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Development of Spectrophotometric Method for The Determination of Mesalazine as Pure Form and in Tablets

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Abstract

This method involves development of a highly sensitive spectrophotometric procedure to estimate mesalazine as pure substance and in the tablets. This particular procedure was focused on the diazotization of mesalazine with an excess quantity of sodium nitrite ($NaNO_2$) in an acid medium using HCl solution to produce a corresponding diazonium compound which reacts with 8-hydroxyquinoline reagent in an alkaline solution of NaOH to yield read-orange azo dye which is soluble in water and showed maximum absorption peak at the wavelength of 500 nm against the blank solution. The calibration graph was linear and compatible to Beer's law over the concentration range from 0.25 to 12.5 µg/ml with an exceptional determination coefficient (R^2= 0.9994) and apparent molar absorptivity 2.88×10^4 $L.mol^{-1}cm^{-1}$. The limits of detection (LOD) and quantitation (LOQ) were premeditated and found to be 0.2023 and 0.6524 µg/ml, correspondingly. A relative error percent (accuracy) and the relative standard deviation (RSD%) was also calculated and found to be in the range -3.84% - 2.70%, and 0.17% - 1.94%, correspondingly. No interferences were observed from other ingredients that may be exist in the tablets. The stoichiometry of the resulting azo dye has been examined and the experimental results revealed that the mole ratio of mesalazine to 8-hydroxyquinoline is 1:1. The advised procedure was successfully applied for the determination of mesalazine in its pharmaceutical form (tablets).

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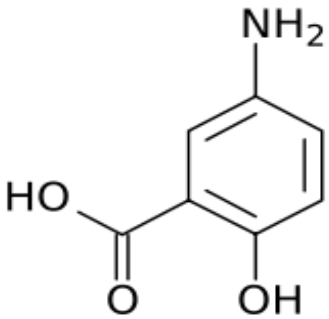
1. Introduction

Mesalazine (MESZ) or mesalamine, is a light grey powder. It is practically not soluble in alcohol but, it is little soluble in water. It can be dissolved in dilute hydrochloric acid and sodium hydroxides solutions (British Pharmacopoeia, 2013). MESZ was chemically known as 5-amino salicylic acid. It has melting point of 280°C. The chemical structure of MESZ was exemplified in the following (Scheme 1) (Cartwright, 2016).



Scheme 1. Mesalazine

($C_7H_7NO_3$)

M.Wt.= 153.135 g/mol

MESZ was used in the treatment of chronic bowel and ulcerative colitis which is a gathering of autoimmune diseases that cause large intestine inflammation (Zayed & Farrag, 2016). The anti-inflammatory mesalazine effect was achieved by the process of inhibiting the conversion of arachidonic acid in the mucosa, by stopping cyclooxygenase enzyme (Zawada et al, 2017). Also, the MESZ can be used to treat hyperemesis gravidarum in pregnant women as a second choice (Pasternak et al, 2013).

A variety of diverse spectrophotometric approaches have been issued for the approximation of MESZ in its pharmaceutical preparations. Most of the aforementioned procedures involved oxidative coupling reactions with thymol in the existence of sodium meta periodate (Salih & Mohammed, 2020), phenothiazine in the presence of potassium sulphate (K_2SO_4) as oxidant (Shehab & Muhammed, 2020), pyrocatechol and K_2SO_4 (Shihab, 2011), histidine in the presence of N-bromosuccinimide (NBS) (Zakaria, 2019), 8-hydroxyquinoline and N-(1-naphthyl) ethylenediamine (NNED) in alkaline solution (Zakaria, 2013). Others depended on the diazotization reaction of MESZ and coupling with some reagents for instance, 2,6-dihydroxytoluene (Aziz & Sultan 2019), resorcinol (Madhavi et al, 2011) and phlorogycinol (Hamdon et al, 2012). A condensation reaction was also applied to estimate MESZ via spectrophotometric methods by using reagents of salicylaldehyde (Anumolu et al, 2019) and p-dimethylaminocinnamaldehyde (Sama et al, 2011). Charge transfer reactions with alizarin red sulphonate (Altayib et al, 2014) and p-bromanil (Al-Ramadhani & Al-Mtioti, 2019), as well as ion-pair complex formation between the MESZ and bromothymol blue (Nair et al, 2015) have also been employed for the estimation of MESZ.

