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Spectrophotometric Determination of Mesalazine

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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 3.3682×10^3 $l.mol^{-1}.cm^{-1}$. The recommended method has been successfully applied to the assay of MESA in pharmaceutical preparations.

Keywords: spectrophotometry, oxidative coupling, mesalazine, histidine.

التقدير الطيفي للميزالازين

الملخص

يتضمن البحث طريقة طيفية بسيطة وسريعة لتقدير الميزالازين (MESA). تعتمد الطريقة على تفاعل الاكسدة والاقتران للميزالازين مع الهستدين(HIS) في وسط قاعدي باستخدام ن_بروموسكسيناميد (NBC) بوصفه عاملا" مؤكسدا" اذ يتكون معقدا" يكون ذائبا" بالماء ومستقرا" ويعطي اعلى امتصاص عند الطول الموجي الاعظم 459 نانوميتر. كانت حدود قانون بيير في مدى التراكيز 50-750 مايكروغرام ميزالازين /20مل (2.5 -37.5 مايكروغرام/ مل) وقيمة الامتصاصية المولارية 3.3682×10^3 لتر.مول$^{-1}$.سم$^{-1}$. تم تطبيق الطريقة المقترحة بنجاح لتقدير الميزالازين في مستحضراته الصيدلانية.

الكلمات الدالة: طيفي، اقتران تأكسدي، ميزالازين، هستدين.

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 $Fe(NO_2)_3$ in presence of HCl (Moharana *et al*., 2011), Ortho-Chloranil (Al-Enizzi *et al*., 2012), 1,5-diphenyl carbazide (1,5-DPC) (Hamdoon, 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 eqution of straight line, the molar absorbtivity was 3.368×10^{3} l.mol^{-1}.cm^{-1}.

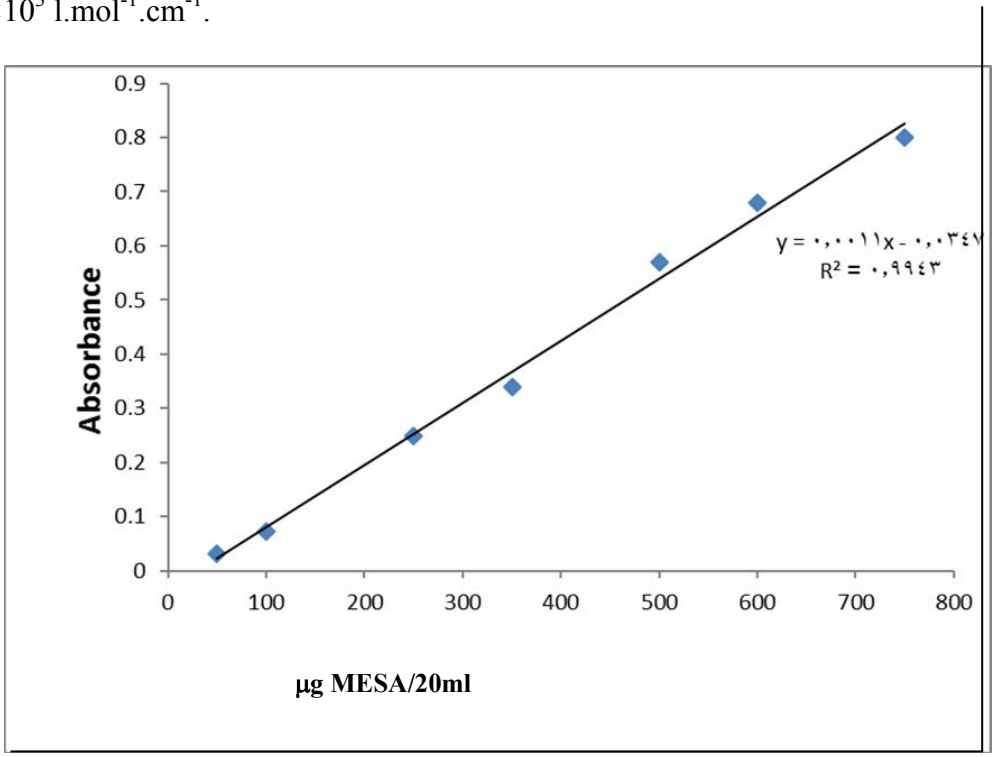


Fig. 1: Calibration graph for determination of MESA using the proposed method.

