| Interferences | Recovery 2.5 µg of MER/µg of Interferences |       |      |
|---------------|--------------------------------------------|-------|------|
|               | 50                                         | 100   | 150  |
| Starch        | 100.3                                      | 99.7  | 99.1 |
| Glucose       | 100.1                                      | 100.3 | 98.9 |
| Arabic Gum    | 99.6                                       | 98.3  | 97.5 |
| Lactose       | 99.4                                       | 98.5  | 99.8 |

#### Application of the method:

To test the applicability of the present method, it is applied to the estimation of MER in pharmaceutical preparations. The results are listed in Table(9) indicating a good applicability of the method. The performance of the proposed method was assessed by calculation of the t-test compared with the literature method [16] for 95% confidence level with eight degrees of freedom. The results showed that the t – value was less than the tabulated value = 2.306, so, there was no significant difference between the proposed and literature method for MER (Table 10).

Table 9: Application of method

| Pharmaceutical preparation                        | µg MER present/10 ml | µg MER measured/10 ml | Recovery %* | RSD % |
|---------------------------------------------------|----------------------|-----------------------|-------------|-------|
| MER Injection (10 mg/2 ml Astrazeneca UK limited) | 10                   | 10.23                 | 102.32      | 0.512 |
|                                                   | 25                   | 25.58                 | 102.32      | 0.198 |
|                                                   | 50                   | 51.16                 | 102.32      | 0.276 |

\* Average of five determinations .

Table 10: The results of t-test analysis

| Pharmaceutical preparation                        | Recovery %     |                                   | t.exp |
|---------------------------------------------------|----------------|-----------------------------------|-------|
|                                                   | Present method | Literature method <sup>[16]</sup> |       |
| MER Injection (10 mg/2 ml Astrazeneca UK limited) | 98.6           | 99.2                              | 1.84  |

## Comparison of Method

Table 11: Comparison of the method

| Analytical parameters                                       | Present method       | Literature method [3] | Literature method [6]          |
|-------------------------------------------------------------|----------------------|-----------------------|--------------------------------|
| Method                                                      | Charge-transfer      | Charge-transfer       | Ion –pair                      |
| Reagent                                                     | DDQ                  | NQS*                  | -Bromothymol and --bromocrysol |
| Temperature (°c )                                           | At room temperature  | -----                 | -----                          |
| $\lambda_{\max}$ (nm)                                       | 345                  | 449                   | -420<br>-418                   |
| Medium of method                                            | pH 7                 | Alkaline              | pH 3                           |
| Color of the dye                                            | Light orange         | Dark yellow           | -Blue<br>-purple               |
| Barr's law range (ppm)                                      | 0.625 -12.5          | 20-120                | 12.5-62.5                      |
| Molar absorptivity (l.mol <sup>-1</sup> .cm <sup>-1</sup> ) | $2.3812 \times 10^4$ | $7 \times 10^4$       | $1.43 \times 10^3$             |
| Pre-separation                                              | Non                  | -----                 | Solvent extraction             |
| Stability of the color                                      | 45 min.              | 60                    | -----                          |
| Application of the method                                   | Injection powder     | Injection powder      | Injection powder               |

\*NQS: 1,2 naphthoquinone -4-sulphonic acid sodium salt

From table(13) the present method is sensitive, need not to any extraction steps and it is applicable for determination of MER in pharmaceutical preparation.

### Conclusion

A sensitive new procedure for determination of meropenem has been established, the method is

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simple, accurate, economic, need not to any separation steps and ,or adjustment of temperature, and the method was applied for determination of meropenem in pure and dosage form with good accuracy.

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## تقدير الميروبينيم بالطريقة الطيفية في المستحضرات صيدلانية

ندى احمد خليل ، ولادة حميد ابراهيم

كلية الصيدلة ، جامعة الموصل ، الموصل ، العراق

### الملخص

تم تقدير الميروبينيم بطريقة سهلة وحساسة في النموذج النقي وفي مستحضراته الصيدلانية. الطريقة تعتمد على تفاعل انتقال الشحنة وتكوين معقد بين المركب الدوائي الميروبينيم والكاشف 3,2- ثنائي كلورو 6,5- ثنائي سيانو 4,1- بنزوكوينوناذ يتكون معقد ملون يعطي أعلى امتصاص عند الطول الموجي 345 نانومتر تتبع الطريقة قانون بير في مدى التركيز (0.625-12.5) مايكرو غرام / مل. كانت قيمة معامل الامتصاص المولاري ( $2.3889 \times 10^4$ ) لتر.مول<sup>-1</sup>سم<sup>-1</sup> ودلالة ساندل 0.0161 مايكرو غرام. سم<sup>-2</sup> كان الانحراف القياسي النسبي أفضل من  $\pm 3.32\%$  وتراوح الخطأ النسبي بين 0.97- إلى 0.60 %.

تم تطبيق الطريقة بنجاح في تقدير الميروبينيم في مستحضراته الصيدلانية (الحقن).