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Development of spectrophotometric method for determination of mesalazine in pharmaceutical preparation

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Abstract

A simple, rapid, sensitive and accurate spectrophotometric method for the determination of mesalazine in pharmaceutical preparation has been developed. The method is based on the reaction of mesalizine, with excessive nitrite, in an acidic medium, to produce the corresponding diazonium salt. After the removal of residual nitrite with sulphamic acid, the diazonium salt was coupled with N-(1-naphthyl)ethylenediamine (NEDA) in acidic medium to give a violet colored azo dye which has maximum absorption at 552 nm, the colored chromogen was stable for twenty four hours. Beer's law is obeyed in the concentration range of 0.5-40.0 µg/ml (R^2 = 0.999) with limit of detection (LOD) being 0.113 µg/ml. The molar absorptivities were $1.5.10^4$ $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.0263 $\mu g.cm^{-2}$. Recoveries of mesalazine from excipients were 95.4 -102.3 %. No interference was observed from common excipients present in pharmaceutical formulations. The proposed method has been applied successfully to determine mesalazine in pharmaceutical preparation as tablets (Pentasa and Sunmesacol).

Keywords. Mesalazine, spectrophotometry, NEDA, Bratton-Marshall reagent, Pharmaceutical Preparation.

1. INTRODUCTION

Mesalazine (mesalamine, 5-aminosalicylic acid, 5-ASA) is used to treat inflammatory bowel diseases, especially non-specific ulcerative colitis and Crohn's disease. Drugs based on both mesalazine parent substance and its prodrugs (sulfasalazine, olsalazine, balsalazide) are often used for this. Mesalazine is metabolized *in vivo* by acetylating enzymes that produce *N*-acetylmesalazine (*N*-acetyl-5-aminosalicylic acid) (Fig. 1) [1, 2].

A number of analytical methods have been reported for the determination of 5-ASA in pharmaceutical dosage forms and biological fluids including spectrofluorometric, micellar electrokinetic chromatography, differential pulse voltammetry, HPLC, LC/MS/MS and spectrophotometric [3-6]. However, the most widely used and applied methods are those based on spectrophotometry due to its sensitivity, specificity and simplicity. Spectrophotometric methods are based on three principles: (i) diazotization of an organic amine and subsequent coupling with reagents such as 8-hydroxiquinolin, α-naphthol, resorcinol, phloroglycinol, diphenylamine…to form an azo dye [7-9], (ii) nucleophilic reaction of benzofurazan and benzofurocxan reagents (4-chloro-5.7 dinitrobenzofurazan, 7-chloro-4,6 dinitrobenzofurocxan, 5.7-dichloro-4, 6-dinitro-benzofuroxan) with mesalazin in polar media forms a stable product with a strong red color [2], and (iii) Schiff bases are formed by a condensation reaction between mesalazine and an aldehyde or ketone such as vanillin, cinnamaldehyde, p-dimethyl-aminobenzaldehyde [6, 10].

However, the disadvantages of using these methods are that the reaction is often narrow linearity range, requiring heating or extraction, long time for the reaction to complete, use of non-aqueous systems, low stability of the colored product formed. Spectrophotometric methods based on diazotization and coupling principle are sensitive and specific. N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDA) is a simple diamine reported as a coupling agent in spectrophotometric analysis of thiols, aromatic amines, sulfonamides, aminophenols, dinitroanilines, and chloroanilines. It is widely used for the determination of drugs and pharmaceutical containing free primary aromatic amino group.

The objective of the investigation reported in this paper was to evaluate a simple spectrophotometric method for the determination of mesalazine. Based on the diazotization of 5-ASA and coupling with N-(1-naphthyl)ethylenediamine

dihydrochloride (NEDA) reagent and applying the method to the determination of 5-ASA in pharmaceutical preparation.

