Indirect Spectrophotometric Determination of Mefenamic Acid using Safranin Dye

Maha M Al-Taee*, Mohammed Salim Al-Enizzi

†Chemistry Department, College of education for Girls ,Mosul University. Mosul. Iraq;
*e-mail: mohammmed.salim@uomosul.edu.iq

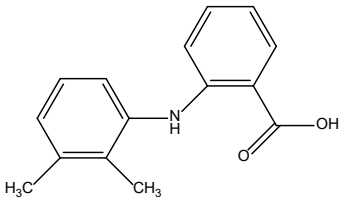
Received: December 31, 2023; Accepted: January 21, 2024
DOI: 10.17721/moca.2024.38-44

A simple, accurate, and rapid indirect spectrophotometric method has been developed for the determination of mefenamic acid in its pure form and in pharmaceutical preparations. The method is based on the oxidation of the mefenamic acid by N-bromosuccinimide (NBS) (excess) and estimating the amount of unconsumed NBS by safranin dye in the presence of the acid and the surfactant SDS in the aqueous medium at λ_{max} = 527 nm. Regression analysis of Beer–Lambert's plot proves excellent correlation in the concentration ranges 0.025 – 5 μg mL^{-1} with a correlation coefficient 0.993. A molar absorptivity, Sandell sensitivity, and limits of detection (LOD) and quantification (LOQ) were calculated to be 2.05×10^4 L mol^{-1} cm^{-1}, 0.0117 μg cm^{-2}, 0.254 μg mL^{-1} and 0.847 μg mL^{-1}, respectively. The possibility of quantitative determination of mefenamic acid in pure form and in pharmaceutical preparations in the form of syrup and capsules with average recoveries 102.57%, 100.88%, and 100.57%, and RSD ≤1.69%, ≤1.42% and ≤1.89% also, respectively, are shown.

Keywords: spectrophotometry, mefenamic acid, safranin dye, oxidation

Introduction

Mefenamic acid (MFA) 2-[(2,3-dimethyl phenyl) amino] benzoic acid, It is a non-steroidal anti-inflammatory drug that belongs to the anthranilic acid class [1] Commercially known as (ponstan) or (ponstel) [2], it is used medically as an antipyretic and is prescribed as a pain reliever, it is used for some common diseases such as Osteoarthritis and Rheumatoied arthritis [3], it has a stronger ability to relieve pain than aspirin and is less dangerous in terms of stomach ulcers [4], mefenamic acid white solid that is insoluble in water and slightly soluble in methyl chloride and alcohol [5].The mefenamic acid was determined by sensitive methods [6-12],chromatography [13-16], and other methods [17-20].

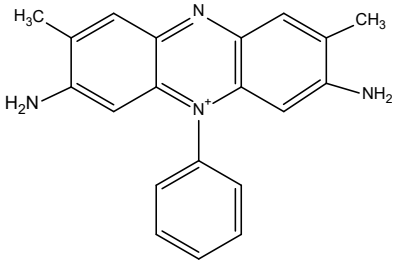


Mefenamic Acid ($C_{15}H_{15}NO_2$)
2-(2,3-Dimethylphenyl) aminobenzoic acid
M.wt = 241.3g/mol

Figure 1. Chemical structure of mefenamic acid.

Safranin Dye. This substance is also known as basic red, it is a biological stain. dye used in histology and cytology, It is used as a contrast dye in some staining protocols to color the cell nucleus red.

This type is known as dimethyl safranin because it contains two methyl groups. There is also trimethylsafranin, which contains an additional methyl group and is mainly used in biological staining applications. Safranin is also used as an indicator in redox reactions in analytical chemistry [21-22]. Some drugs were determinated spectrophotometrically using safranin dye [22-30].



$C_{20}H_{19}ClN_4$
M.wt = 350.85g/mol

Figure 2. Chemical composition of safranin dye.

Matherials and methods

Apparatus: Shimadzu UV-1800 Double-beam spectrophotometric was used for absorbance measurements with 1cm matched glass cells.

Reagents and chemicals The reagents and chemicals used were of a high score of Purity. as in Table 1.

Table 1. Reagents and chemicals used.

