EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 7
328-335
Year: 2015

COLORIMETRIC DETERMINATION OF MESALAMINE IN PURE AND DOSAGE FORMS

Safaa Mohamed Abdel Hamide* **and Nagat A. Abbas***

*Lecturer at National Organization of Drug Control and Research.

***Author for Correspondence: Safaa Mohamed Abdel Hamide**
Lecturer at National Organization of Drug Control and Research.

Article Received On 12/10/2015 **Article Revised On 02/11/2015** **Article Accepted On 22/11/2015**

ABSTRACT
Three simple, sensitive, economic, rapid and reproducible spectrophotometric methods I, II, III are described for determination of mesalamine in pure drug as well as in tablet dosage forms. Method I is based on the reaction of mesalamine with vanillin in phosphate buffer medium with pH equals 7, forming yellow colored Schiff s base complex with absorption maximum at 380 nm. Method II describes, the reaction of mesalamine with ninhydrin in dimethylformamide forming pink colored complex with absorption maximum at 550 nm. Method III is based on the reaction of mesalamine with P-benzoquinone giving yellow colored complex with absorption maximum at 390 nm, under optimum conditions, mesalamine could be quantified in the concentration range of (100-400)µg/ml, (50-300)µg/ml and (70-300)µg/ml for methods I, II and III ,respectively the correlation coefficients are 0.9999, 0.9998 and 0.9995 for methods I, II and III respectively which show the linearity of the response against concentration. The methods were validated as per ICH guidelines. The developed and validated colorimetric methods are suitable for assay determination in pure and pharmaceutical formations which are more useful with respect to less analysis time.

KEYWORDS: Spectrophotometry, mesalamine, vanillin, ninhydrin, P-benzoquinone, validation.

INTRODUCTION
Mesalamine (united States Adopted Name) is also known as Mesalizine (international Nonproprietary name/British Approved Name) or 5-amino salicylic acid (5-ASA) or chemically designated as 5-amino-2-hydroxy benzoic acid. Mesalamine is an anti inflammatory drug[1] used to treat inflammatory bowel disease, such as ulcerative colitis[2] and mild to moderate crohn's disease.[3] Local intestinal flora split sulfasalazine into sulphapyridine and 5-aminosalicylate.[4] The chemical formula of Mesalamine is $C_7H_7No_3$ with the molecular weight of153.135 $g.mol^{-1}$ and molecular structure is shown below. Mesalamine is a tan to pink crystalline powder, relatively insoluble in chloroform, ether, n-hexane and ethyl acetate, freely soluble in dilute hydrochloric acid and alkali hydroxides.[5] Mesalamine is available in tablet dosage form and is an official drug of USP. A number of analytical methods have been developed for the analysis of mesalamine in pure form and pharmaceutical dosage forms. The therapeutic importance of mesalamine has necessitated the development of analytical methods for its determination in dosage forms in compliance with good manufacturing standards. The drug is officially listed in 2000 United States pharmacopoeia and the official method of its determination with high performance liquid chromatography (HPLC) using mobile phase containing tetrabutylammonium hydrogen sulphate as an ion pairing agent.[6] Various other techniques such as UV-spectrophotometry[7-15], potentiometric titration[16], high performance liquid chromatography (HPLC) combined with UV fluorescence[17-20] and electrochemical (Ec) detection[21], visible spectrophotometric methods, because of simplicity, cost effectiveness, sensitivity, selectivity, fair accuracy and precision, have remained competitive in the area of chromatographic techniques for pharmaceutical analysis. Few visible spectrophotometric methods are found in the literature for the assay of mesalamine, in a method reported by Rafeel et al.[16] Hydrogen donating ability of mesalamine to 1, 1-diphenyl -2-picrylhydrazyl radicals is used as a basis for its determination in pharmaceutical dosage forms. Patel et al.,[15], reported three methods for the assay of mesalamine in tablet dosage forms with Bratton-Marshall reagent, p-dimethylaminobenzaldelyde and Gibb's reagent. Reaction of mesalamine with potassium iodide and potassium iodide was used by Navya Sloka et al.,[7], for determining mesalamine, the reported visible spectrophotometric methods suffer from one or the other disadvantage such as poor accuracy and precision, using relatively expensive reagents, heating, control of temperature narrow range of linear response and low molar absorptivity considering this drawback, there was a need to develop more advantageous spectrophotometric

methods, for the determination of mesalamine in pharmaceutical form as well as in pure form the aim of this work is to describe three sensitive, selective, accurate, precise, simple and economical methods for the determination of mesalamine in pure form as well as in pharmaceutical dosage forms. The proposed methods were validated as per the guidelines of international conference on harmonization ICH.[22]