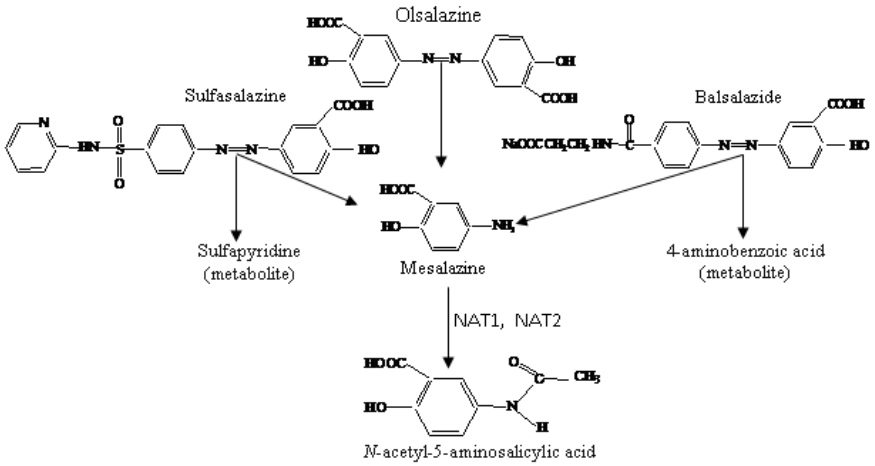

Figure 1: Metabolism of mesalazine and its prodrugs in in humans body [2]

2. EXPERIMENTAL

2.1.Chemicals and equipment

A Biochrom Model SP-60 double beam, UV-VIS spectrophotometer (Biochrom Ltd., UK) with 1.0 cm matched quartz cells was used for absorbance measurements.

Mesalazine (Sigma-Aldrich, Germany, certified to be 99.0 %) and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) (Maya - R, China, certified to be 99 %) were used. All other chemicals and solvents used were of analytical reagent grade.

Commercial samples of tablets namely Pentasa (Ferring, Switerland) and Sunmesacol (Sunpharma, India) containing 500 and 400 mg of mesalamine, respectively were purchased from local pharmacy market and employed in the study.

2.2. Standard solutions

A stock solution of mesalazine (1 mg/ ml) in absolute ethanol. The working standard solution of mesalazine containing 50 µg/ml was prepared by dilution.

Hydrochloric acid solution, 1 N. This solution was prepared by diluting 8.5 ml of the concentrated acid to 100 ml with distilled water. Sodium nitrite solution, 1 %. This solution was prepared by dissoslving 1 g of sodium nitrite in 100 ml distilled water in a volumetric flask. Sulphamic acid solutions, 3 %. This solution was prepared by dissolving of 3 g of sulphamic acid in 100 ml distilled water. NEDA solution, 0.1 %. This solution was prepared by dissolving 0.1 g of NEDA in distilled water in a 100 ml volumetric flask.

Pentasa and Sunmesacol tablets solution 1 mg/ml. Weigh and mix the contents of ten tablets (each one contains 500 mg 5-ASA for Pentasa and 400 mg 5-ASA for Sunmesacol), an accurately weighed amount of powder equivalent to 0.1 g 5-ASA was dissolved in 10 ml absolute ethanol and 30 ml distilled water, after filtration of the solution, the volume of filtrate was completed to 100 ml with distilled water in a volumetric flask. The working Pentasa and Sunmesacol tablet solution of mesalazine containing 50 µg/ml was prepared by dilution.

2.3. Procedure and calibration graph

Into a series of 10 mL volumetric flasks, volumes of 5-ASA working standard solution equivalent to 0.5-40 µg/ml were transferred. To each flask, 2.0 ml of hydrochloric acid (1 N) and 0.5 ml of sodium nitrite (0.3 % w/v) were added and a reaction time of 5 minutes at 0-5 °C was given for completion of the reaction. Next, 0.5 ml of sulphamic acid (3 % w/v) was added to each flask with gentle shaking and after 3 minutes, 5 ml of NEDA reagent (0.1 %w/v) was added, and kept for 5 minutes. Finally the volume in each flask was brought up to the 10 ml mark with distilled water. The absorbances of violet-colored chromogen were measured at 552 nm against the reagent blank and and a calibration graph was constructed. The colored chromogen was stable for twenty four hours.

2.4. Statistical analysis

The limit of detection (LOD) and quantification (LOQ) of the method are given by $3.\frac{SD}{b}$ and $10.\frac{SD}{b}$ respectively, relative standard deviation (RSD (%))= $\frac{SD}{\bar{X}}.100$; where SD is the standard deviation, b is the slope of the calibration curve equation, $\bar{X}$" is the average value of the measurement. The % recovery of the added pure drug was calculated as:

% Recovery = [(Ct–Cs)/Ca] x 100

where Ct is the total drug concentration measured after standard addition; Cs, drug concentration in the formulation sample; Ca, drug concentration added to the formulation

Calculation and processing of data were done using the programs Origin Pro 8.0 and Statistica 7 (US).