Chemical name	Molecular structure	Molecular weight (g / mol)	Company
Mefenamic acid	$C_{15}H_{15}NO_2$	241.3	S.D.I- Iraq
Safranin dye	$C_{20}H_{19}ClN_4$	350.85	BDH
N-bromo-succinimide (NBS)	$C_4H_4BrNO_2$	177.99	BDH
Sulphuric acid	H_2SO_4	98.08	Scharlau
Surfactant (SDS)	$C_{12}H_{25}NaSO_4$	288.372	SIGMA-AL-DRICH

Preparation methods: Mefenamc acid (prepared 100 and diluted to 50 μg mL^{-1}): 0.01 g of the drug was dissolved in an amount of ethanol, then the volume was completed to 100 mL with distilled water in a 100 mL volumetric flask, then diluted to 50 μg mL^{-1}.

Safranin (100 μg mL^{-1}): 0.01g from dye was dissolved in 100 ml distilled water.

N-bromosuccinimide (NBS) (1×10^{-2} M): 0.1779 g was dissolved in 100 mL of distilled water, and prepared another oxidizing agents in the same concentrations.

Sulphuric acid (1 M): 5.63 mL was taken from the concentrated acid (density= 1.83 g/mL, 95%) and diluted to 100 mL by distilled water.

Surfactants (0.1%): dilute solutions of surfactants were prepared by dissolving 0.1 g of each substance in 100 ml of hot distilled water.

Mefegen capsules analysis (500 mg): Five capsules of the pharmaceutical preparation were weighed and the equivalent of the weight of one capsule was taken and dissolved in an amount of ethanol. Then the solution was filtered and the volume was added to 100 mL of distilled water to obtain 5000 μg mL^{-1}, A solution it was prepared at a concentration of 50 μg mL^{-1}, and then the concentrations (0.5, 1.5, 2.5) μg mL^{-1} of mefenamic acid were taken.

Mefedad (oral suspension) analysis (50 mg/5 mL): The proposed methods were applied to the oral suspension of mefenamic. The syrup was analyzed by taking 5 mL of the drug solution and diluting it into 100 mLl of distilled water to obtain a solution with a concentration of 500 μg mL^{-1}, from which 50 μg mL^{-1} were prepared. different volumes (0.1, 0.3, 0.5) mL were taken to obtain concentrations (0.5, 1.5, 2.5) μg mL^{-1} of mefenamic acid.

Results and discussion

A primary study, the absorption spectrum and the standard curve of the dye: The absorption spectrum of the dye at a concentration of 100 μg mL^{-1} was studied by taking increased sizes of dye (0.1-3.0) mL in a 10 mL volumetric bottle to obtain the optimal amount that gives the best absorption at a wavelength of 527 nm. As shown in Fig.3.

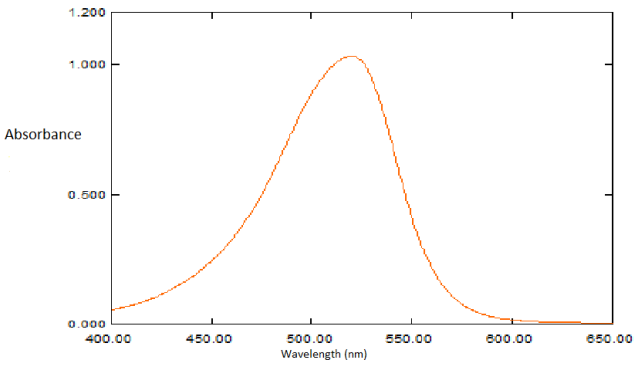


Figure 3. Absorption spectrum of safranin dye.

2 mL of the dye solution were taken, which is equivalent to 20 μg mL^{-1} of dye and its use in subsequent studies falls within the standard calibration curve for dye, Figure 4.

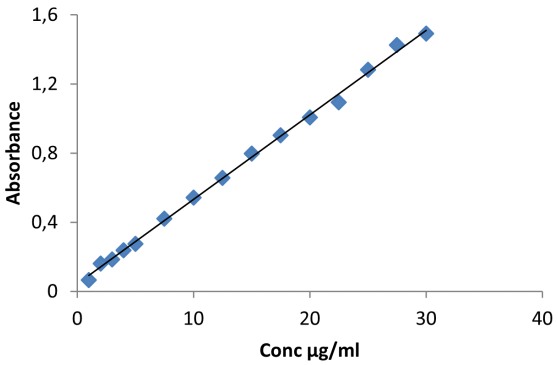


Figure 4. The standard calibration curve for dye.