Numerous techniques have also been used for estimating MESZ in some biological liquids and pharmaceutical forms which included; cyclic voltammetry using sodium dodecyl sulfate modified carbon paste electrode (Tanuja et al, 2018), RP-HPLC (Rao & Sekhar, 2013), fluorescence probe (Guang et al, 2015) and electrochemical oxidation method for MESZ at poly (glutamic acid) modified glassy carbon electrode (Kumar et al, 2017).

This investigation describes the ideal conditions to develop a sensitive spectrophotometric method to determine MESZ in water via coupling of 8-hydroxyquinoline with diazotization MESZ in an alkaline solution of NaOH to form an orange water

soluble azo dye and to explore its applicability in tablets.

2. Materials and Methods

The chemical materials and reagents used in this research displayed a considerably high degree of purity and were acquired from the BDH, Fluka and Merck companies.

Stock solution of MESZ (500 µg/ml): A 0.0500 g of MESZ was weighted and set in nearby 5 ml of DW and with the identical solvent the volume was completed up to 100 ml in a calibrate flask and saved in dark bottle.

MESZ standard solution (50 µg/ml = 3.265×10^{-4} M) was prepared by taking a suitable volume of the stock solution and diluted by DW in a calibrated flask.

Solution of 8-hydroxyquinoline solution *(*1% w/v*):* A 1.00 g of 8-hydroxyquinoline reagent was dissolved in a small quantity of DW and the same solvent was used to bring the volume to 100 ml in a calibrated flask. The solution was then saved in a brown container.

Solution of sodium nitrite (0.2% w/v) (2.898×10^{-5} M): A 0.2000 g of $NaNO_2$ was dissolved in a minimal quantity of DW and the volume was then completed to 100 ml with the identical solvent.

HCl solution (1M): It was prepared by diluting 8.47 ml of concentrated hydrochloric acid to 100 ml with DW.

Solution of sulphamic acid (0.5%): A 0.50 g of the sulphamic acid was weighed and dissolved in a 100 ml DW using a calibrated flask.

Solution of sodium hydroxide (1M): The solution was prepared by diluting 100 ml of the standard solution of NaOH (10 M) (BDH) with DW to 1 L and placed in a plastic bottle.

2.1. Analysis of MESZ in tablets

Solution of Awasalazine and Pentasa tablets (50 µg/ml)

Five tablets of Awasalazne (400 mg MESZ exist in each tablet) or Pentasa (500 mg MESZ in each tablet) were finely grinded, mixed will and weighted exactly to quantity comparable to 0.010 g of MESZ and then was dissolved with 5 ml ethanol and completed to 40 ml with DW with slight heating. The solution was then filtered and completed with DW to 100 ml using a calibrated flask. The working solution of MESZ (50 µg/ml) was prepared by diluting with DW.

3. Results

3.1. Preliminary investigations

On treatment of MESZ with an excess quantity of $NaNO_2$ solution in presence of HCl solution the corresponding diazonium ion was formed. The relating diazonium ion was then coupled with 8-hydroxyquinoline in a basic solution of NaOH to produce colored azo dye. (Figure 1)