3. RESULTS AND DISCUSSION

3.1. Principles of the method

The method is based on diazotization of mesalamine with nitrous acid, to form diazotized mesalamine (1). The residual nitrite (as nitrous acid) which was undesirable due to its side reaction, such as, nitrosation of coupling agent, was removed by sulphamicacid (2), followed by its coupling with NEDA reagent to form a violet colored chromogen (3) with maximum absorption at 552 nm; it obeyed the Beer's law in the concentration range of 0.4-40 µg/ml. The reaction mechanism is shown in figure 2.

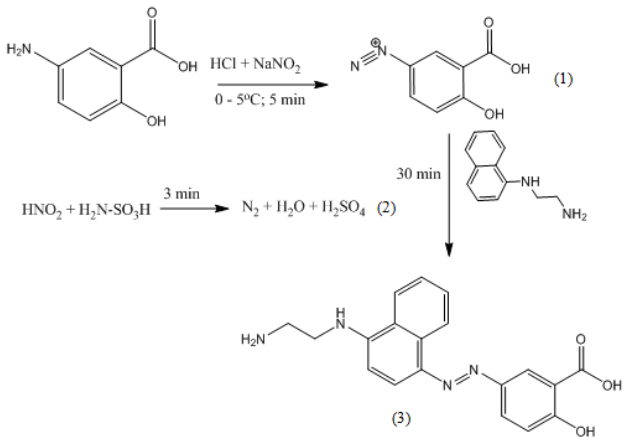

Figure 2: The reaction mechanism

3.2. Study of the optimum reaction conditions

The various parameters affecting the colour intensity of the dye have been studied and optimum conditions are selected.

3.2.1. Choice of coupling agent

Different coupling agents are used for the reaction with diazotized mesalazine. The results in table 1 indicated that NEDA gave the highest intensity with a good colour contrast for coloured product.

Table 1: Selection of coupling agent

Reagents 0.1 %	Absorbance	λ_{max}(nm)	$\Delta\lambda^{*}$nm
α-napthylamine	0.148	381	83
8-hydroxyl quinoline	0.16	407	114
Phenol	0.186	318	40.5
Diphenylamine	0.28	341	44.5
Aniline	0.38	330	70
NEDA	0.47	552	82

$^{*}\Delta\lambda$= colour contrast = $\lambda_{maxS} - \lambda_{maxB}$, where S = The dye, B = Blank.

3.2.2. Effect of acids on the diazotization

The effect of the amount of different acid (week and strong) for the diazotization of 5-ASA, has been investigated. The results indicated that 2.0 ml of 1 N HCl produces the highest intensity for the dye, so it has been selected in the subsequent experiments (figure 3).

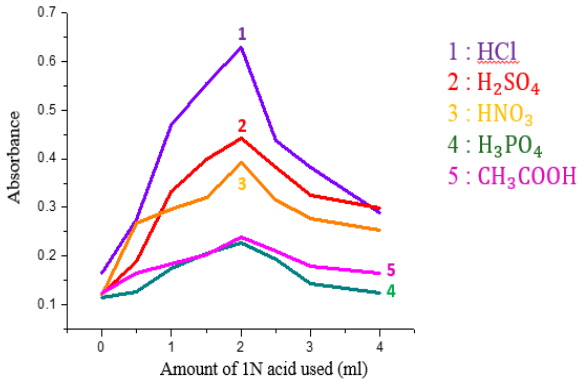

Figure 3: Effect of diazotization acid on absorbance

3.2.3. Effect of nitrite amount and time

The colour reached maximum intensity when using 0.5 ml of 1 % (w/v) sodium nitrite solution

within 5 minutes reaction time (table 2). It seems that diazotization of 5-ASA was fast.

3.2.4. Effect of sulphamic acid amount and time

The presence of unreacted nitrite was undesirable in diazotization reaction. Therefore, it should be removed by sulphamic acid which quickly reacts with nitrite. The results indicated that 0.5 ml of 3 % sulphamic acid solution with 3 minutes standing time was considered to be the most suitable (table 3) and therefore was selected subsequently.