Setting the optimal conditions

Choosing the type of oxidizing agent: 2 mL of the safranin dye solution (100 μg mL^{-1}) was taken, then 1 mL of the oxidizing agent 1×10^{-2} M and 1 mL of hydrochloric acid were added in a 10 mL volumetric flask. The solutions were diluted with distilled water and leave it for 10 minutes at room temperature, then the absorption was measured at a wavelength of 527 nm against blank solutions. The table shows that the best oxidizing agent is NBS. The results are shown in Table 2.

Table 2. The effect of various oxidizing agent

Oxidizing agent 1×10^{-2} M	Absorbance
$CuSO_4.5H_2O$	0.796
KIO_4	0.926
NCS	0.958
NBS	**0.036**
$KBr.KBrO_3$	0.506
$K_2Cr_2O_7$	1.00
$KMnO_4$	Turbit

Different volumes of the oxidizing agent: The effect of the volume of the oxidizing agent NBS 1×10^{-2} M was studied, different volumes of (0.0-1) mL were used, and it was found that 1 mL is the best volume, as it gives the best dye retention. The results are shown in Fig.5.

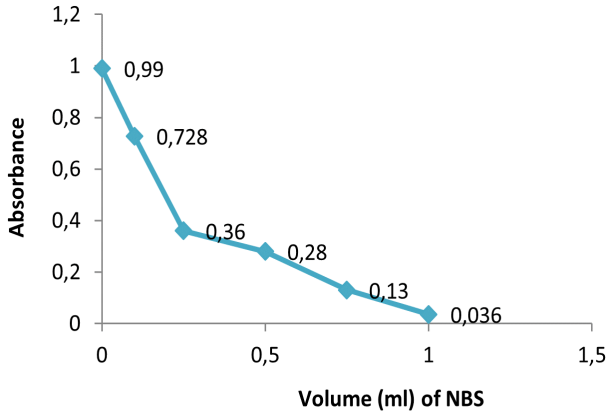


Figure 5. Effect of different amounts of oxidizing agent.

Different types of acids: 1 mL of different types of acids (1 M) was used and added to 1 ml of the drug compound with 1 mL of oxidizing agent and left for 5 minutes, 2 mL of the dye was added and left for 10 minutes, then the absorbance was measured at 527 nm. The results appear in Fig.6.

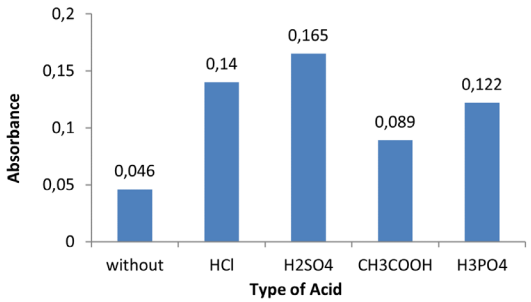


Figure 6. Effect of different types of acids.

Different amounts of acid (H_2SO_4): Different amounts (0-1.5) mLl of the acid used H_2SO_4 were added in order to find out the optimal amount. It was found that 1 ml is the best and gives the highest absorption, as in Table 3.

Table 3. Different amounts of sulfuric acid.

Volume (ml) of H_2SO_4 (1M)	Absorbance
Without	0.041
0.25	0.106
0.5	0.129
0.75	0.147
1	**0.166**
1.25	0.127
1.5	0.118

Effect of oxidation time: The time required for oxidation was studied by leaving the solutions after dissolving the drug compound (50 $\mu g\,mL^{-1}$), then adding 1 ml of the oxidizing agent (1×10^{-2} M) and 1 mL of sulfuric acid (1M), then the solutions were left for different periods of time, then adding 2 mL of safranin dye 100 $\mu g\,mL^{-1}$. After that, it was diluted with distilled water to the mark, then the solution was measured at a wavelength of 527 nm against its blank solutions. The results are shown in Table 4.

Table 4. Effect of oxidation time.