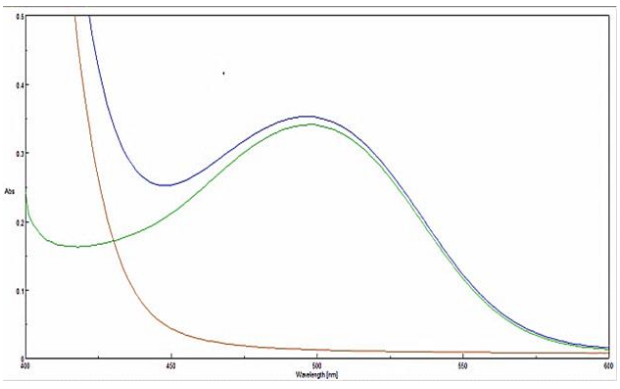


Figure 1: Initial spectrum for the resulting dye

3.2. Study of optimum condition

Effect of acid type and its amount on absorbance

Acidity is necessary for the completion of diazotization process. Therefore, the influence of varying quantities (0.5-3.0 ml) of several acids (1M) (such as H_2SO_4, HCl, HNO_3, HCOOH and CH_3COOH) on absorbance of the resulting azo dye was examined. The results are illustrated in Figure 2 and revealed that 1.0 -1.3 ml of 1M HCl solution exhibit maximum absorbance conforming to other acids. Therefore, 1ml of 1M HCl with standing time for 2 minutes to complete the diazotization reaction were established for the subsequent experiments.

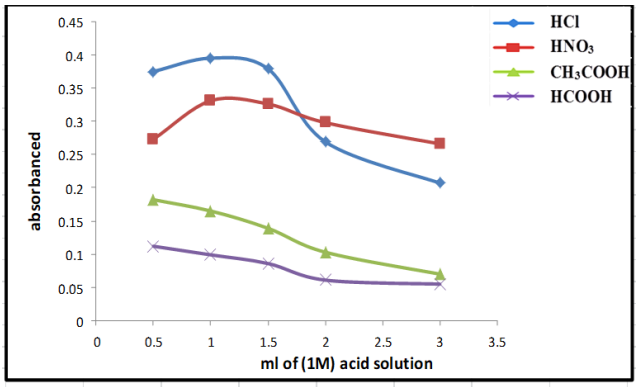


Figure 2: Effect of various acids on absorption of the resulting azo dye

Effect of sodium nitrite amount and the time on absorbance

The influence of several quantities of $NaNO_2$ solution from 0.2 to 2.0 ml at different waiting times 1-7 minutes on absorbance was investigated. The results are nominated in Figure 3 and indicated that the diazotization reaction of MESZ was accomplished after 5 minutes after the addition of 1.5 ml of 0.2% $NaNO_2$ solution, because this amounts of $NaNO_2$ give the highest absorbance. Therefore, 1.5 ml has been chosen for the next experiments.

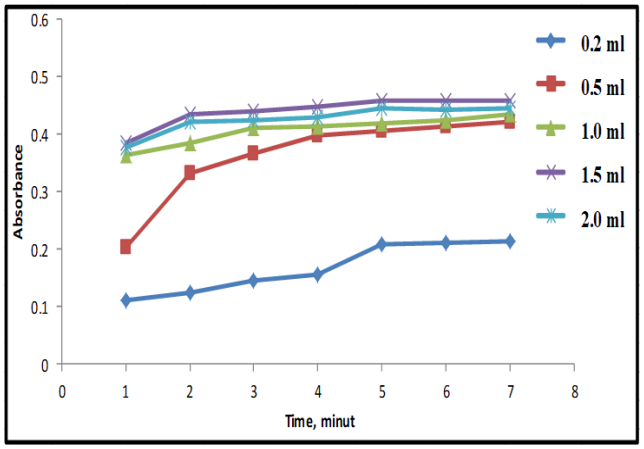


Figure 3: Effect of sodium nitrite amount and time on absorbance

Influence of sulphamic acid amount

The influence of several quantities 0.1-0.3 ml of sulphamic acid (0.5%) on the absorbance of the resulting azo dye have been also studied. Figure 4 show that 0.2 ml of sulphamic acid (0.5%) with occasional shaking for 4 minutes was sufficient to remove the excess of $NaNO_2$ (Clayden et al, 2001) Therefor, 0.2 ml of 0.5% sulphamic acid was followed for the all subsequent experiments.

