Spectrophotometric Determination of Mefenamic acid in pure and Pharmaceutical Preparations

B A Saleem1* and L A Alnuaimy1
1Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

Corresponding author: basmasaleem@uomosul.edu.iq

Abstract. Chromate ions CrO42- have been used in three simples, rapid, an accurate spectrophotometric method. These methods were suggested to estimate mefenamic acid in its pure form and pharmaceutical preparations. These methods were different from each other with reactants components, and they are similar as, indirect methods and depends on oxidation – reduction reaction. So that, Method 1, based on oxidation reduction reaction between mefenamic acid and chromate ion in presence of acidic medium and sodium oxalate to form a water soluble, and stable pinkish-violet colored complex with 1,5-diphenylcarbazide as a reagent which was measured at $\lambda_{\text{max}}$ 545 nm. Method 2 depends on oxidizing mefenamic acid with chromate ions in presence of sodium oxalate as a catalyst agent and acidic medium to form with indigo carmine $\lambda_{\text{max}}$ at 610 nm. water soluble and stable blue complex. Finally, method 3 based on the reaction between mefenamic acid and chromate ion in presence of sodium oxalate and acidic medium to form with chrome azurol S a red complex measured $\lambda_{\text{max}}$ at 512 nm. These three methods have been followed Beer’s law at (2-40,1.2-40 and 2-36) $\mu$g/mL methods 1,2 and 3 respectively, while molar absorptivity and Sandall’s sensitivity $0.84 \times 10^4$, $0.54 \times 10^4$ and $1.08 \times 10^4$ Lmol$^{-1}$cm$^{-1}$ and 0.0287, 0.0446 and 0.0223 $\mu$g.cm$^{-2}$ for methods 1,2 and 3 respectively. The suggested methods were applied successfully to determination of mefenamic acid in pharmaceutical formulation with good recoveries.

Keywords: Mefenamic acid, Chrome azurol S, Indigo carmine, 1,5-Diphenylcarbazide, Oxidation, Oxidation-reduction reaction.

1. Introduction
Mefenamic acid (Mef) also known as Ponstan as a tradition name, while, chemically known as [2-(2,3-dimethyl phenyl) amino] benzoic acid according to IUPAC. Mef. is non-steroidal medication and anti-inflammatory drug. The general uses of Mef acid was treated inflammatory diseases, (Fig.1)[1-4]. The investigative literature reveals that there are not many methods of drug estimation. There are several techniques proven in the literature for estimating Mef. So that Mef has been estimated using high-performance liquid chromatography [5-8], as well as using some spectrophotometric methods [9-20].

![Figure 1. Chemical structure of Mefenamic acid](image)
The reagents used in this project were (a) indigo carmine (Ind), (b) 1,5-Diphenylcarbazide (Dpc) and (c) Chrome azurol S (Chz) fig 2. Ind was chemically named as 5,5'-indigosulfonic acid disodium salt. Dpc almost used with chromium ion as it was forming a violet very stable compound. Finally, Chz was a red – brown powder, used for estimation of cations like europium (III) and samarium(III), [21-26]. In fact, the suggestion of these three indirect spectrophotometric methods was very necessary to assay Mef. in pure form and in some pharmaceuticals preparation. Because of the medicinal importance of Mef. in our daily life. In addition to the paucity of the spectrophotometric methods used to estimate Mef. Particular Faculties or courses may have their own guidelines for different aspects of presentation, so always check your own course documentation or with course tutors. The following is general advice on the presentation of courses assignments which is usually, but not always, appropriate.

![Chemical structures](image)

**Figure 2.** Chemical structure of a-Ind., b- Dpc. and, c- Chz

2. **Experimental**

2.1. Apparatus:
JASCOV–630UV-Visible spectrophotometer with 1 cm matched cells were used for measurements of absorbance, also measurements of pH carried out using HANA pH.

2.2. Chemical materials:

Mefenamic acid solution, 100μg.mL⁻¹: 0.01 g of Mef (SDI-Iraq) was dissolved in 5 mL of ethanol as a solvent, then the volume was completed to 100 mL with distilled water using for this purpose a 100 mL volumetric flask.

Chromate solution, 4.31×10⁻⁴ M: 0.021 g of potassium chromate (Fluka) dissolved in distilled water in 250 mL volumetric flask.

Indigo carmine solution, 1.2 ×10⁻³ M0.1399 g of indigo carmine (BDH) was dissolved in distilled water, then the volume completed with distilled water to 250 mL volumetric flask.
1,5-Diphenylcarbazide, 1×10⁻³ M: 0.0605 g of 1,5-Diphenylcarbazide (BDH) was dissolved in 5 mL of acetone, then the volume completed with distilled water to 250 mL using a volumetric flask.
Chrom azurol S, 1×10⁻³ M: 0.1513 g of indigo carmine (BDH) was dissolved in distilled water and completed the volume with distilled water to 250 mL volumetric flask.

