Spectrophotometric Determination of Mefenamic acid using Metol reagent by Oxidative Coupling Reaction

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Abstract: A simple, sensitive, and accurate spectrophotometric method for the determination of Mefenamic acid is described. The method is oxidative coupling reaction of Mefenamic acid with metol using potassium periodate as an oxidizing agent in alkaline medium to form a water-soluble product, that is stable and has a maximum absorption at 533 nm. Beer’s law is obeyed in a concentration range of 2.4-24 µg/ml Mefenamic acid with a molar absorptivity of 7.2x10³ L/mol.cm. The proposed method has been successfully applied to the determination of Mefenamic acid in various pharmaceutical preparations.

1. Introduction:
Mefenamic acid is 2-[(2,3-dimethyl phenyl) aminobenzoic acid. MA an anthranilic acid derivative is a member of the fenamate group of non-steroidal anti-inflammatory drugs (NSAIDs). It exhibits anti-inflammatory, analgesic, and antipyretic activities. It is used for the relief of mild to moderate pain. It is also indicated for the treatment of rheumatoid arthritis. Similar to other NSAIDs, MFA inhibits prostaglandin synthetase [1]. The drug is official in British Pharmacopoeia and the assay is based on the non-aqueous titrimetric method [2]. A literature survey reveals that various analytical techniques were used for the determination of MA such as UV spectrophotometry [3-6], High-performance liquid chromatography [7-10]. Therefore, the need for a fast, low cost and selective method is obvious, especially for the routine quality control analysis of pharmaceutical products containing Mefenamic acid. The proposed method was successfully applied to the determination of Mefenamic acid in bulk pharmaceutical, tablets, and capsules. The results obtained by the proposed method were in excellent agreement with those given by the official method [2], proving that the method is a reliable alternative for the analysis of Mefenamic acid in pure form and pharmaceutical preparations.

2. Material and Methods
All absorption measurements were obtained by using a double beam Shimadzu 1800, Kyoto, Japan UV-Visible Spectrophotometer with 40 mm matched quartz cells and the pH measurements were performed by using i-Trans, BP 3001, Singapore pH meter.

3. Results and discussion

3.1. The general principle of the method.
The principle of the method is to associate the solution of the drug Mefenamic acid with the metol reagent in the presence of potassium and sodium hydroxide, as it is produced in a violet color that gives the highest absorption at the wavelength of 533 nm versus the blank.

3.2. Preparatory.
When adding 2 ml of potassium Periodate solution with a concentration of 10⁻² molar to 1 ml of Mefenamic acid solution with a concentration of 300 mg/ml and then adding 3 ml of a solution of metol 10⁻² molar in the presence of 2 ml of sodium hydroxide at a concentration of 0.1 molar as a
purple-colored product is formed. And the volume was completed in a 25 ml volumetric flask up till the mark with water. The absorption spectrum of the colored product was measured as it gave $\lambda_{\text{max}}$ 533 nm versus the imaging solution.

3.3 Control the optimum conditions for the reaction.

At the beginning of setting the optimal conditions, the following experiments were fixed using 1 ml of Mefenamic acid solution with a concentration of 300 mg/ml at a final volume of 25 ml. The absorption of the solutions was measured at the wavelength of 533 nm opposite the imaging solution using glass cells width 1 cm.

3.3.1. Coupling detector.

3 ml of each of the reagents used with a concentration of $10^{-2}$ molar was taken in a 25 ml volumetric flask. Each contains 1 ml of drug solution of Mefenamic acid at a concentration of 300 mg/ml in the presence of 3 ml of potassium Periodate solution at a concentration of $10^{-2}$ molar and added to it. 2 ml of sodium hydroxide with a concentration of 0.1 molar s. The absorption spectrum of the colored product and the results are measured as in Table 1.