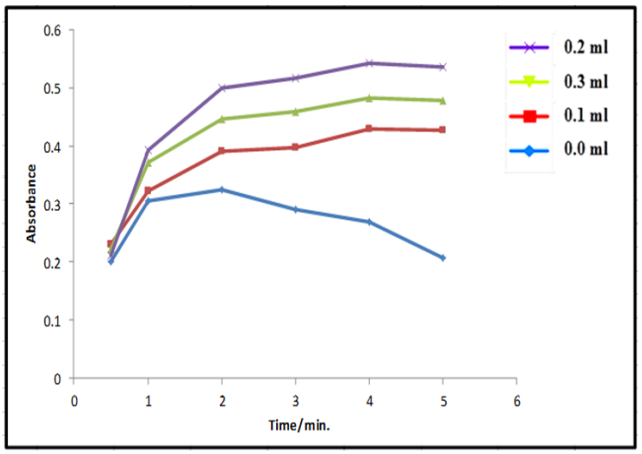


Figure 4: Influence of sulphamic acid quantities and the time on absorption

Effect of 8-hydroxyquinoline amount

The influence of several quantities from 0.5 to 2.5 ml of 8-hydroxyquinoline solution on the magnitude intensity of absorption of the producing dye was examined.

The data in Figure 5 reveal that the quantity 2.0 ml of 8-hydroxyquinoline are enough to be the ideal amount for showing high absorbance with excellent determination coefficient (R^2 = 0.9996) for coupling reaction with diazonium compound of MESZ, therefore, 2ml of 8-hydroxyquinoline (1%) it has been selected for subsequent investigation.

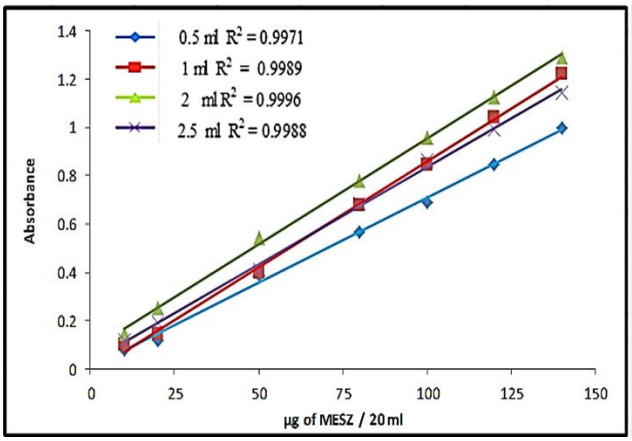


Figure 5: Effect of 8-hydroxyquinoline amount on absorbance

Effect of coupling reaction time

The influence of time on the coupling of diazotized MESZ with 8-hydroxyquinoline reagent was carried out by measuring the absorbance of azo dye at room temperature at different time before the addition of DW to complete the final volume. The results Figure 6 illustrate that the coupling reaction of diazotized MESZ with 8-hydroxyquinoline requires at least 7 minutes to give high intense colour and high absorbance values.

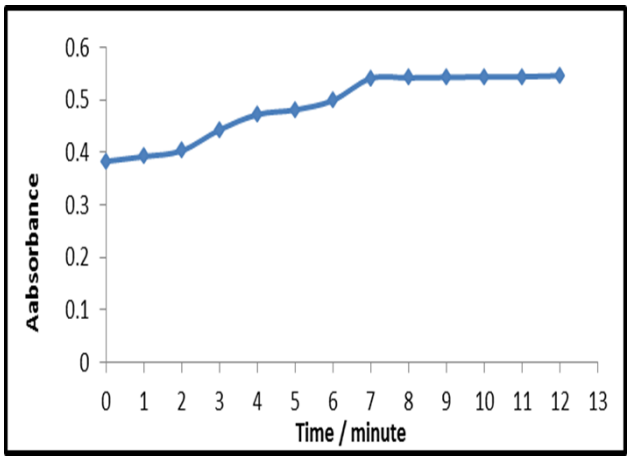


Figure 6: Influence of coupling reaction time on absorbance

Effect of base type and its amount

To obtain an intense color of azo dye, the coupling reaction of diazotized MESZ with 8-hydroxyquinoline was performed in an alkaline solution. Therefore, the influence of several quantities from 0.5 to 3.0 ml of various weak and strong bases (1M) on the intensity of absorption of the dye was performed.