Sodium oxalate solution, 0.01 M: 0.1340 g of sodium oxalate (Fluka) was dissolved in 100 mL of distilled water a 100 mL volumetric flask.

HCl solution, 1 N: An appropriate dilution of concentrated acid used for preparing hydrochloric acid solution with 1N concentration in 100 mL distilled water.
Dosage form of Ponstan: Ponstan capsule (250 mg, SDI-Iraq) and Ponstan suspension (50 mg / 5 mL Al-Anam pharma-Ind., Baghdad-Iraq). Five capsules were weighed and mixed well, then 0.01 g of ponstan was dissolved in ethanol and then added with water. Distilled into 100 mL in a volumetric flask, to prepare 100 μg of Ponstan's solution.

Preparation of the suspension: Mix 100 mL of the suspension with 100 mL of ethanol and then dilute with distilled water to 250 mL using a volumetric flask, dilute 2.5 mL of this solution to 100 mL to prepare a 100 μg mL⁻¹ solution.

3. Results and Discussion:

3.1. Effect of pH

To estimate the suitable acid for oxidation reduction process, several kinds of acids have been studied such as (H₂SO₄, HCl and CH₃COOH). In order to select the effective kind of acid at the optimum pH value, (1-4) mL of 1 N acids was added to the mixture of 100μg/mL drugs, then, 4.31×10⁻⁴ M chromate solution, 2 mL of oxalate ion 0.01 M and 2 mL of reagents in a final volume 25 mL. Finally, the measurements were carried out at λₘₐₓ 545, 610 and 512 nm. Figure 3 show that hydrochloric acid was the optimum kind of acids and 2 mL of HCl was selected for the subsequent experiment.

![Figure 3. Kind of acids](image)

3.2. Effect of oxidation agent amount

The proposed method based on using an excess amount of chromate (VI) ions as oxidizing agent. Mef. reduced chromate (VI) to chromate(III) in acidic medium of 1N hydrochloric acid and sodium oxalate as a catalyst of oxidation process, then the residual amount of chromate was reacted with reagents (Ind, Dpc. and Chz.) Depending on this fact (0.5-4.0) mL of chromate ion solution 4.31×10⁻⁴ M was studied. It was found from the experimental work which was reported in Fig.4 that the best amount of chromate is 1.5 mL.
3.3. Effect of sodium oxalate amount

The catalyst sodium oxalate was added to catalyze the oxidation process between chromate ion Mef. [29]. Therefore, the effect of addition of various amounts of oxalate ion (1.0-4.0) mL has been studied and the results shown in Fig. 5 indicating that 2 mL of sodium oxalate was an optimum amount for the subsequent experiments.

3.4. Effect of reagents amount

To check the effective amount of reagents amount, 25 mL volumetric flask containing (50-750) µg of Mef., 2 mL of 1N HCl, 1.5 mL of chromate ions amount, 2 mL of sodium oxalate 0.01 M and (1-4) mL reagents (Ind, Dpc. and Chz.), after diluting that flasks with distilled water and measuring the absorbance against reagent blank at λ max 545, 610 and 512 nm. The optimum amounts were 2 mL of 1.0×10^{-3} M Dpc, 1.52×10^{-3} M Inc and 1.0×10^{-3} M Chz. Therefore, these amounts were selected for the subsequent experiments.

3.5. Effect of surfactants

This study was checked by adding a various kind of surfactant to the reaction mixture for each method, such as, CPC cetylpyridinium chloride as a cationic surfactant, SDS sodium dodecyl sulfate as an anionic surfactant, and triton X-100 as a non-ionic surfactant. This study
was experimentally no effect results, so that, this study was neglected from the subsequent experiments.