Table 2: Effect of nitrite amount and time on absorbance

ml of 1% (w/v) $NaNO_2$ solution	Absorbance/minute standing time						
	0	1	2	3	5	7	10
0	0.012	0.093	0.029	0.018	0.154	0.112	0.201
0.5	0.137	0.303	0.361	0.411	0.433	0.331	0.303
1.0	0.213	0.28	0.38	0.332	0.388	0.303	0.287
1.5	0.356	0.366	0.337	0.319	0.374	0.211	0.32
2.0	0.305	0.350	0.349	0.376	0.406	0.304	0.271
2.5	0.056	0.065	0.048	0.04	0.179	0.14	0.237

Table 3: Effect of sulphamic acid and time on absorbance

ml of 3% (w/v) sulphamic acid solution	Absorbance/minute standing time						
	0	1	2	3	5	7	10
0	0.002	0.017	0.019	0.02	0.013	0.015	0.011
0.1	0.003	0.029	0.034	0.063	0.042	0.021	0.014
0.3	0.006	0.023	0.055	0.266	0.063	0.044	0.023
0.5	0.024	0.148	0.274	0.401	0.320	0.291	0.029
0.7	0.054	0.146	0.231	0.265	0.177	0.151	0.019
1.0	0.044	0.185	0.235	0.305	0.271	0.203	0.188
1.5	0.057	0.164	0.244	0.294	0.221	0.162	0.114
2.0	0.062	0.102	0.199	0.274	0.207	0.192	0.142

3.2.5. Effect of NEDA amount

The effect of NEDA amount on the colour intensity of the dye has been studied. From the result it can be observed that 5 ml of 0.1 % NEDA was the most suitable amount which gives the highest value of absorbance for the azo-dye formed (table 4).

Table 4: The effect of NEDA amount

Amount of 0.1 % NEDA (ml)	Absorbance / µg of 5-ASA		
	5	10	15
0.5	0.031	0.150	0.336
1.0	0.081	0.279	0.668
2.0	0.315	0.506	1.089
3.0	0.364	0.685	1.694
4.0	0.475	0.913	1.87
5.0	0.518	1.006	2.301
6.0	0.500	0.966	2.151
7.0	0.477	0.853	1.941

3.2.6. Final absorption spectra

When 5-ASA was treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 552 nm. characteristic of the violet dye. The reagent blank shows no absorption at this wavelength (Fig. 4).

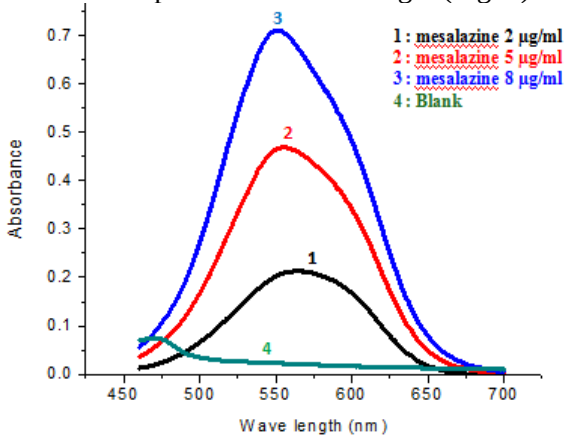

Figure 4: Absorption spectra of 5-ASA-NEDA and blank

3.3. Comparison with other spectrophotometric methods

The proposed method compares favorably with other reported methods. As shown in table 5 the proposed method is more sensitive than other methods in that it needs no heating and the product is stable for a longer time.

3.4. Interference

The extent of interference by some excipients magnesium stearate, glucose, lactose, glycine and starch which are often found in pharmaceutical preparations was studied by measuring the absorbance of solutions containing 5 µg/ml of mesalamine and 500, 1000 and 2000 µg/ml of excipients in the final volume of 10 mL. It was found that these excipients do not interfere in the present method. The range of recovery is between 95.4 and 102.3 %. We consider that this variation is acceptable.