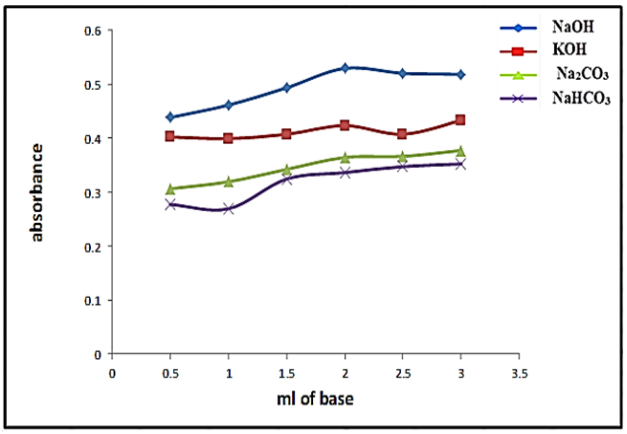


Figure 7: Effect of alkaline solution on absorbance

The results in Figure 7 explain that the solution of sodium hydroxide (1M) show high sensitivity than the other bases, and the results also illustrate that 2 ml of

sodium hydroxide (1M) is the optimal amount for the reaction therefore, it was selected for subsequent investigation.

Colour stability of the azo dye formed

Under optimal conditions, the time effect on the permanency of the colour of the resulting azo dye was performed at 499 nm by studying two diverse amounts of MESZ (50 and 150 µg /20ml).

The results in Figure 8 reveal that the colour stability of the resulting azo dye reaches its maximum absorbance at laboratory temperature and remains stable for at least one hour.

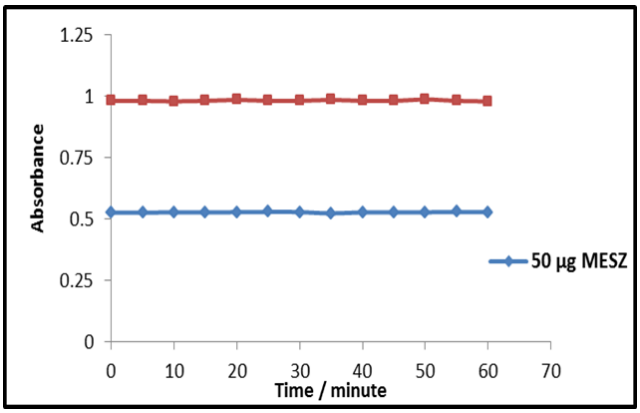


Figure 8: Stability of azo dye with time

Final absorption spectrum

Under optimum conditions, the coupling of 8-hydroxyquinoline with the diazotized MESZ in an alkaline solution of NaOH an orange colored, water soluble azo dye was formed that showed a peak with high absorbance at wavelength of 499 nm, where as the blank solution shows a minor absorbance at the same wavelength (Figure 9).

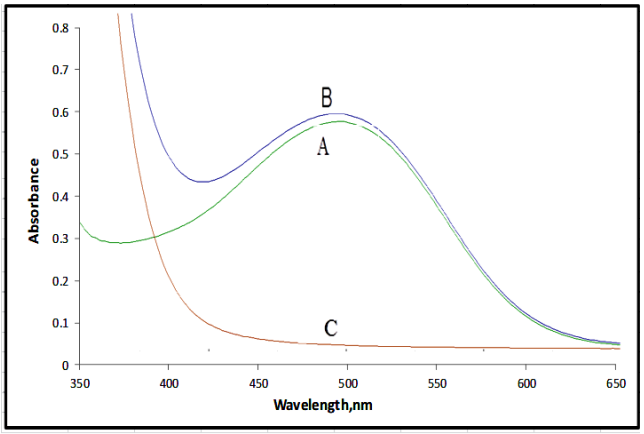


Figure 9: Absorption spectra of 2.5 µg/ml MESZ treated agreeing to the suggested method vs.(A) blank, (B) DW and (C) blank recorded vs. DW