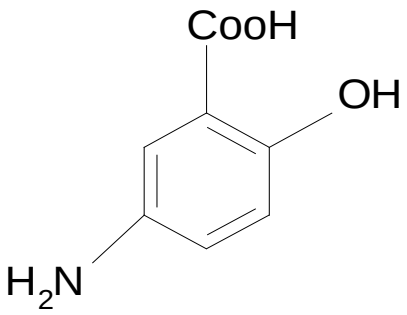


Fig. Structure of mesalamine.

EXPERIMENTAL

Apparatus

1. Shimadzu 1660 UV recording spectrophotometer with 1cm cell.
2. Hanna PH meter for adjusting the PH value.

Materials and reagents

1. Mesalamine pure sample was obtained from Mena pharma Egypt and commercial forms (Tablets was obtained from local market).
2. Phosphate buffer was prepared by mixing 36ml of 0.2 M sodium hydrogen phosphate with 64 ml of 0.1 M citric acid and the pH-value was adjusted to the desired value (pH=7.0) by using 0.2 M sodium hydroxide.
3. Vanillin reagent was prepared by mixing 2 (g) of vanillin powder with 0.3 ml of acetaldehyde and volume was completed to 5o-ml with ethyl alcohol.
4. Ninhydrin reagent: 0.2% w/v of ninhydrin in dimethylformamide (DMF) and should by freshly prepared.
5. 0.1 M ethanolic solution of P-benzoquinone.
6. 0.1 M Phosphate solution (NaHpo4+NaH_2 Po4) adjusted to pH= 7.0 by sodium hydroxide solution.

Standard solution of Mesalamine

Solution of 4.0mg/ml was prepared by dissolving 400mg of mesalamine in 100 ml distilled water; the solution was store in cool and dark place. Diluted solutions were prepared by appropriate dilution.

General Procedures

Method I, (using vanillin reagent)

Into 10-ml measuring flask, different aliquots of mesalamine solution (0.1-1.0) ml were transferred to provide final concentration of (40-400) μ g/ml, to each flask 1.5 ml of vanillin and 3.0 ml of phosphate buffer (pH=7.0) were successively added and set aside at room temperature for 20-minutes, the volume was made up to mark with distilled water and absorbance was measured. It was found that the colored complex formed has absorption maximum at max = 380 nm against blank prepared similarly omitting drug, calibration curve was plotted as a function of absorbance against concentration of drug.

Method II (using ninhydrin reagent)

Into 10.0 ml test tubes, different aliquots of mesalamine solution (0.10-1.0)ml were transferred to provide concentration of (50-300) μ g/ml, to each tube added 3.0 ml of ninhydrin in dimethyl formamide was added, the test tubes were heated on water bath at 60 ± 5°C for 15-minutes. Then test tubes were cooled to room temperature and the contents of each tube were transferred slowly to 10-ml measuring flask, and the volume was made up to mark with distilled water and the absorbance of colored complex was measured, it was found that the colored complex absorbed maximally at max = 550 nm against a blank the standard calibration graph was plotted as a function of absorbance against concentration.

Method III: (p-benzoquinone)

Into set of 10-ml test tubes, different aliquots of mesalamine solution of (0.1-1.0) ml were transferred to provide concentration of range of (70-300) μ g/ml, to each tube added 2.0 ml P-benzoquinone and 4.0 ml of phosphate solution of pH-7.0, then the tubes were placed in water bath at 70 ± 5°C for 30-minutes, then the tubes were cooled to room temperature and the contents of each tube were transferred carefully to 10-ml measuring flask and volume was completed to mark with distilled water and absorbance of reaction mixture was measured it was found that the colored product absorbed maximally at λ_{max}= 390 nm against a blank prepared similarly omitting drug the calibration standard curve was constructed as a function of absorbance VS concentration.

Procedures for tablets

An accurately weighed amount of powder, equivalent to 400 mg of powder was dissolved in water then filtered. The procedure was continued as described above.

