Spectrophotometric Determination of Mesalazine

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(Received 13/9/2018 ; Accepted 25/10/2018)

ABSTRACT
Mesalazine (MESA) is determined by a simple and rapid visible spectrophotometric method. This method is depend on oxidative coupling reaction of mesalazine with histidine (HIS) in alkaline media using N-bromosuccinimide (NBS) as oxidizing agent to form a water soluble and stable product, that it has a maximum absorption at 459 nm. Beer’s law is followed in a concentration range of 50 to 750 µg /20ml (2.5-37.5 µg /ml) with a molar absorptivity of 3.3682×10³ l.mol⁻¹. cm⁻¹. The recommended method has been successfully applied to the assay of MESA in pharmaceutical preparations.

Keywords: spectrophotometry, oxidative coupling, mesalazine, histidine.

INTRODUCTION
Mesalazine (MESA), also named mesalamine, its chemical name is 5-amino-2-hydroxy benzoic acid. The powder or crystals of MESA has a white or light grey or light pink color(British pharmacopia, 2013). It is soluble in dil.acidic and alkaline medium, fairly insoluble in chloroform, ether, ethyl acetate and n-hexane. (Moharana et al., 2011).

MESA has been determined by different kinds of analytical techniques in various formulations and some biological liquids these involve: HPLC (Darak et al., 2012), RP-HPLC (Rao and Sekhar, 2013), UHPLC–MS/MS (Banda et al., 2016), electrochemical studies by CV technique (Tanuja et al., 2018) and spectrofluorimetric technique (Elbashir et al., 2015). Also, MESA has been estimated by various spectrophotometric methods in pure form and drugs formulations by various reagents for example 1,2-Naphthoquinone-4-sulphonate (NQS), p- dimethyl amino cinnamaldehyde (PDAC) (Gurupadayya et al., 2010), a solution of Fe(NO₂)₃ in presence of HCl (Moharana et al., 2011), Ortho-Chloranil (Al-Enizzi et al., 2012), 1,5-diphenyl carbazide (1,5-DPC) (Hamdonn, 2018), 8-hydroxyquinoline and N-(1-naphthyl)ethylenediamine (Zakaria, 2013), sodium nitroprusside with hydroxylamine hydrochloride (Al-Sabha and Habeb, 2015). Also MESA has been estimated in a Ultraviolet region (Mhatre et al., 2013).
The suggested method gives good results for estimation MESA in pure and drugs formulations by oxidation with N-bromosuccinimide then coupling the product with histidine in alkaline medium, the formed colored complex prove to be intense, water-soluble and stable.

**EXPERIMENTAL**

**Instruments**

The UV Spectrophotometer was used (JascoV-630) and a pair of silica cells were used for all experiments, also the pH of solution was estimated by pH meter type HANA.

**Analytical reagents were used in this work**

**Standard MESA solution, 500 μg.ml⁻¹**. A 0.05g of MESA (Fluka) was dissolved in 10 ml of absolute ethanol and diluted to 100 ml distilled water using a volumetric flask.

**Histidine(HIS) solution, 0.01M.** A 0.1551g of HIS was dissolved in 100 ml distilled water using a volumetric flask.

**N-bromosuccinimide(NBS) solution, 0.015M.** Accurate weight of 0.2669 g of NBS was dissolved in 100 ml distilled water using a volumetric flask.

**Sodium hydroxide solution, 1N.** A concentrated solution (10N, fluka) was diluted to 1000 ml distilled water in a volumetric flask then transported to plastic container.

**Pharmaceutical preparation.** An accurate weight (equivalent to 0.05g MESA) of the powder for ten tablets was dissolved in 10 ml absolute ethanol and the volume completed to 100ml by distilled water in a volumetric flask.

**General method and calibration graph**

To 20 ml volumetric flasks, 0.1-2 ml of MESA solution (500 μg/ml) were transported, then 1 ml of HIS (0.01 M), 0.5 ml of NBS (0.015M) and 1 ml NaOH (1N) were added. The solutions were left to stand for 15 minutes before completing the volumetric flasks with distilled water. The measured absorbance's against the reagent blank were done at 459 nm and Beer's law was applied from 50-750 μg MESA / 20ml Fig. (1). From the equation of straight line, the molar absorbtivity was 3.368×10³  l.mol⁻¹.cm⁻¹.

**Fig. 1: Calibration graph for determination of MESA using the proposed method.**
RESULTS AND DISCUSSION

All factors affected on the color development for 500µg MESA in 20 ml were investigated.