3.3. Suggested method and calibration curve

To a sequence of 20 ml calibrated flasks, 0.1-7 ml of 50 µg/ml pure MESZ solution and 2 ml of 0.1% $NaNO_2$ was added and followed by 1ml of 1M HCl solution. The solutions were mixed thoroughly and set aside constant at room temperature for 2 minutes. After that 0.2 ml of 0.5% sulphamic acid solution was then added and mixed well. A 2 ml of 8-hydroxyquinoline reagent (1%) was added, mixed well and left for 7 minutes. Finally, 2 ml of sodium hydroxide solution (1M) was added and the volumes of all calibrated flasks were completed by DW. The absorbance of the sample solutions was measured at 499 nm versus blank solution prepared in the same manner but without the drug. A relationship between the absorbance and the concentration was plotted to obtain a straight line cover the concentration range from 5 to 250 µg MESZ/20 ml (Figure 10). The molar absorptivity (Ɛ) and the Sandell's sensitivity were calculated and found to be 2.88×10^4 l/mol.cm. and 0.0065 µg/cm^2 respectively.

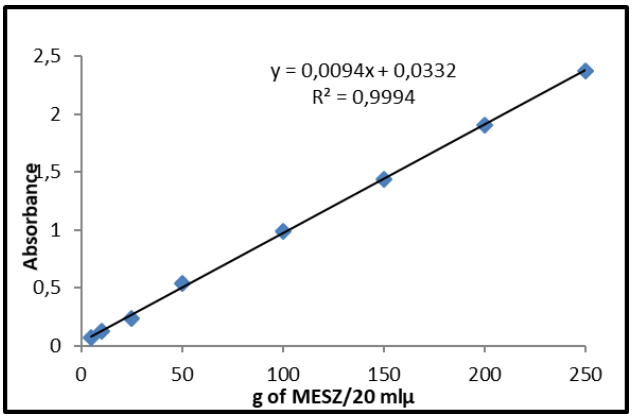


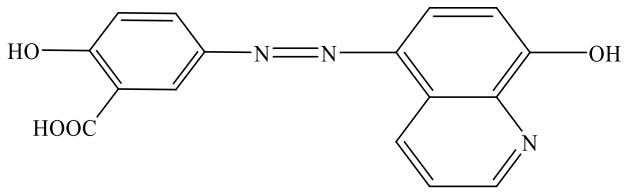
Figure 10: The calibration curve for MESZ determination

4. Discussion

Stoichiometry of the azo dye

The stoichiometry of the resulting azo dye was studied under established conditions by using the continuous variation and molar ratio methods (De Levie, 1997). The acquired results in Figure 11 show that the dye was produced by (1:1) a combination ratio of diazotized MESZ and 8-hydroxyquinoline.

According to the results obtained in Figure 11, the chemical structure of the azo dye can be represented as in (Scheme 2).