ESULTS AND DISCUSSION

Mesalamine had low absorption value in UV spectrophotometry and so low sensitivity. The aromatic amino group in mesalamine reacted with many colored reagent such as vanillin, ninhydrin, b-benzoquinone forming colored product.

Optimization of the reaction conditions

Method I (vanillin method)

Vanillin reagent was used for estimation amino group containing compounds.[23-24] This reagent contains an aldehyde group that can be reacted with amino group via condensation reaction forming colored Schiff base product which absorbed at 380 nm, phosphate buffer of

pH=7 required to achieve reaction and obtain color. Trials were made up to study effect of buffer, time and temperature, it was found that pH= 7.0 and 30 minutes at room temperature was found to be optimum conditions that gave maximum color intensity and maximum absorption at 380 nm. The change in type of buffer, pH-value and concentration of buffer lead to change in λ_{max} the suggested reaction pathway between mesalamine and vanillin is shown in scheme 1. The complex formed between mesalamine and vanillin was formed by the ratio of 1:1 as indicated by the molar ratio and continuous variation methods. Trials were made up to study effect of buffer, time and temperature as follow:

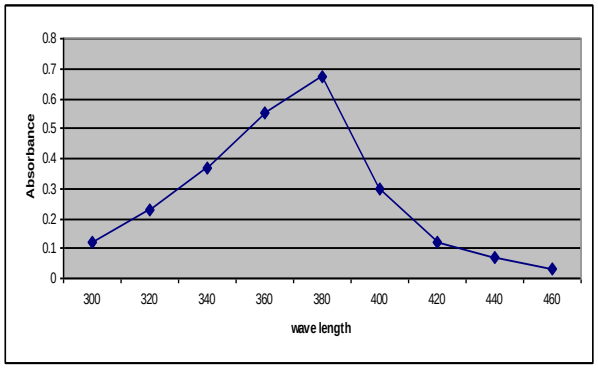


Fig. 1. Absorption curve for reaction of mesalamine and vanillin.

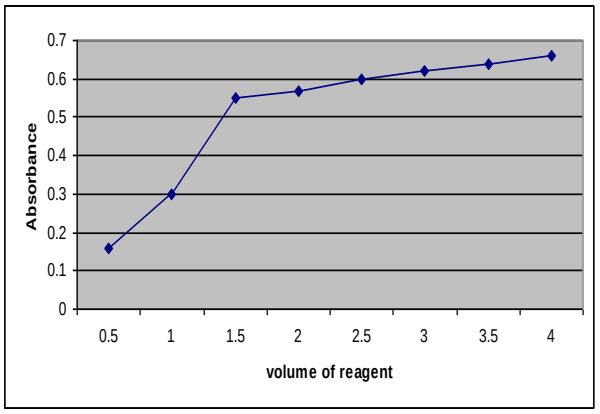


Fig. 2. Effect of volume of vanillin reagent at λ_{max} = 380.

1. Effect of type of buffer

Different types of buffers were used like acetate buffer, borate buffer, universal buffer and phosphate buffer, results showed that phosphate buffer was the best one, which gave the highest color intensity and highest color absorbance of formed chromogen.

2. Effect of value of buffer solution

By taking different values of phosphate buffer from (5.0-10.0), results exhibited that the pH-value equals 7.0 was found to be the best one that gave high color intensity and high absorbance values.

3. Effect of volume of buffer solution

By taking different volumes of phosphate buffer ranging from (1-5) ml, results revealed that 3.0 ml of phosphate buffer of pH=7.0 and 1.5 ml of vanillin reagent were the best values, as it gave highest absorbance value.