Time/ min	1.0	3.0	5.0	7.0	**10**	15
Abs.	0.126	0.141	0.165	0.179	**0.191**	0.180

The table above shows that 10 minutes is enough time to complete the oxidation process, so it was used in subsequent experiments.

Studying the effect of surfactant: Different types of surfactants were studied, including positive, negative, and neutral, by adding 1 ml of each substance at a concentration of 0.1% to the reaction mixture. The results obtained from the experiments indicate that the use of SDS has a role in increasing absorption and stabilizing the product, and Fig. 7 it shows its positive effect, so it was used.

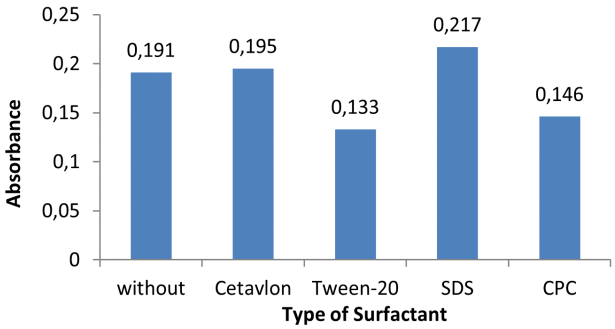


Figure 7. Study of surfactants.

Studying different amounts of surfactants: increasing amounts of SDS (0.0-2) mL were taken and it was found that 1 mL gives the best absorption in Table 5.

Table 5. Effect different amounts of SDS.

Volume (mL) of SDS 0.1%	Without	0.5	**1**	1.5	2
Absorbance	0.191	0.206	**0.215**	0.200	0.184

The effect of temperature and stabilization time: Different temperatures were studied on the formation and stability of the product. It was found that the absorbance decreases at high temperatures. The formation time of the product is 25 minutes for more than 100 minutes. It was found that the colored product it gives the best absorption at a temperature of 50 C˚ and the results are shown in Fig.8.

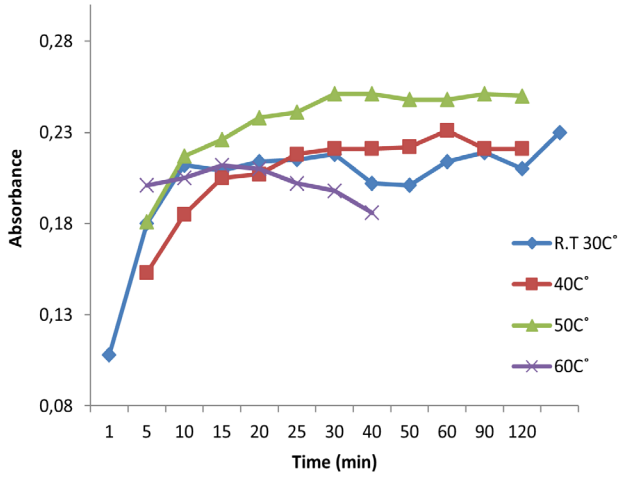


Figure 8. The effect of temperature and stabilization time.

Absorption spectrum: The absorption spectrum was taken after fixing the optimal conditions for the reaction, as in Fig.9.

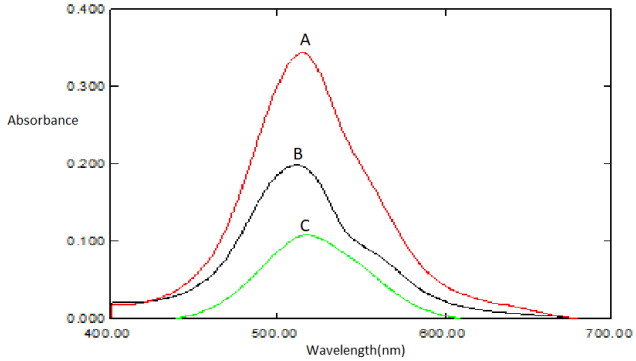


Figure 9. Final absorption spectrum for the determination of mefenamic acid. **A**: Absorption spectrum of 2 µg mL^{-1} of mefenamic acid versus distilled water. **B**: Absorption spectrum of 2 µg mL^{-1} of mefenamic acid versus blank solution. **C**: Absorption spectrum blank solution versus distilled water.

The optimal conditions are summarized in Table 6.