Scheme 2: The chemical composition of the azo dye

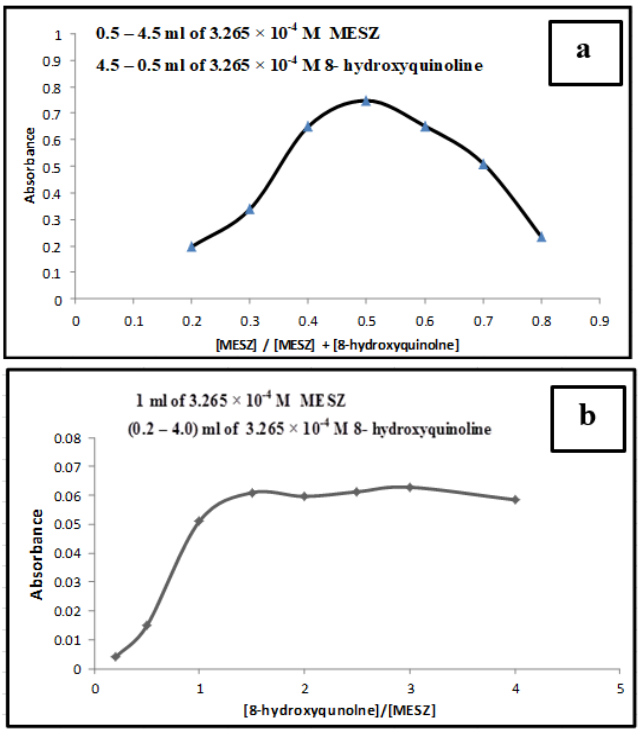


Figure 11: Plots of (a) A continuous variation and (b) molar ratio of the resulting azo dye.

Statistical analysis

The collection of data obtained by applying the proposed method such as Beer's law limits, molar absorption (εmax), accuracy (% recovery), precision (RSD), conditional stability constant, LOD and LOQ values are listed in Table 1. These results indicate that the proposed method Sensitive and precise. The linearity of the method was also proven by calculating the regression equation and the corresponding estimation factor (R^2), which represents good linearity for the proposed method and these data are presented in Table 1.

Application

The applicability of the proposed procedure for the assessing MESZ in two type of tables (Pentasa and AwaSalazine) was applied for four different amounts

20, 50, 100 and 200 µg of MESZ in a total volume 20 ml of each tablet. The results are listed in Table 2.

Table 1. Optical characteristics and statistical data for the proposed method

Parameter	Data
Beer's law range	0.25- 12.5 µg/ml
λmax	500 nm
εmax	2.82 ×10⁴ l/mol.cm
Range of recovery*	96.2% - 102.7%
Relative error range*	- 3.84% - 2.7%
RSD*	≤ 1.94 %
Sandall's sensitivity ,	0.00434 µg/cm²
Determination coefficient (R^2)	0.9994
Average of stability constant (K)	0.37×10⁷ l/mol
LOD ,	0.202 µg/ml
LOQ ,	0.652 µg/ml
Sandell sensitivity	0.00531

*Average of five determinations

Evaluation of recommended procedure

We have verified the efficacy of the proposed procedure according to the standard addition methods and demonstrated that the recommended method can be effectively useful to assay the MESZ without any effect of foreign species. The findings are shown and listed in Figure 12 and Table 3.

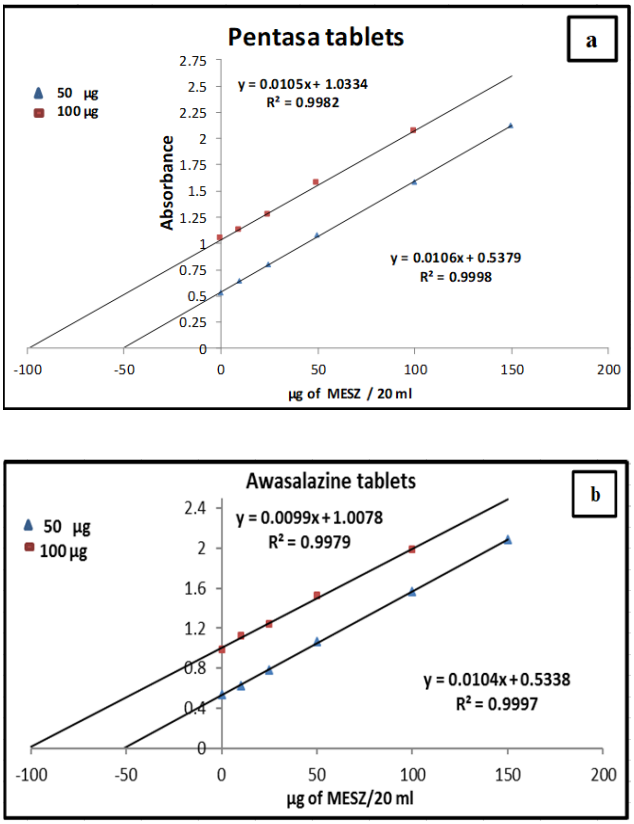


Figure 12: Calibration graphs of standard addition methods for analysis of MESZ in (a) Pentasa and (b) Awasalazne tablets