| Table 1. Coupling detector |
|---------------------------|
| Reagent Conc.10^{-2} M | $\lambda_{\text{max}}$ nm | Absorbance |
|-------------------------|-----------------|-------------|
| N,N-Dimethyl-p-phenylene diamine dihydrochloride | 495 | 0.158 |
| 2- aminothiazol | 497 | 0.118 |
| 4- Nitroaniline | 495 | 0.113 |
| Metol | 533 | 0.656 |

From the results are shown in the table above, when the metol was chosen as a coupling reagent as it gave the highest absorption of the colored output at the wavelength of 533 nm and this wavelength was chosen in subsequent experiments

3.3.2. Effect of pairing detector amount

The effect of the coupling reagent quantity was studied by taking different sizes 2.5-0.5 ml of the metol reagent solution at a concentration of $10^{-2}$ molar with different sizes 0.5, 1, 1.5 ml of the drug solution of Mefenamic acid at a concentration of 300 mg/ml final concentration (6,12,18 mg). In the presence of 2 ml of the oxidizing agent solution at a concentration of $10^{-2}$ molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 molar s and complete the volume up till the mark in the 25 ml volumetric flask with water. Then the absorption of the solutions was measured at the wavelength of 533 nm and the results are listed in Table 2.

| Table 2. Effect of pairing detector amount |
|-------------------------------------------|
| ml of Reagent 1x10^{-2} M | Abs/ml of Mefenamic Acid 300µg/ml |
|----------------------------|----------------------------------|
|                            | 6 | 12 | 18 |
| 2                         | 0.205 | 0.454 | 0.610 |
| 2.5                       | 0.330 | 0.635 | 0.697 |
| 3                         | 0.390 | 0.656 | 0.742 |
| 3.5                       | 0.402 | 0.601 | 0.668 |
| 4                         | 0.245 | 0.569 | 0.696 |
| 4.5                       | 0.237 | 0.551 | 0.627 |
It was noted from the findings shown in the table above that the volume of 3 ml of a solution of metol with a concentration of $10^{-2}$ molar gives the highest absorption of the colored output so it was adopted in subsequent experiments.

3.3.3. The best oxidizing agent.

2 ml were taken from each of the oxidizing agent solutions used at a concentration of $10^{-2}$ molar and added 1 ml of mefenamic acid solution at a concentration of 300 mg/ml and 3 ml of a metol solution reagent with a concentration of $10^{-2}$ molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 Molar in a 25 ml volumetric flask and complete the volume up to the mark with water. Then the absorption of the solutions was measured at the wavelength of 533 nm, and the findings are shown in Table 3.

| Oxidizing agent 10^{-2}M | Abs. | max\(\lambda\) |
|--------------------------|------|--------------|
| N-Bromosuccinimide       | 0.085| 499          |
| Potassium Periodate      | 0.654| 533          |
| Ammonium Persulfate      | 0.097| 320          |
| Potassium thiosulfate    | 0.279| 539          |

As the above table shows that the oxidizing agent of potassium Periodate with a concentration of $10^{-2}$ molar gave the highest absorption of the product, therefore potassium Periodate was adopted as the best oxidizing agent in subsequent experiments.

3.3.4. Effect of oxidizing agent amount.

The amount of potassium Periodate with a concentration of $10^{-2}$ molar was studied by adding volumes 1-3.5 ml to a 25 ml volumetric flask containing 1 ml of Mefenamic acid concentration of 300 mg/ml and 2 ml of metol reagent solution at a concentration of $10^{-2}$ Molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 molar. Then the volume was completed up to the mark in a 25 ml volumetric flask with water. Absorption was measured at 533 nm wavelength and the results are listed in Table 4.

| ml of 10^{-2}M of potassium Periodate | Abs. |
|---------------------------------------|------|
| 1                                     | 0.502|
| 1.5                                   | 0.536|
| 2                                     | 0.658|
| 2.5                                   | 0.539|
| 3                                     | 0.389|
| 3.5                                   | 0.377|

It was noted from the above findings shown in the table that the best volume gives the highest absorption is 2 ml and was relied upon in subsequent experiments.