**Principle of the Method**

The method included two steps:

1- Oxidation of MESA by NBS to produce MESA derivative (DMESA)

![Chemical structure of MESA and DMESA](image)

2- The coupling of DMESA with histidine in alkaline medium to produce orange dye.

![Chemical structure of DMESA, histidine, and orange dye](image)

Choosing of Oxidizing Agent

The best one of oxidizing agents which give the highest intensity was selected after studying different types of available oxidizing agents (Table 1)

**Table 1: Selection of oxidizing agent**

| Oxidizing agent (1ml of 0.015M)soln. | Absorbance | Δλ |
|-------------------------------------|------------|----|
| NaIO₄                               | 0.262      | 166|
| KIO₃ (Bad result)                   |            |    |
| K₂CrO₄ (Bad result)                |            |    |
| K₂Cr₂O₇ (Bad result)               |            |    |
| NCS                                 | 0.215      | 195|
| NBS                                 | 0.408      | 168|
| Ammonium cerium(IV) sulfate (Bad result) turbid |          |    |

Δλ = λ_{max} S - λ_{max} B  \quad S = \text{Dye}  \quad B = \text{Blank}

Results illustrated in Table 1 show that NBS gave the highest intensity and a good color contrast for colored product.
The medium of Present Reaction

The primarily experiment has shown that reaction of MESA with HIS in presence of NBS needs alkaline medium, therefore various types of bases were studied (Table 2).

Table 2: Choosing suitable base

| Base (1ml of 1N) | Absorbance | ∆λ |
|-----------------|------------|-----|
| NaOH            | 0.404      | 167 |
| KOH             | 0.385      | 134 |
| Na₂CO₃          | 0.296      | 129 |
| NaHCO₃          | 0.202      | 135 |

Results in (Table 2) show that a certain alkaline medium was needed and NaOH gave the best results with volume equal to 1 ml (Table 3).

Table 3: Effect of base amount on absorbance

| NaOH solution (ml of 1N) | Absorbance | pH   |
|-------------------------|------------|------|
| 0                       | 0.385      | 6.30 |
| 0.5                     | 0.409      | 12.27|
| 1                       | 0.430      | 12.63|
| 1.5                     | 0.375      | 12.75|
| 2                       | 0.369      | 12.86|
| 3                       | 0.336      | 12.92|

Effect of HIS Reagent Concentration

The effect of HIS amount on the color intensity of the dye has been studied. From the results, it can be observed that 1 ml of 0.01M HIS is the most suitable amount which gave the highest intensity of color and highest value of correlation coefficient (Table 4).

Table 4: Effect of HIS amount

| HIS solution (ml of 0.01M) | Absorbance/µg of MESA | R   |
|---------------------------|------------------------|-----|
| 0.5                       | 0.054 0.105 0.121 0.168 0.223 0.9875 |
| 1                         | 0.095 0.210 0.296 0.434 0.482 0.9922 |
| 1.5                       | 0.122 0.210 0.247 0.399 0.447 0.9816 |
| 2                         | 0.148 0.213 0.23 0.328 0.368 0.9825 |

Effect of NBS Amount on Absorbance

The effect of various volumes of NBS solution (0.015M) on the color intensity has been studied. A 0.5 ml of NBS was the optimum amount which gave the highest intensity of color and highest value of correlation coefficient (Table 5).

Table 5: Effect of NBS amount on absorbance

| NBS solution(ml of0.015M) | Absorbance/µg of MESA | R   |
|----------------------------|------------------------|-----|
| 0.3                        | 0.125 0.133 0.284 0.262 0.285 0.8696 |
| 0.5                        | 0.151 0.196 0.363 0.496 0.644 0.9885 |
| 1                          | 0.085 0.198 0.302 0.439 0.463 0.9848 |

The Effect of Time on Oxidation of MESA

Only 15 minutes was needed to complete the oxidation process before completing the volume with distilled water (Table 6).
The results in Table (7) showed that no effect of surfactant on the intensity (Table 7).

### Table 7: Effect of surfactant

| Surfactant Solution | I* | II | III | IV |
|---------------------|----|----|----|----|
| CTAB 1×10^{-3}M     | 0.189 | 0.208 | 0.277 | 0.383 |
| SDS 1×10^{-3}M      | 0.545 | 0.560 | 0.473 | 0.367 |
| Triton x-100 1%(wt/v) | 0.544 | 0.527 | 0.509 | 0.391 |
| Without             | 0.564 |    |    |    |

I* MESA+S+HIS+NBS+NaOH
II MESA+HIS+S+NBS+NaOH
III MESA+HIS+NBS+S+NaOH
IV MESA+HIS+NBS+NaOH+S

**The Best Order of Addition**

The optimum order of reagent addition be followed as given under the general procedure because it gives highest color intensity, otherwise a loss in color intensity occurred (Table 8).