Table 5: Comparison of the proposed method with other spectrophotometric methods

Parameters	Proposed method	[9]	[10]
λ_{max} (nm)	5525	510	440
Reagents	NEDA	α-Napthol	PDAB
Beer´s limit (µg/mL)	0.5-40	1-15	50-500
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.50×10^4	1.48×10^4	3.74×10^2
Sandell's sensitivity ($\mu g/cm^2$)	0.02633	0.01030	0.415
Stability of colored products (h)	24	3	5
Regression equation (Y = bx + a)			
Slope (b)	0.0851	0.5578	0.0237
Intercept (a)	0.0985	0.0152	0.0041
Correlation coefficient (R^2)	0.999	0.9975	0.9980
Relative standard deviation (%)	0.615	0.9216	0.00436
LOD (µg/mL)	0.113	0.0059	0.699
LOQ (µg/mL)	0.342	0.0179	1.84

Table 6: Effect of interferences

Foreign compounds	Recovery (%)		
	500	1000	2000
Magnesium stearate	97.0	100.1	100.4
Glucose	99.2	98.4	96.1
Lactose	102.3	102.0	101.9
Glycine	97.2	95.4	100.7
Starch	97.2	100.3	95.5

3.5. Application of the method

The results of analysis of marketed formulation are shown in table 7. The relative standard deviation values are below 2 % indicating the precision of the method. The validations of the proposed methods were further confirmed by recovery studies. The % recovery varies from 98.52±0.712 to 99.68±0.692 indicated high accuracy of methods. The high % recovery value indicates non interference from excipients used in formulations.

4. CONCLUSION

The proposed methods are found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of mesalamine in pharmaceutical preparation.

Table 7: Results of analysis of marketed tablets

Brand name of tablet dosage form	Labeled amount (mg/tablet)	Amount found by proposed methods	Recovery (%)	RSD (%)
Pentasa	500	498.45	99.68±0.692	0.694
Sunmesacol	400	394.09	98.52±0.712	0.722

REFERENCES

1. P. G. Javier, G. Fernando, M. José, M. P. José. *Role of 5-Aminosalicylic Acid (5-ASA) in Treatment of Inflammatory Bowel Disease*, Digestive Diseases and Sciences, **47(3)**, 471-488 (2002).
2. S. Y. Garmonov, Z. C. Nguyen, I. F. Mingazetdinov, L. M. Yusupova, N. S. Shitova, R. N. Ismailova, V. F. Sopin. *Spectrophotometric determination of mesalazine in urine for assessing the acetylation phenotype in vivo in humans*, Pharma. Chem. J., **45(12)**, 48-51 (2011).
3. K. Kanchanamala et al. *Simultaneous quantification of mesalamine and its metabolite N-Acetyl mesalamine in Human plasma by LC-MS-MS and its application to a bioequivalence study*, British Journal of Pharmaceutical Research, **4(13)**, 1568-1590 (2014).
4. J.Qin, X Di, X Wang, Y Liu. *Development and validation of an LC-MS/MS method for the determination of mesalazine in beagle dog plasma and its application to a pharmacokinetic study*, Biomed Chromatogr, **29(2)**, 261-7 (2015).
5. Theia'a N. Al-Sabha, Nagham N. Habeeb. *Spectrophotometric Determination of Mesalamine Using Sodiom Nitroprusside as Chromogenic Reagent*, Eur. Chem. Bull, **4(8)**, 384-388 (2015).
6. Fatima Altayib Alasha Abdalla and Abdalla Ahmed Elbashir. *Development and Validation of Spectrophotometric Methods for the Determination of Mesalazine*, Medicinal Chemistry, **4(3)**, 361-366 (2014).
7. V. Madhavi, V. Panchakshari, T. N. Prathyusha, C. B. Sekaran. *Spectrophotometric determination of mesalazine in bulk and tablet dosage forms based on diazo coupling reaction with resorcinol*, Int. J. Pharm. Sci. Rev., Res., **11(1)**, 105-109 (2011).
8. Enam A. Hamdon, Safaa A. Zakaria, Nassim M. AL-Hamdany. *Spectrophotometric Determination of Mesalazine with Phloroglycinol in Pharmaceutical Preparation*, Tikrit Journal of Pure Science, **17(2)**, 133-137 (2012).
9. Bala Sekaran Chandra, et al. *Simple and sensitive spectrophotometric methods for the analysis of mesalamine in bulk and tablet dosage forms*, Quim. Nova, **34(6)**, 1068-1073 (2011).
10. Patel KM, et al. *Development and Validation of Spectrophotometric Methods for the Estimation of Mesalamine in Tablet Dosage Forms*, J. Young Pharm, **2(3)**, 284-288 (2010).

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