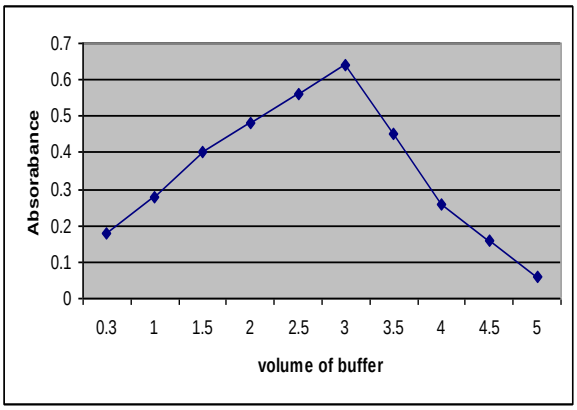


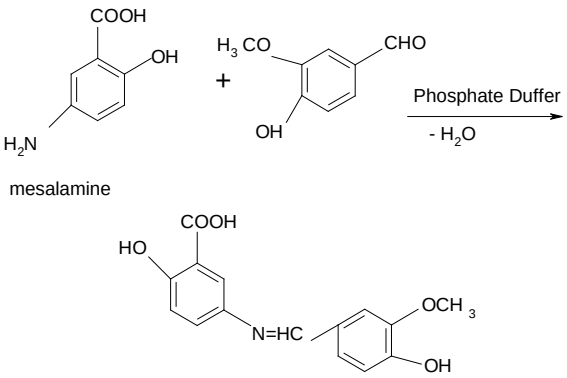
Fig. 3. Effect of volume of phosphate buffer on reaction of mesalamine and vanillin at λ_{max} = 380.

4. Effect of time

This effect can be done by leaving reaction mixture at different time intervals ranging from (10-60) minutes, results showed that maximum color intensity and absorbance value were given at 20-minutes.

5. Effect of temperature

This effect was made by heating reaction mixture at different temperature intervals ranging from room temperature, 30, 40, 50, results showed that temperature decreased color of complex and decreased value of measured absorbance of color complex, so formation of colored complex was obtained at room temperature after 30 minutes.



Scheme 1. The suggested reaction path way of mesalamine and vanillin.

Method II (ninhydrin reagent)

Ninhydrin reagent reacts with compounds containing amino group via condensation reaction.[25-27] So mesalamine reacted with ninhydrin forming colored complex, which absorbed maximally at 550 after heating in water bath for 20 minutes at 60°C. To optimize reaction conditions, several parameters must be studied

like concentration and volume of reagent, time, temperature and solvent.

The validity of method can be determined. The optimum conditions were established by varying one variable and observing its effect on the absorbance of the colored product.

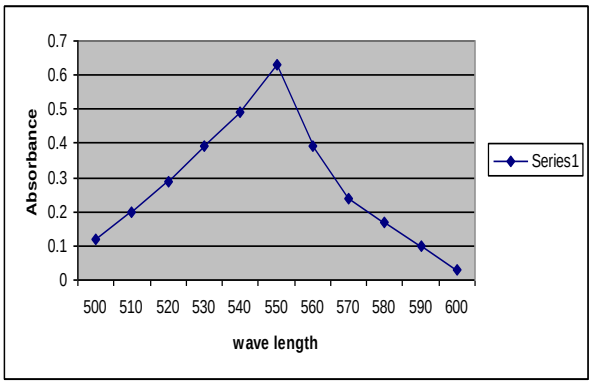


Fig.4. Absorption curve of reaction of mesalamine and ninhydrin.

Mesalamine was capable to react with ninhydrin only at higher temperature, maximum color intensity was obtained by heating on water bath at 60°C ± 5°C for 20-minutes and prolonged heating decreased the color intensity due to dissociation of colored product. The developed color was stable for at least 2-hours. Different dilution solvents like water, ethanol, methanol, isopropanol, acetone and dioxane were tried but water gave the best results. To optimize the reaction conditions different factors must be studied as follow.

1. Effect of volume of reagent

This effect was done by taking different volume of ninhydrin from (0.5-5.0) ml and measuring absorbance in each volume, the results showed that 3.0 ml of reagent was the best as it gave the highest absorbance of formed complex.

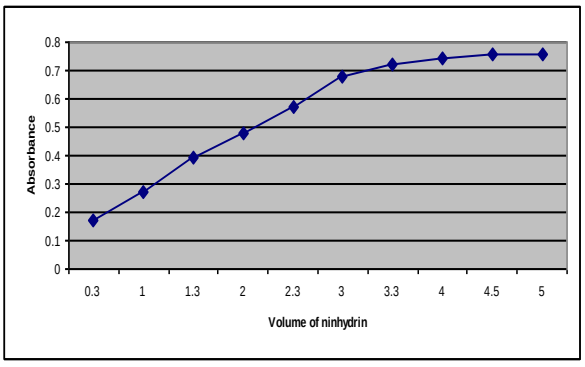


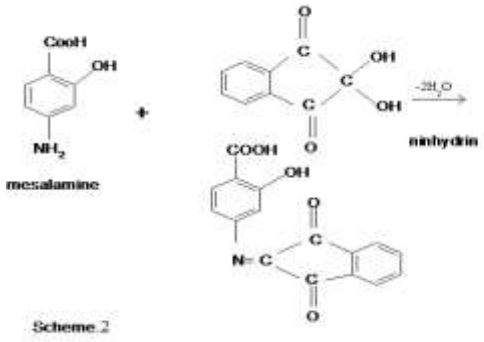
Fig. 5. Effect of volume of ninhydrin at λ_{max} = 550.