3.3.5. Effect of the base

This study was carried out with various volumes ranging from (0-4 ml) from the NaOH base solution with 0.1 molar concentration to 1 ml of the Mefenamic solution with a concentration of 300 mg/ml in the presence of 2 ml of the oxidizing agent potassium Periodate solution at a concentration of $10^{-2}$ molar then added to it 3 ml of solution for the metol reagent at a concentration of $10^{-2}$ molar and complete the volume up to the mark in a 25 ml volumetric flask with water. Absorption was measured at 533 nm wavelength and the acidic function of solutions and results was measured as in Table 5.
Table 5. Effect of the base

| ml of NaOH (0.1 M) | Abs. | pH |
|-------------------|------|----|
| Without           | 0.387| 6.7|
| 1                 | 0.464| 10.6|
| 1.5               | 0.611| 10.7|
| 2                 | 0.658| 11.1|
| 2.5               | 0.520| 11.3|
| 3                 | 0.467| 11.4|
| 3.5               | 0.343| 11.5|
| 4                 | 0.318| 11.4|

So, the above table shows that when using 2 ml of the base that gives the highest value of absorption (acid function 11.15) in subsequent experiments.

3.4 Stability of the reaction output.

This study was carried out by taking three different volumes (0.5, 1, and 1.5 ml) of Mefenamic acid solution with a concentration of 300 mg/ml, its final concentrations are 6, 12, and 18 mg/ml, respectively, and adding 2 ml of the metol solution with a concentration of $10^{-2}$ molar and adding 2 ml of potassium Periodate solution with a concentration of $10^{-2}$ molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 molar concentration in a 25 ml volumetric flask and complete the volume to the mark of the mark with water, then the absorption was measured for each model against the blank solution, and the results are shown in Table 6.

Table 6. Stability of the reaction output.

| Time (minute) | The absorbance of Mef., µg/ml |
|--------------|-------------------------------|
|              | 6    | 12   | 18   |
| 5            | 0.211| 0.487| 0.752|
| 10           | 0.222| 0.513| 0.755|
| 15           | 0.246| 0.578| 0.757|
| 20           | 0.355| 0.656| 0.763|
| 25           | 0.380| 0.611| 0.766|
| 30           | 0.405| 0.619| 0.771|
| 35           | 0.255| 0.638| 0.770|
| 40           | 0.250| 0.633| 0.764|
| 50           | 0.247| 0.591| 0.776|
| 60           | 0.239| 0.588| 0.775|

It was found that the absorption value of the colored product remains stable for not less than 60 minutes and is an appropriate time to complete many of the measurements and results as in the table above.

3.5 Solvent effect

The effect of some solvents was studied on the product formed from the reaction of 1 ml Mefenamic acid at a concentration of 300 mg/ml and in the presence of 2 ml of potassium Periodate at a concentration of $10^{-2}$ molar and 3 ml of metol reagent at a concentration of $10^{-2}$ molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 molar The volume was supplemented with
different solvents in a 25 mL volumetric flask up to the mark. The absorption spectrum was taken for each solution versus its phototoxic solution. The results are shown in Table 7 and Figure 1.

### Table 7. Solvent effect

| Solvent   | Absorbance | \(\lambda_{\text{max}}\) |
|-----------|------------|---------------------------|
| Ethanol   | 0.593      | 541                       |
| Acetone   | 0.671      | 544                       |
| Methanol  | 0.577      | 543                       |
| Water     | 0.655      | 533                       |

As the results are shown in the above table and Figure 1 that water is the appropriate medium for reaction and gives the highest absorption at a wavelength of 533 nm as well as for its availability and cheapness it was used in all experiments.