3.6. Effect of order of addition

The effect of changed the sequences of reactants addition were studied. Table 1 indicates that the sequence I gives the best absorption for method 1, sequence IV for method 2 and sequence VI for method 3 therefore, these sequences have been adopted in subsequent experiments.

| Reaction component | Order number | Absorbance |
|--------------------|--------------|------------|
| Mef+Ox+H+S+Dpc     | I            | 0.303      |
| Mef+H+S+Ox+Dpc     | II           | 0.273      |
| **Method 2**       |              |            |
| Mef+Ox+H+S+Inc     | III          | 0.335      |
| Mef+H+S+Inc+Ox     | IV           | 0.115      |
| **Method 3**       |              |            |
| Mef+Ox+H+S+Chz     | V            | 0.319      |
| Mef+Chz+Ox+H+S     | VI           | 0.094      |

**Mef**: Mefenamic acid, **Ox**: Chromate ions, **S**: Sodium oxalate, **H**: Acid, **Inc**: Indigocarmin, **Dpc**: 1,5-Diphenylcarbazide, **Chz**: Chrom azurol S

3.7. Effect of time

The colored of the resulted complex was formed immediately and developed for 2-3 min., this experimental result was recorded when the stability of the colored complex was studied under the optimum conditions, and measured for different time periods as observed from Fig.6

![Figure 6. stability of the colored complex](image)

4. Beer’s law and final absorption spectra
The calibration curve of Mef resulted when absorbance was measured at (545, 610 and 512 nm) for 25 mL series calibrated flasks, which contained an increasing amount (10-1000) µg/mL of Mef. and 2 mL of 1N hydrochloric acid, 1.5 mL of chromate solution 4.31×10⁻⁴ M, 2 mL of 0.01 M sodium oxalate as a catalyst, and 2 mL of (1.0×10⁻³ M Dpc, 1.2×10⁻³ M indigo carmin and 1.0×10⁻³ M Chz). The flasks were then diluted with distilled water to the mark, mixed well and measured the absorbance at (545, 610 and 512 nm) against the reagent blank. The linearity of the suggested method was followed Beer's law from 2-40 and 1.2-40 and 2-36 µg/mL of Mef., also, molar absorptivity and Sandall sensitivity were 0.84×10⁴ and 0.594×10⁴ and 1.08×10⁴ l.mol⁻¹.cm⁻¹, 0.0287, 0.0446 and 0.0223 µg.cm⁻², and for method 1, 2 and 3 respectively.(Fig. 7).

Figure 7. Calibration curve of mfenamic determination using three suggested methods

The final spectrum of Mef. under the optimum conditions were drawing as fig (8-10)

Figure 8. Absorption spectrum of 100 µg/mL Mef. treated according method 1 and measured against, a: reagent blank,  b: reagent blank against distilled water
Figure 9. Absorption spectrum of 100 µg/mL Mef. treated according method 2 and measured against, A: blank, B: distilled water and C: reagent blank against distilled water

Figure 10. Absorption spectrum of 100 µg/mL Mef. treated according method 3 and measured against, A: blank, B: distilled water and C: reagent blank against distilled water

5. Accuracy and precision

The accuracy and precision of the calibration curve for Mef. for three concentrations (100, 300 and 500) µg.mL\(^{-1}\) have been checked, the results are reliable as shown in Table 2

| Amount of Mef taken, µg/25mL | Recovery*, % | Relative standard deviation (RSD)*, % |
|-----------------------------|--------------|--------------------------------------|
| Method 1                    |              |                                      |
| 100                         | 99.28        | ± 0.0022                              |
| 300                         | 98.86        | ± 0.0021                              |
| 500                         | 98.49        | ± 0.0021                              |
| Method 2                    |              |                                      |
6. Mole ratio

Depending on continuous variations method, the reaction ratio of Mef. to chromate (Mef/Mef + Chromate) were determined. The results in Figure 11 indicate that 1:1 is the ratio of Mef. to chromate.

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|       |       | ±       |
|-------|-------|---------|
| 100   | 99.98 | ± 0.0022|
| 300   | 98.93 | ± 0.0021|
| 500   | 98.96 | ± 0.0021|
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* Average of 10 determinations.

**Figure 11.** Job’s methods for mefenamic acid

According to job’s method, the reaction between Pns, Vot and chromate ions were suggested [29,30]:

![Chemical reaction diagram]

Moreover, the probable mechanism between indigocarmin and chromate in presence of sodium oxalate in acidic medium has been suggested [30] as follows:
7. Effect of interferences:

The selectivity and efficiency of the suggested the three methods were studied using foreign chemicals that are usually found in dosage forms as (gum acacia, lactose, starch, menthol and glucose) as indicating in Table 3 which exhibited that there is no effect of interferences in the three methods.