Table 2. Application of the method

Pharmaceutical Preparation	Certified Value	MESZ Found (µg)	Relative Error (%)*	Recovery (%)*	Measured Value	RSD *	t*
Pentasa tablet (Turkey)	500 mg/tab.	20.80	4	104.00	520 mg/tab.	2.17	---
		51.11	2.42	102.42	512.1 mg/tab.	1.83	1.88
		101.28	1.28	101.28	506.4 mg/tab.	1.94	1.79
		198.96	-0.52	99.48	497.4 mg/tab.	3.01	---
AwaSalazine tablet (Iraq)	400 mg/tab.	20,54	2.70	102.7	410.8 mg/tab.	1.54	---
		49.26	-1.48	98.52	394.08 mg/tab.	1.83	1.91
		96.16	-3.84	96.16	384.64 mg/tab.	0.98	1.68
		195.74	-2.13	97.87	391.48 mg/tab.	2.62	---

*Average of five determinations

Table 3: The results of standard addition method

Pharmaceutical Preparation	MESZ Present (µg)	MESZ Measured (µg)	Recovery (%)
Pentasa tablet	50	50.74	101.49
500 mg MESZ/tablet (Turkey)	100	98.42	98.42
AwaSalazine tablet	50	51.32	102.65
400 mg MESZ/tablet (Iraq)	100	101.79	101.79

Table 4. Compare the proposed method

Parameter	Present Method	Literature Method*
Type of reaction	Diazotization and coupling	Charge transfer complex
Reagent	8-hydroxyquinoline	p-bromanil
Λ_{max}(nm)	500	346
Medium of reaction	Basic	Basic
Beer's law range (ppm) (µg/ml)	0.25-12.5	0.48-12
Molar absorptivity (L/mol.cm)	$2.82x10^4$	$6.5x10^3$
RSD (%)	≤1.94	≤1.7
Colour of the producte	orange	orange
Correlation coefficient (R^2)	0.9994	0.9987
Recovery (%)	96.2-102.7	≥98.04
Relative error (%)	-3.84-2.70	-1.1- -0.29
K (l/mol)	$0.37x10^7$	--------
Sandell sensitivity (µg/cm^2)	0.00531	0.0235
LOD (µg/ml)	0.202	0.053
LOQ (µg/ml)	0.652	0.176

* (Al-Ramadhani and Al-Mtioti, 2019)

Compare the proposed method

Some spectroscopic analytical variables of the proposed method for sulfadiazine estimation were compared with the same variables of other spectroscopic methods, and the results were recorded in Table 4.

5. Conclusion

A spectrophotometric method was used for estimating MESZ through diazotization coupling reaction. The method was based on the coupling of 8-hydroxyquinoline reagent in alkaline solution of NaOH with diazotized MESZ to produce a water soluble azo dye which showed maximum absorption at 500 nm. The procedure is easy, swift, sensitive, low-cost and highly selective. In addition, it does not involve temperature, control of pH or solvent

extraction steps. It is also precise enough to be effectively accepted as an alternative to the existing colorimetric methods with an acceptable result. The linearity of calibration curve covers the concentration range from 0.25 to 12.5 µg/ml with an exceptional molar absorptivity 2.88×10^4 $L.mol^{-1}cm^{-1}$. The limits of detection and quantitation were premeditated and found to be 0.2023 and 0.6524 µg/ml, correspondingly. A relative error percent (accuracy) was also calculated and found to be in the range -3.84% - 2.70%. The development approach was successfully applied for the analysis of MESZ in tablets.

Conflicts of interest

There is no conflict of interest.

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