Table 6. Summary of the optimal conditions.

Optimal condition	
λ_{max} (nm)	527
Amount of NBS 1×10^{-2} M (mL)	1
Amount of H_2SO_4 1M (mL)	1
Safranine 100 µg mL^{-1} (mL)	2
Amount of SDS (0.1%) (mL)	1
Tempreture	50 C°

Calibration Curve: Increasing amounts (0.05-1) mL of mefenamic acid (50 µg mL^{-1}) were added to (10 mL) volumetric flasks, then 1 mL of oxidizing agent (1×10^{-2} M), 1 mL of SDS (0.1%), and 1 ml of sulfuric acid (1 M), then the solutions were left for 10 minutes, then add 2 ml of safranin dye (100 µg mL^{-1}), and complete the volume to the mark with distilled water. The solutions were left after dilution at a temperature of 50 C° for 25 minutes, then all solutions were measured against the blank solution at a wavelength of 527 nm, as in Fig.10. Follow Beer's law with in the concentration range (0.25-5) µg mL^{-1}.

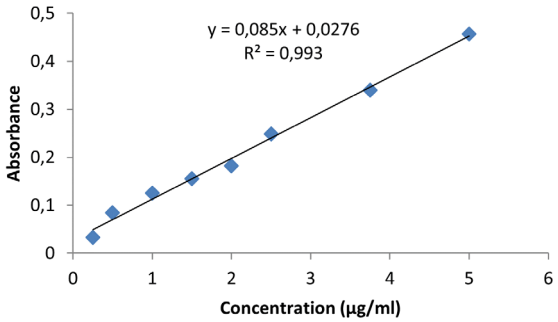


Figure 10. Calibration curve for determination of mefenamic acid.

Proposed chemical reaction are shown on Figure 11[12, 31].

Accuracy and precision of the method: Three concentrations (0.5, 1.5, 2.5) µg mL^{-1} of mefenamic acid (50 µg mL^{-1}) were used to verify the accuracy and precision of the method by taking five readings for each of them as in Table 7, which means that the method has high accuracy and precision.

RSD % = S/$\overline{X}$ ×100, $\overline{X}$ = Σxi/N,

Recovery % =O / T × 100,

Relative Error = O-T/T×100, where S - standard deviation, $\overline{X}$ - Arithmetic mean, N - numbered of reads , O - measured value T - true value.

Table 7. Study of accuracy and precision.

Amount added (µg/ml)		Recovery, %	Average Recovery, %	RSD %
Taken	Found			
0.5	0.52	104.00	102.57	1.69
1.5	1.52	101.33		0.90
2.5	2.56	102.40		0.95

Application of the suggested method for the determination of the drug compound on pharmaceutical preparations.

Table 8 shows the extent of the efficiency and success of the developed method for its application to pharmaceutical preparations. The recovery in the capsule ranged from (96.8-103.6)% and in the syrup ranged from (98.00-104)%.

The standard addition method to pharmaceutical preparations: To verify and prove the efficiency and success of the proposed method, The standard method of addition to pharmaceutical preparations was applied for mefenamic acid in capsule and syrup form. Fig 12,13 shows the application of the standard addition to pharmaceutical preparations when taking (1.0 and 2.0) µg mL^{-1} from capsule and syrup.