Table 3. Effect of interferences.

| Interferences, μg | Recovery (%)/100 μg of Mef. added | Method 1 | Method 2 | Method 3 |
|-------------------|-----------------------------------|---------|---------|---------|
|                   | 100 μg | 300 μg | 750 μg | 100 μg | 300 μg | 750 μg | 100 μg | 300 μg | 750 μg |
| Acacia            | 98.69  | 98.91  | 100.32 | 99.43  | 98.96  | 98.91  | 100.49 | 99.43  | 99.94  |
| Menthol           | 99.47  | 99.94  | 98.62  | 99.74  | 99.94  | 99.43  | 100.61 | 99.84  | 100.62 |
| Starch            | 99.26  | 99.29  | 100.32 | 99.26  | 99.29  | 100.58 | 100.61 | 99.84  | 100.62 |
| Glucose           | 98.53  | 98.83  | 99.93  | 98.62  | 98.83  | 98.63  | 100.38 | 100.11 | 99.78  |
| Lactose           | 99.78  | 99.63  | 98.45  | 99.37  | 99.78  | 99.43  | 100.38 | 100.11 | 99.78  |
| Fructose          | 99.61  | 98.79  | 98.65  | 99.45  | 99.61  | 98.97  | 100.72 | 99.45  | 99.61  |

8. Application of the method:

The current method for estimating Mef. in its pharmaceutical preparations was applied with good recoveries as in Table 4.

Table 4. Application of the method

| Drugs                        | μg Mef present/25mL | μg Mef measured/25mL | Recovery, (R) % | Relative Error, (R.E) % |
|------------------------------|---------------------|----------------------|-----------------|------------------------|
| Ponstidin capsule (250mg),   | 100                 | 100.03               | 100.43          | ± 0.0019               |
| SDI-Iraq                     | 300                 | 300.29               | 98.14           | ± 0.0051               |
| Mefastan (50 mg/5 mL) Al-     | 500                 | 499.34               | 99.17           | ± 0.0032               |
| Pharma-Ind. (Baghdad-Iraq)   | 100                 | 99.83                | 99.47           | ± 0.0024               |
|                              | 300                 | 299.56               | 99.24           | ± 0.0069               |
|                              | 500                 | 498.47               | 98.76           | ± 0.0041               |
The calculations of t-test [30] in table 5, it did not exceed that the theoretical values for five degrees of freedom \((N_1+N_2-2=5)\) at the 95% confidence level when the proposed method has been compared with literature method [10,11 and 15] as shown in Table 5.

### Table 5. The results of t-test analysis

| Drug | Pharmaceutical preparation | Method 1 | Method 2 | Method 3 |
|------|-----------------------------|---------|---------|---------|
| Ponstidin capsule (250mg), SDI-Iraq | Capsule | ± 1.4251 | ± 0.6079 | ± 0.9940 |

9. Comparison of the proposed method:
The accuracy and sensitivity of the present three methods measured with analytical variables by adopting the current method and comparing it with those established in literatures as shown in Table 6.

### Table 6. Comparison of the proposed method with the literatures

| Analytical parameters | Present method | Literature method |
|-----------------------|----------------|-------------------|
| | 1 | 2 | 3 | [11] | [15] |
| Reaction | Oxidation reduction chromate | Oxidation reduction chromate | Oxidation reduction chromate | Oxidation formation Fe III | ------ |
| Oxidant agent | 1,5-diphenylcarbazide | Indigo carmine | Chrome azurol S | o-phenanthroline | 1,2-Naphthoquinone-Sulfonic sodium |
| Reagent | Acidic | Acidic | Acidic | ------ | basic |
| Medium | 545 | 610 | 512 | 510 | 450 |
| \(\lambda_{max} \text{ (nm)}\) | Room temperature | Room temperature | Room temperature | ------ | 30 |
| Temperature \(\circ\text{C}\) | 120 | 120 | 120 | ------ | 35 |
| Stability period (min.) | 2-40 | 1.2-40 | 2-36 | 0.06 – 0.1 | 0.5-10.0 |
| Beer's law range (µg/mL) | 0.84×10^4 | 0.54×10^4 | 1.08×10^4 | 1.6×10^4 | 3.40×10^4 |
| Molar absorptivity \((1.\text{mol}^{-1}.\text{cm}^{-1})\) | 0.0287 | 0.0446 | 0.0223 | 0.0150 | 0.0071 |
| Sandell's sensitivity \((\mug.\text{cm}^2)\) | Pinkish-violet | Blue | Red | Red | Brownish yellow |
| Color of the product | Pharmaceutical preparation | Pharmaceutical preparation | Pharmaceutical preparation | Pharmaceutical preparation | |
| Application of the method | | | | | |
10. Conclusion

In the present work, a method has been proposed to quantify both ponstan which depends on oxidation of chromate in acidic medium in presence of sodium oxalate as a catalyst of oxidation process, to form with indigocarmin, 1,5-diphenyl carbaside and chrome azurol S, a blue colored complex measured at 545, 610 and 512 nm. The proposed methods have been applied successfully to determination of mefenamic acid in pharmaceutical formulation with good recoveries.

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