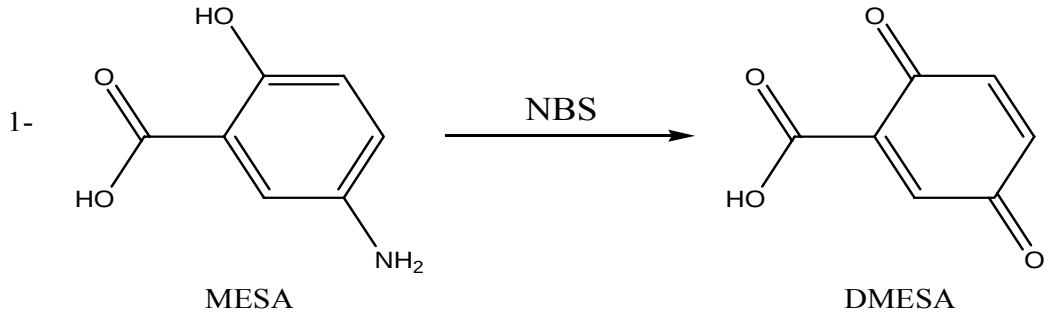
RESULTS AND DISCUSSION

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

Principle of the Method

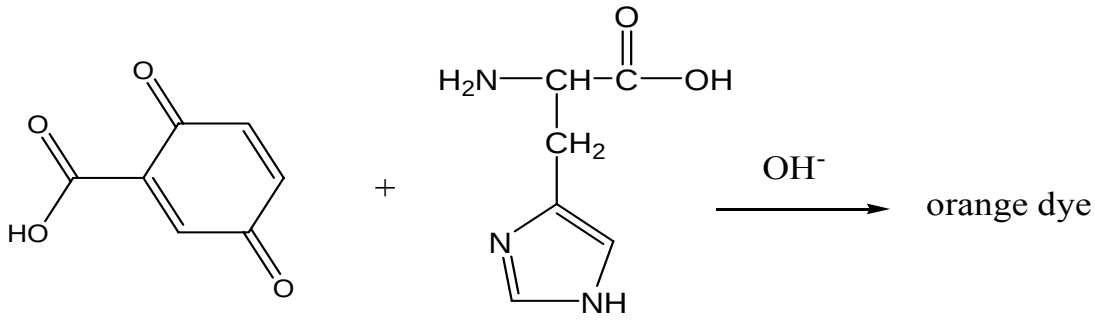
The method included two steps:

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



MESA DMESA

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



DMESA His

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
$NaIO_4$	0.262	166
KIO_3	(Bad result)No color contrast	
K_2CrO_4	(Bad result)No color contrast	
$K_2Cr_2O_7$	(Bad result)No color contrast	
NCS	0.215	195
NBS	0.408	168
Ammonium cerium(IV) sulfate	(Bad result) turbid	

$\Delta\lambda = \lambda_{max}$ S-λ_{max} B S = Dye B=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, therefor various types of bases were studied (Table 2).

Table 2: Choosing suitable base

Base (1ml of 1N)	Absorbance	Δλ
NaOH	0.404	167
KOH	0.385	134
Na_2CO_3	0.296	129
$NaHCO_3$	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)	0	0.5	1	1.5	2	3
Absorbance	0.385	0.409	0.430	0.375	0.369	0.336
pH	6.30	12.27	12.63	12.75	12.86	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					
	200	300	400	500	600	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					
	200	300	400	500	600	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).

Table 6: Effect of time

Time, minutes	0	5	10	15	20
Absorbance	0.478	0.502	0.533	0.569	0.552

Effect of Surfactant

The results in Table (7) showed that no effect of surfactant on the intensity (Table 7).

Table 7: Effect of surfactant

3 ml Surfactant Solution	Absorbance/order of addition							
	I*		II		III		IV	
	A	Δλ	A	Δλ	A	Δλ	A	Δλ
CTAB 1×10^{-3}M	0.189	262	0.208	179	0.277	159	0.383	161
SDS 1×10^{-3}M	0.545	169	0.560	170	0.473	169	0.367	169
Triton x-100 1%(wt/v)	0.544	170	0.527	172	0.509	170	0.391	172
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	Abs./ min. standing time								
	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).