![Figure 1. Solvent effect C: Ethanol; B: Methanol; A: Water; D: Acetone.](image)

#### 3.6. Final absorption spectrum.

The final absorption spectrum was measured by using 1 ml of Mefenamic acid at a concentration of 300 mg/ml and with 2 ml of potassium Periodate at a concentration of \(10^{-2}\) molar and 3 ml of a metol reagent solution at a concentration of \(10^{-2}\) molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 molar and the temperature is 20°C and the time is stable for not less than 60 minutes and complete the volume up to the mark in a 25 ml volumetric flask with water, where the absorption spectrum of the purple color product was measured against the phototoxic solution that gives the highest absorption at the wavelength of 533nm while giving The solution shows slight absorption versus distilled water at the same wavelength Figure 2.

![Figure 2. Final absorption spectrum.](image)
Figure 2. The final absorption spectrum for determination of Mefenamic acid. SW: The absorption spectrum of Mefenamic acid solution opposite distilled water; SB: The absorption spectrum of Mefenamic acid solution versus imaging solution; BW: The absorption of the sodium solution versus distilled water

3.7. Optimization and calibration method

A series of 25 ml volumetric flask containing increasing volumes of 0.2-0.2 ml of Mefenamic acid solution at a concentration of 300 mg/ml final concentrations 2.4-24 mg/ml and with 2 ml potassium Periodate at a concentration of 10⁻² molar and 3 ml of the solution The metol reagent at a concentration of 10⁻² molar and 2 ml of sodium hydroxide solution at a concentration of 0.1 molar and diluted with distilled water up to the mark limit, and with a stable time of at least 60 minutes. The spectrum was calculated and the absorption of the solutions was measured against the blank solution at the wavelength of 533 nm, and Figure 3 represents the titration curve that follows the Peer Law 2.4-24 mg/ml, and the correlation coefficient of r was given as 0.9949 and the molar absorption value was calculated as Its value was 7.2×10³ L/mol.cm and the Sandal indication value is 0.03311 mg/cm².

Figure 3. Calibration curve for determination of Mefenamic drug with methyl solution reagent in the presence of the oxidizing agent potassium Periodate in the oxidative coupling method

\[
\text{Absorbance} = 0.0302x + 0.2608 \\
R^2 = 0.9899
\]
The absorption spectra were measured for these solutions as in Figure 4.

Figure 4. Spectra of absorption of standard solutions

The proposed equation:

3.8. Accuracy and compatibility

The above optimal conditions were used to test the accuracy and compatibility of the method, and this was done by taking six readings and taken for three different quantities 2.4, 7.2, 12 mg/ml of Mefenamic acid within the limits of the Bear law in the titration curve in terms of calculating the regression rate and relative standard deviation (RSD,) in Table 8 It was found that the method is of high accuracy (100.01% retrieval rate) and with high compatibility, where both the retrieval rate, its rate, and the standard deviation were calculated mathematically as follows:
### Table 8. Accuracy and compatibility

| Mefenamic con. µg/ml | Recovery% | Recovery% | RSD% |
|----------------------|-----------|-----------|------|
| 2.4                  | 100.46    | 100.01    | 0.58 |
| 7.2                  | 99.86     |           | 0.38 |
| 12                   | 99.71     |           | 0.24 |

### 3.9. Detection limit and quantitative limit

The detection limit was calculated by measuring the absorbance of the solution to the lowest concentration of 1.2 mg/ml in the titration curve within the limits of the Beer Law. In optimum conditions, the detection limit was 0.03 mg/ml.

The quantitative limit is calculated by measuring the absorption of the solution to the lowest concentration of 1.2 mg/ml in the titration curve within the limits of the Law of Beer. In optimum conditions, the quantitative limit was 0.1 mg/ml.

### 4. Applications

The method could be applied to pharmaceutical preparations containing Mefenamic acid, which is the pharmaceutical preparation for mifrelic tablets and capsule for Mefenamic Acid (Capsule 500µg) Mefril (Tablet)

#### 4.1. Direct addition method

Three different concentrations of grain solution and capsule were taken: 4.8, 9.6, 12, 16.8 mg/ml. The solutions were treated with the same steps as when preparing the titration curve, and absorption was measured at the wavelength of 533 nm versus the imaging solution. Six measurements were calculated for each concentration and then completed. The calculation of retrieval and results is listed in Tables 9 and 10.