2. Effect of Temperature

This effect was done by taking different temperatures ranging from (30,40,50,60,70c/and measuring absorbance of reaction at each temperature, the results showed that the temperature equals to 60° was the best as it gave the maximum absorbance.

3. Effect of heating time

This effect was done by heating reaction at 60°C for different time intervals ranging from (5-30°) minutes, experimental data showed that 15-minutes was the best time for giving the highest color intensity and maximum absorbance.



Method III (P- benzoquinone reagent)

P- benzoquinone reagent was used for determination amino group containing compounds.[28] Scheme3 shows the possible reaction pathways predicted from the literature and from the results of the present work, where the primary amino group of mesalamine condenses with the carbonyl group of P-benzoquinone releasing water molecule with forming condensation product.

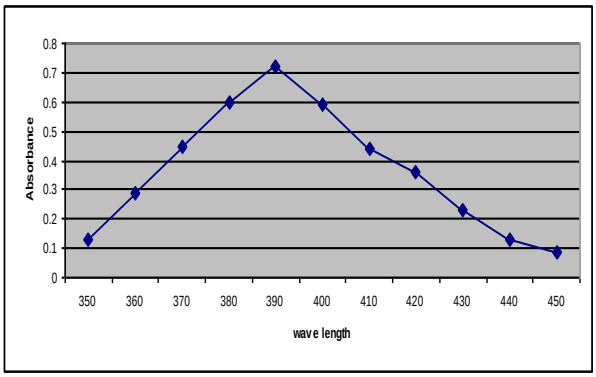
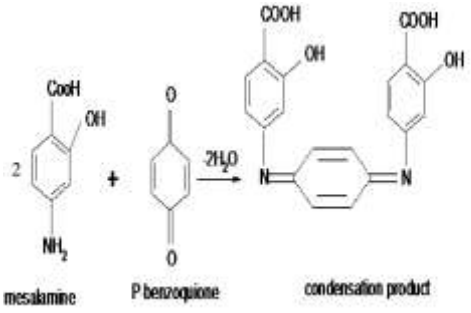


Fig.6. Absorption curve of reaction of mesalamine with P- benzoquinone.



Scheme 3. Reaction pathway of Mesalamine and P-benzoquinone.

Under the reaction conditions used, which are heating to 70°C ± 5°C for 30-minutes. It was clear that the product of reaction between P-benzoquinone and mesalamine absorbed maximally at 390 nm the absorpimetric properties of the colored species as well as the effect of different parameters on the color development were extensively studied to determine optimal conditions of the assay procedures. The reaction was studied as a function of the volume of the reagent, selectivity of the solvent, reaction time and stability. The optimum conditions were incorporated into general procedures. The factors affecting the reaction were studied as follow.

1. Effect of volume of reagent

By taking different volumes of reagent, ranging from (0.5-3.0) ml experimental data showed that 2.0 ml was the best as it gave maximum absorbance.

2. Effect of type and volume of buffer

By taking different types of buffer solutions, results showed that phosphate buffer of pH equals 7.0 was the suitable buffer due to giving maximum color intensity and by using different volumes of phosphate buffer ranging from (1-6), results exhibited that 4.0 ml of phosphate buffer of pH = 7.0 was the best one for obtaining maximum absorbance.

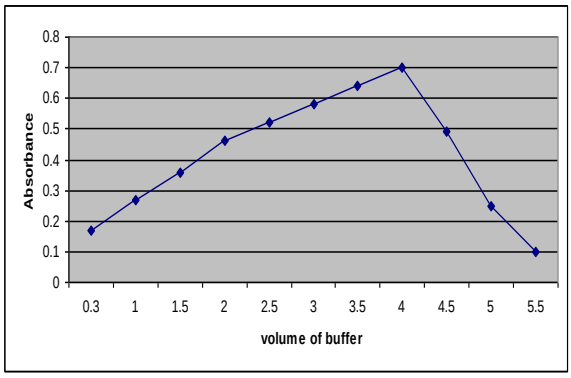


Fig.7. Effect of volume of buffer on reaction of mesalamine with P- benzoquinone at λ_{max} = 390.