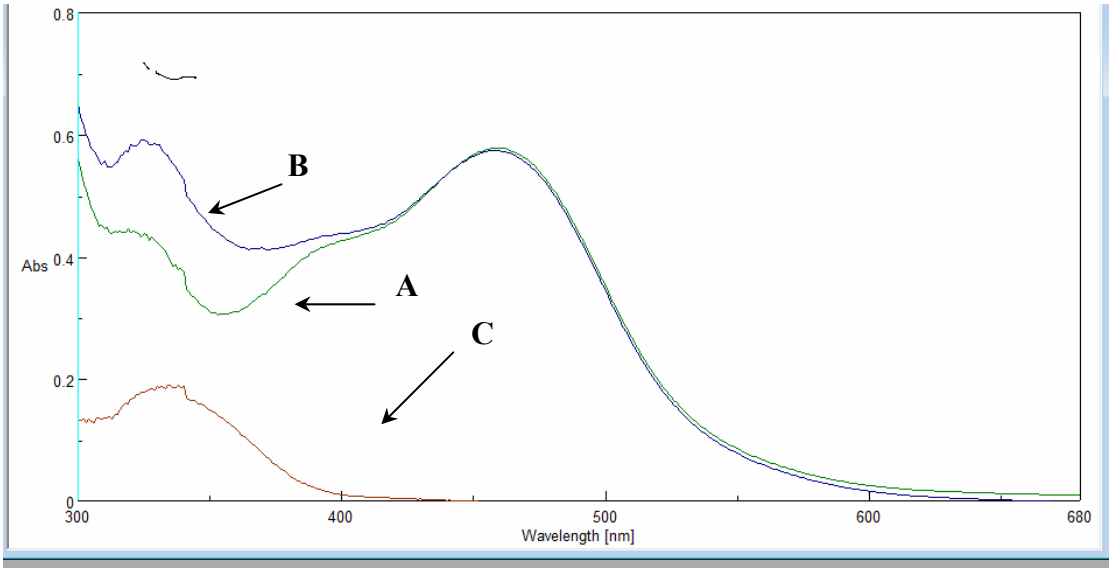


Fig. 2: Absorption spectrum of the colored product 500 µg MESA (A) against blank, (B) against distilled water and (C) blank against distilled water.

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.

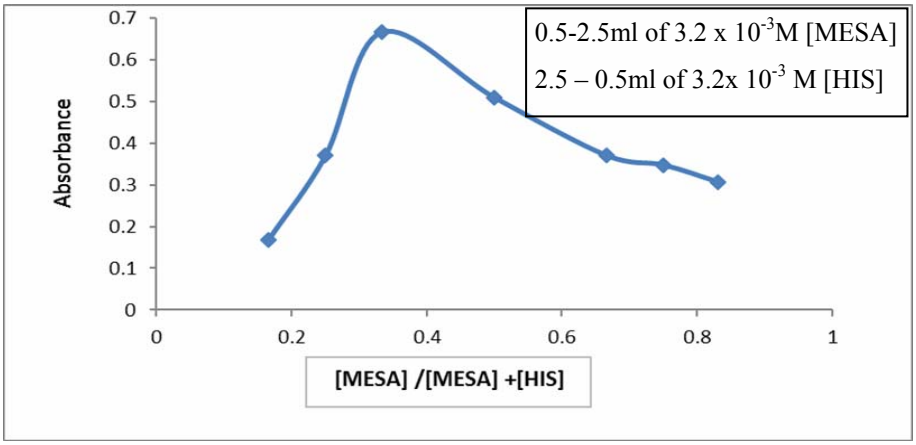
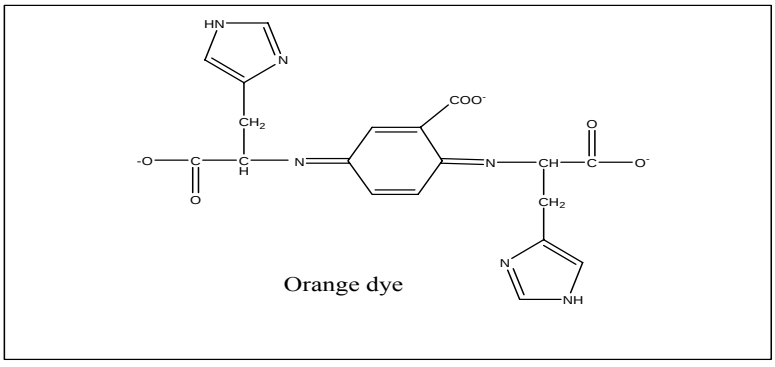


Fig. 3: Job' plot for MESA-HIS colored product

Therefore, the probable colored product have the below structure:



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	200	193.8	96.9	3.1	±0.40
500 mg	400	397.3	99.3	0.7	±0.17
Ferring	500	495.5	99.1	0.9	±1.25
Mezelazin tablets	200	202.3	101.1	-1.1	±0.87
400 mg	400	396.5	99.0	1.0	±0.37
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 absorptivity $l.mol^{-1}.cm^{-1}$	3368.2	3685	29480
Stability of the color (minutes)	240	65	60
Medium of method	Alkaline	Acidic	Alkaline
Reagent	Hisitidine	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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