#### Table 9. Direct addition method in pills.

| Mefenamic Acid took µg/ml | Mefenamic Acid found µg/ml | RE % | Recovery % | Average Recovery |
|---------------------------|---------------------------|------|------------|-----------------|
| 1.2                       | 1.17                      | 0.46 | 100.46     |                 |
| 4.8                       | 4.83                      | -0.33| 99.66      | 99.84           |
| 9.6                       | 9.58                      | -0.60| 99.39      |                 |
| 14.4                      | 14.34                     | -0.13| 99.869     |                 |

The above table shows the success of the proposed method for estimating mefenamic in the pharmaceutical preparation containing it has reached the value of the rate of recovery of 99.84% in the pills.

#### Table 10. Direct addition method in the capsule.

| Mefenamic Acid took µg/ml | Mefenamic Acid found µg/ml | RE % | Recovery % | Average Recovery |
|---------------------------|---------------------------|------|------------|-----------------|
| 1.2                       | 1.21                      | -0.25| 99.74      |                 |
| 4.8                       | 4.93                      | -0.28| 99.71      | 100.03          |
| 9.6                       | 9.66                      | 0.20 | 100.20     |                 |
| 14.4                      | 14.38                     | 0.46 | 100.46     |                 |

The effectiveness of the proposed Mefenamic process in the pharmaceutical preparation containing it achieved a recovery rate of 100.03% in the capsule.

#### 4.2. Standard addition method.
To indicate the efficiency and accuracy of the proposed method and prove that this method is free of interference, as the standard addition method was applied in the estimation of Mefenamic acid pills and the capsule of Mefenamic acid, as it included the addition of fixed quantities 1.2 _2.4 mm of the pharmaceutical preparation solution at a concentration of 300 mg/ml in a chain Volume volumes of 25 ml were added, then increasing volumes 0.2, 0.4, 0.6, and 0.8 ml were added from the standard solution of Mefenamic acid at a concentration of 300 mg/ml, and the above solutions were treated in the same way that was used when preparing the titration curve, as it was measured Absorption of all these solutions versus the blank solution at wavelength 533 nm, and the results are shown in Table 11 and 12 and Figure 5 and 6.

Table 11. Standard addition method in the tablet

| Conc. of Mefenamic acid (taken) mg/ml | Conc. of Mefenamic acid (found) mg/ml | Recovery, % |
|-------------------------------------|--------------------------------------|-------------|
| 1.2                                 | 1.31                                 | 100.12      |
| 2.4                                 | 2.8                                  |             |

Table 12. Standard addition method in the Capsule

| Conc. of Mef. (taken) µg/ml | Conc. of Mef. (found) µg/ml | Recovery % |
|----------------------------|-----------------------------|------------|
| 1.2                        | 1.4                         | 100.44     |
| 2.4                        | 2.7                         |            |

Figure 5. Standard addition curve for determination of Mefenamic acid in tablet

Figure 6. Standard addition curve for determination of Mefenamic acid in the capsule
As it was evident from the results shown in the above table that the standard addition method is well compatible with the direct method within acceptable limits of error, which indicates that the method is acceptable.

5. Conclusions

The development of a sensitive, simple, and accurate spectrophotometric method for measuring mefenamic acid through oxidative coupling reactions. The process was based on the mefenamic acid reaction with the metol reagent in the presence of the potassium potassium agent in the basic medium. The final color violet substance shall be produced at a temperature of 20-35 ° C, with the maximum absorption at 533 nm wavelength. The law of the Bear's is within the range 1.2-24 μg / ml and the coefficient of correlation was 0.9949. The value of the molar absorption was 7.2x103 L / mol.cm and the index of sandel was 0.03311 μg / cm². The limit value for detection was 0.19 μg / ml. The process was extremely accurate and precision, The recovery value was 100.67%, and the standard deviation value was 1.56%. The process was used successfully in pharmaceutical preparations for the determination of mefenamic acid in tablets and capsule.

6. References

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