3. Effect of temperature

By taking different temperature ranging from (30-85) C° the results showed that 70±5C° was the best one.

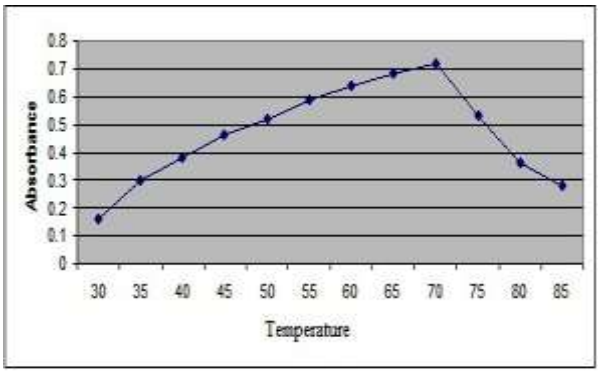


Fig.8. Effect of temperature on reaction of mesalamine with P- benzoquinone at λ_{max} = 390.

4. Effect of heating time

By taking different time intervals ranging from (10-40) minutes, results showed that 30-minutes, were the suitable one.

Validation of the proposed methods

Under the described experimental conditions, standard calibration curves for reaction mesalamine with vanillin, ninhydrin and P-benzoquinone were plotted as a function of absorbance of colored product against concentration of mesalamine, conformity with Beer's law was clear in the concentration range of the final dilution as shown in Table. 1.

The proposed methods were tested for linearity, accuracy and precision. By using the above procedures, linear regression equations were obtained. The regression plots showed a linear dependence of the absorbance over beer's law ranges which are given in Table. 1. The table also shows the results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the molar absorptivities, Sandel sensitivity and correlation coefficients obtained by the linear least squares treatment of the results.

In order to determine the accuracy and precision of the above methods. Solutions containing three different concentrations of mesalamine were prepared and analyzed in five replicates. The analytical results were obtained from this investigation are summarized in Table. 2. the mean relative standard deviation (RSD) and the mean standard analytical error (SAE) could be considered to be very satisfactory.

The proposed methods for determination of mesalamine were applied to commercial formulations together with the reference method[5-6] these determinations were carried out on the same batch of samples. The results obtained showed that the calculated t-and F- values did not exceed the theoretical values at 95° confidence limits for five degree of freedom table.3, from which we can conclude that the proposed methods did not differ significantly from reference methods.

Table. 1. optical characteristics and statistical data of the regression equations for the determination of mesalamine using the proposed methods.

Parameters	Methods.1. (using vanillin)	Method. II. (using ninydrin)	Method. III (using P-benzoquinone)
Color of product produced	Yellow	Violet-pink	yellow
λ_{max} nm	380	550	390
Linearity range, μ g/ml	(100-400) μ g/ml	(50-300) μ g/ml	(70-300) μ g/ml
Molar absorptivity L. mol^{-1}. cm^{-1}	$4.47x10^2$	$3.22x10^2$	$4.83x10^2$
Sandells sensitivity μ $g.cm^{-1}$	0.376	0.342	0.370
Regression equation slop, (b)	0.363	0.345	0.355
Intercept, (a)	0.0091	0.011	0.005
Relative standard deviation (RSD)	0.80	0.67	0.76
Correlation coefficient (r)	0.9999	0.9998	0.9995
Limit of detection (LOD)	5.37	5.82	6.42
Limit of quantitation (LOQ)	17.9	19.42	21.4

Table. 2. Evaluation of the accuracy and the precision of the proposed methods.