### Table 8: The order of addition

| Order number | Order of addition       | Abs. |
|--------------|-------------------------|------|
| I            | MESA+HIS+NBS+OH         | 0.569|
| II           | NBS+MESA+HIS+OH         | 0.228|
| III          | NBS+HIS+MESA+OH         | 0.144|
| IV           | MESA+HIS+OH+NBS         | 0.209|
| V            | OH+NBS+MESA+HIS         | 0.485|
| VI           | OH+NBS+HIS+MESA         | 0.092|
| VII          | HIS+NBS+OH+MESA         | 0.357|
| VIII         | MESA+NBS+OH+HIS         | 0.238|

**The stability period**

The experimental results (Table 9) showed that the absorbance remained constant at least for 4 hours.

### Table 9: Effect of color stability time

| µg of MESA | 5   | 10  | 15  | 20  | 30  | 40  | 50  | 60  | 4 hours |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| 250        | 0.270 | 0.270 | 0.270 | 0.270 | 0.270 | 0.270 | 0.270 | 0.270 | 0.270 |
| 500        | 0.571 | 0.570 | 0.570 | 0.570 | 0.571 | 0.570 | 0.572 | 0.573 | 0.573 |
| 600        | 0.687 | 0.687 | 0.687 | 0.685 | 0.686 | 0.685 | 0.686 | 0.686 | 0.686 |

**Final absorption spectrum**

When MESA was treated according to the suggested work, the absorption spectrum, showed a maximum absorption at 459 nm versus the blank solution Fig. (2).
Fig. 2: Absorption spectrum of the colored product 500 µg MESA (A) against blank, (B) against distilled water and (C) blank against distilled water.

The Nature of the Reaction Product

Job's of the continuous variation (Delvie, 1997). Fig. (3) indicate that a colored product has a structure of 1:2 MESA to HIS reagent at 459 nm.

Therefore, the probable colored product have the below structure:

Orange dye
Application of the Method

To test the applicability of the present method, it has been applied to estimate MESA in drug formulation (tablet). On applying proposed procedure, a good recovery, accuracy and precision are obtained as shown in (Table 10).

Table 10: Application of method

| Drug                      | µg MESA present /20ml | µg MESA measured /20ml | Recovery*, % | Relative error*,% | Relative standard deviation*,% |
|---------------------------|-----------------------|------------------------|--------------|-------------------|-------------------------------|
| Pentasa tablets 500 mg    | 200                   | 193.8                  | 96.9         | 3.1               | ±0.40                         |
| Ferring                   | 400                   | 397.3                  | 99.3         | 0.7               | ±0.17                         |
| Mezelazin tablets 400 mg  | 500                   | 495.5                  | 99.1         | 0.9               | ±1.25                         |
| Awa media                 | 500                   | 503.0                  | 100.6        | -0.6              | ±0.40                         |

*Average of four determinations

Comparison of method

Table (11) shows the comparison between the various analytical parameters found in suggested work with other spectrophotometric methods.

Table 11: Comparison with other methods

| Analytical parameters    | Suggested work | Method (1) (Shihab, 2011) | Method (2) (Zakaria, 2009) |
|--------------------------|----------------|---------------------------|-----------------------------|
| λ_max (nm)               | 459            | 530                       | 471                         |
| Beer’s law range(ppm)    | 2.5-37.5       | 0.4-10                    | 0.4-12                      |
| Molar absorbptivity Lmol⁻¹.cm⁻¹ | 3368.2        | 3685                      | 29480                       |
| Stability of the color (minutes) | 240            | 65                        | 60                          |
| Medium of method         | Alkaline       | Acidic                    | Alkaline                    |
| Reagent                  | Histidine      | Pyrocatechol              | Resorcinol                  |
| Type of reaction         | Oxidative coupling | Oxidative coupling | Diazotisation              |
| Nature of the dye        | 1:2            | 1:1                       | 1:1                         |
| Application part         | Determination of MESA in tablets | Determination of MESA in tablets and capsules | Determination of MESA in capsules |

The proposed method is a simple, rapid, sensitive, more stable and can be used to determine MESA in drugs formulations.

CONCLUSION

A simple, sensitive and rapid spectrophotometric method for estimating MESA in aqueous solution has been carried out by the reaction of MESA with HIS in presence of NBS in alkaline medium. The suggested work has been successfully applied to determine MESA in pharmaceutical preparation (Tablets).

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