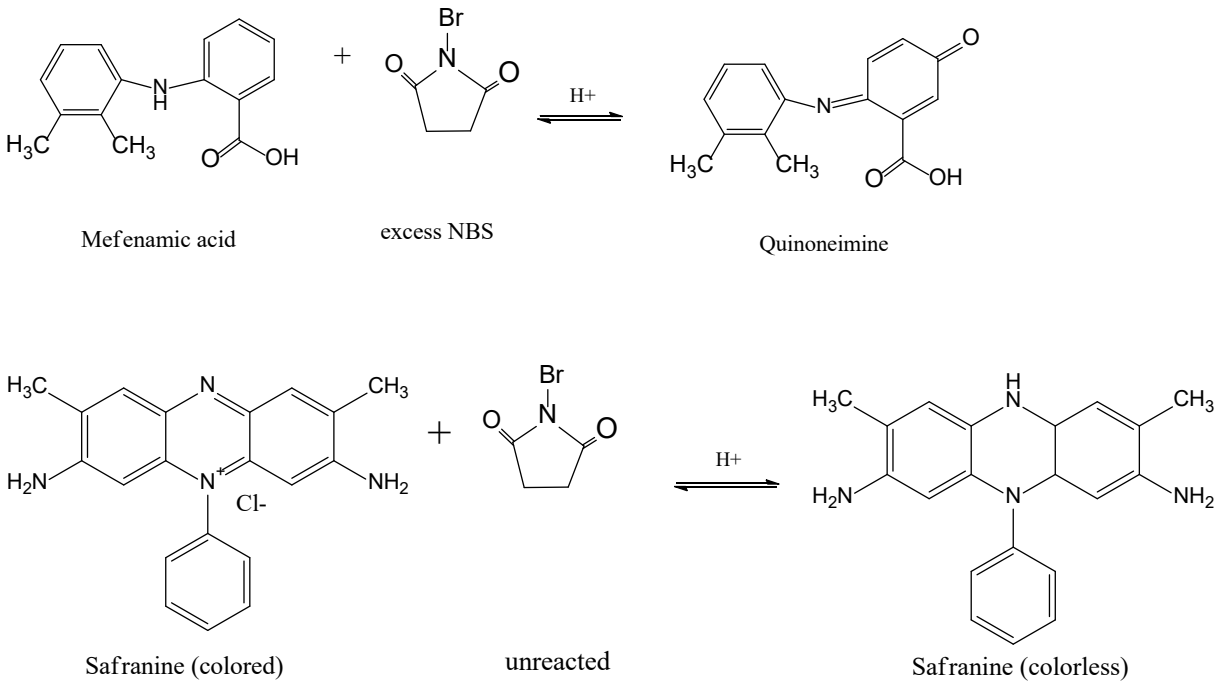


Figure 11. Proposed chemical reactions.

Table 8. Determination of the drug compound in pharmaceutical preparations.

Pharmaceutical preparation	Certified Value	Amount present, µg/mL		Drug content found, mg	Relative Error	Recovery, %	Average Recovery, %	RSD%
		Taken	Found					
Capsule Brussels Laboratories. Ltd	500 mg	0.5	0.484	484.00	-3.2	96.80	100.57	1.89
		1.5	1.52	506.65	1.3	101.30		2.06
		2.5	2.59	518.00	3.6	103.60		0.40
Oral Suspension Wadi Alrafdain company	50 mg \ 5 ml	0.5	0.49	49.00	-2	98.00	100.88	1.42
		1.5	1.51	50.33	0.66	100.66		0.96
		2.5	2.6	52.00	4	104.00		0.34

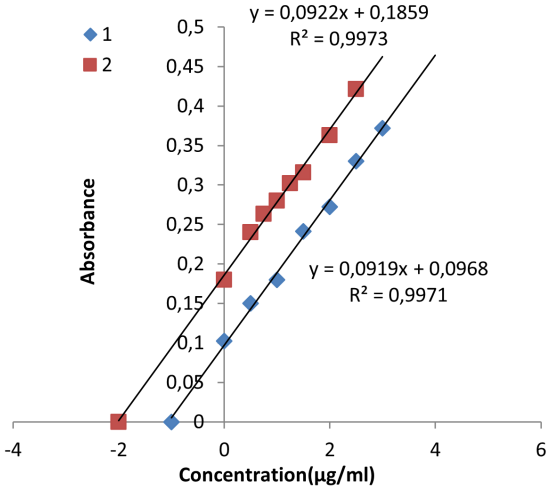


Figure 12. Standard addition to the pharmaceutical preparation (syrup).

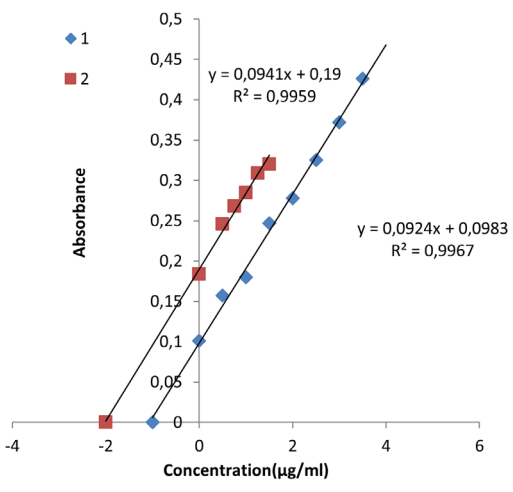


Figure 13. Standard addition to the pharmaceutical preparation (capsule).