Method I	Added	Found ± S.D[a]	RSD %	SE	Confidence limits [b] ± SD%
Method I (using vanillin)	100 200 300	99.55 ± 1.00 200.11 ± 1.01 299.58 ± 1.03	1.02 0.97 0.88	0.10 0.05 0.14	99.55 ± 1.204 200.11 ± 11.192 299.58 ± 1.352
Mean					
Method II. (using ninhydrin)	80 160 240	79.5 ± 1.04 159.34 ± 1.16 239.58 ± 1.25	1.12 0.872 0.946	-0.65 -0.41 -0.17	79.5 ± 1.29 159.39 ± 1.38 239.58 ± 1.42
Mean					
Method. III. (using P-benzoquinone)	100 200 300	100.11 ± 0.71 200.55 ± 0.65 300.12 ± 0.38	0.96 0.84 0.93	0.11 0.27 0.40	100.11 ± 1.22 200.55 ± 1.05 300.12 ± 1.27
Method I	Recovery %				
	99.55% 100.11% 99.18%				
Method. II	99.5% 99.34% 99.58%				
Method. III.	100.11% 100.55% 100.12%				

Table: 3. Determination of mesalamine in tablets using the proposed methods compared to the reference one.

Pharmaceutical preparation	% Recovery ± SDa			
	Method. I. (using vanillin)	Method. II (using ninhydin)	Method. III (P-benzoquinone)	Reference method
	99.95 ± 0.55 t= 0.257(c) F= 0.44(d)	99.88 ± 0.89 t= 0.305(c) F= 0.71(d)	99.66 ± 0.46 t=0.139(c) F=0.135(d)	99.77 ± 1.25

a= spectrophotometer method.
a= Mean ± standard deviation for five determinations
c= tabulated t-value for p = 0.05 and eight degree of freedom is 2.306
d= Tabulated F-value for p = 0.05 = 6.39 for eight degree of freedom.

CONCLUSION

That data given above revealed that the proposed methods were simple, accurate and sensitive with good precision and accuracy, with these methods, one can do analysis with speed at low cost without losing accuracy. The proposed methods can be used as alternative methods to the reported one for the routine determination of mesalamine tablets. This encourages their successful use in routine analysis of these drugs in quality control laboratories.

REFERENCES

1. Daslaning, D.; Patel, M.; Shah, A.; "Design, Development and Characterization of Extended Release Multiunit Particulate of Anti-Inflammatory Drug". Int. J. Pharma. Sic. and Drug Res., 2009; 1(2): 100-102.
2. Kruis, W.; Schreiber, 1.; Theuar.; Brandes.; Schutz.; Howaldt.; Krakamp: Hamling et al.," Low dose balsalazide (1.5 g twice daily) and mesalamine (0.5 g three times daily) maintained remission of ulcerative colitis but high dose balsalazide (3.0 g twice daily) was superior in preventing release", gut, 2001; 49(6): 783-789.
3. Sandborn WJ, Fegan BG. Lichtenstein GR; "Medical management of mild to moderate crohn's disease: evidence-based treatment algorithms for induction and maintenance of remission" Aliment pharmacol. Ther., 2007; 26(7): 987.
4. Finkel, Cubeddu and Clark; lippencott's Illustrated Reviews: Pharmacology, 4th Ed. P. 393.
5. British pharmacopoeia, 2008; II: 1403.
6. United States Pharmacopoeia, 24th Ed, United States pharmacopoeial convention.
7. S. Navya Sloka. B.M. Gurupadayya and CH. Aswaui Kumar; "Sensitive spectrophotometric method for the determination of mesalamine in Bulk and pharmaceutical formulations" Der Pharma Chemica, 2010; 2(4): 389-396.
8. Acharjya, S.K.; Sahu. A.; Das, S.; Sagar, P.; Annapurna, M.M.; "Spectrophotometric methods for the determination of mesalamine in bulk and pharmaceutical dosage forms. J. Pharm. Educ. Res., 2010; 1: 63.
9. A.K. Moharana, M. Banarjee, S. Panda, J.N. Muduli; "Development and validation of UV-spectrophotometric method for the determination of mesalamine in bulk and tablet formulation" Int. J. Pharm. pharm Sci., 2011; 3(2): 19-21.
10. Singh, R.K., P.S. Patel and P. Gupta. "UV spectrophotometric method for the estimation of mesalamine in bulk and its pharmaceutical dosage forms. IJPSR, 2010; 1(3): 44-49.
11. Shah, R.S.; S.A. Shah, M. B. Devani and K.P. Soni, "Spectrophotometric determination of mesalamine and its dosage forms". Indian Drugs, 1994; 31(34): 258.
12. Srinivasa Rao, Narala and K. Saraswathi, "Three simple and sensitive spectrophotometric methods (A,B and C) for quantitative determination of mesalamine in bulk and tablet dosage forms. International journal of research in pharmaceutical and Biomedical Sci., 2010; (1): 10-13.
13. Gatkal, S.H.; Mhatre, P. R. ; Chopade, V.V.; Chandliari, P.D.; " Development and validation of a stability indicating assay method of mesalamine by using different stress degradation conditions. I. J. Pharm. Sci. Res., 2013; 4(1): 401-406.
14. Sasmita Kumari Acharjya, Alok Sahu, Srikanta Das, Radhysagar and M. Mathrusri Annapurna, " Five simple precise and cost effective spectrophotometric methods have been developed for the estimation of mesalamine in bulk and its pharmaceutical preparations. J. pharmaceutical Education and Res., 2010; 1(1): 63-67.
15. Patel, K. M. C.N. Patel, B. Panigrahi, A.S. Parikh and H.N. Patel. "Three simple and sensitive visible spectrophotometric (A, B and C) have been developed for the quantitative estimation of mesalamine in bulk drug and pharmaceutical dosage forms. Pharma analysis, 2010; 2(3): 284-288.
16. Rafeel, J. A.; Jabor, J. R.; Casogrande, R.; Georgetti, S.R.; Bonn, M. F.; Fonseca, M.J.V.; " Potentiometric titration of mesalamine in pure and pharmaceutical dosage forms". Rev, Bras. Cience farm., 2007; 43: 97.
17. Nobilis, M.; Vyliralova, Z.; Sladkova, K.; Lisa, M.; Holcapek, M.; Kvetina, J.; "High Performance liquid-chromatographic determination of 5-amino salicylic acid and its metabolites in blood plasma" J. chromatogr., A, 2006; 1119(1-2): 299-308.
18. Bystrowska, B.; Nowak, J.; Brandys, J.; "Validation of a LC method for the determination of S-aminosabiylic acid and its metabolite in plasma and urine". J. pharm. Biomed. Anal., 2000; 22: 341.
19. Palumbo, g.; Baccbi, S.; Primovera, L; Palumbo, P.; Carlucci, g.; "A validated HPLC method with electrochemical detection for simultaneous assay of 5-amino salicylic acid and its metabolite in human plasma. Biomed. Chromatogr, 2005; 19: 350.
20. Hany, P. W.; and Dash, A.K.; "Simple liquid chromatographic method for the analysis of 5-amino salicylic acid and its degradation products J. chromatogr, 1997; 233-239.
21. Nigovi, B.; and Tmuni B.; "Determination of 5-amino salicylic acid in formulation by differential pulse voltammetry. Journal of pharmaceutical and biomedical analysis, 2003; 169-174.
22. ICH, 1996. Q2B: Text on validation of Analytical procedures Methodology step 4, consonances guidelines. ICH Harmonized Tripartite guidelines.
23. Siddappa, K.; Mallikarjun, M.; Reddy, P. T.; and Tambe, M.; "Spectrophotometric determination of metronidazole through Schiff's base system using vanillin and PDAB reagents in pharmaceutical preparations". Ecl. Quim., SaoPaulo, 2008; 33(4): 41-44.
24. Kudige, N.; Prashanth, Kanakapura Basavaiah and Madihalli S. Raghu "spectrophotometric determination of Zolmitriptan in bulk drug and

pharmaceuticals using vanillin as a reagent" ISRN Analytical chemistry, 2013; (2013).
25. Mubeen, G.; Noor, K.; "Spectrophotometric determination for analysis of metoformine hydrochloride" Indian Journal of Pharmaceutical Science, 2009; 71(1): 100-102.
26. Bali, A.; and Gour, P.; "A novel method for spectrophotometric determination of pregabalin in pure form and capsules", Chemistry and Central Journal, 2011; 5: 59.
27. Hosseinimeha, S.J.; Pourmorda, F.; Moshtaghi, E. Amini, M.; J. "Colorimetic determination of Baclofen with ninhydrin reagent and compared with HPLC method in tablet" Asian. Chem, 2010; 22(1): 522-526.
28. Bab, M.S.; Prasda, M.V.; and Ramu, B, K.; "Development of new visible spectrophotometric methods for quantitive determination of Almotriptan Malate using quinones as chromogenic reagents", J. chem. Sci. Trans., 2012; 1(2): 297-302.