Comparation the method with other methods: The proposed method for determinatin mefenamic acid was compared with other spectroscopic methods. Table 9 summarizes the comparison of the proposed method with other spectroscopic methods, as the results demonstrated the success of the proposed method for determination mefenamic acid in pharmaceutical preparations.

Table 9. Comparison of the method with other methods.

Analytical parameters	Present method	Literature method [7]
λ_{max} (nm)	527	347
Molar absorptivity (L mol^{-1} cm^{-1})	10^4×2.05	10^3×9.04
Sandell's sensitivity (μg cm^{-2})	0.0117	0.0266
Beer's law Range (ppm)	0.25 -5	0.4-48
RSD %	1.4≤	1.151

Conclusion

The pharmaceutical compound mefenamic acid was determinated in its pure form and in pharmaceutical preparations in the form of syrup and capsules. The method was sensitive and accurate, based on the oxidation of the mefenamic acid by *N*-bromosuccinimide (NBS) (excess) and estimating the amount of unconsumed NBS by safranin dye in the presence of the acid and the surfactant SDS in the aqueous medium.

Acknowledgments

We, the authors, extend our thanks and praise to the staff of the University of Mosul for their support and encouragement.

Author's statement

We certify that this research or manuscript is an original work and has not been published anywhere or submitted for publication. All sources for this work have been duly cited. We take full responsibility for the content of this manuscript, including any errors or omissions, and to ensure that all changes are made by the author and that all contributors are properly registered. We realize that any violation of these data may result in this manuscript being removed from publication.

Author's Contributions

Each of the individuals mentioned in the study contributed equally to the work and writing.

References

1. Naveed, S.; Qamar, F., Simple UV spectrophotometric assay of Mefenamic acid. International Journal of Pharma Sciences and Research (2014) , 5(7), 364-366.
2. Goth, A., Medical pharmacology principle and concepts. 9th Edn., the C.V. Mosby Company Printied in United State of America .(1978). 392-400.
3. Craig, C. R.; Stitzel, R. E., Modernpharmacology with clinical applications, 5 th Edn. Little Brown and Company, Boston. (1997) . 329.
4. Medicinal Chemistry., Staff members of the medical chemistry department. Mansoura University Faculty of Pharmacy. (2003). 82.
5. British pharmacopeia on CD-ROM. 3rdvEdn. System simulation Ltd, the stationary office. London. (2016).
6. Othman, N. S.; Awadis, L. S., Spectrophotometric determination of mefenamic acid in pharmaceutical preparations via arsenazo III – cerium III reaction. J. Raf. Sci. (2009) . 20(1): 8-21.
7. Othman, N. S.; Awades, L. S., Spectrophotometric determination of mefenamic acid using the Oxidation Reduction Reaction of Iodide and Iodate Ions. National Journal of Chemistry. (2009). 34,.363-374.
8. Vijayageetha Ragupathy., Shantha Arcot., Spectrophotometric determination of Drotaverine HCl and Mefenamic acid in tablets by chemometric methods. Res.J.Pham.Bio.&Chem.Sci. (2013) .4.2.899-909.
9. Subramanian, N.; Devipriyadharshini , T.; Venkateshwaran, K.; Chandrasekar ,P., Spectrophotometric determination of tranexamic acid and mefenamic acid in tablet dosage form using derivatization technique. int,j.pharm .biomed .res. (2011).2,.1,.26-29.
10. Saleem ,B. A.; Alnuaimy,L. A.; Spectrophotometric Determination of Mefenamic acid in pure and Pharmaceutical Preparations. J.Phys:conf.ser. (2021).**1853** 012048.
11. Rashad, I. Q.; Bakir ,M.H., Spectrophotometric Determination of Mefenamic acid using Metol reagent by Oxidative Coupling Reaction. IOP Conf. Ser.: Mater. Sci. Eng. (2021).**1058** 012081.
12. Alarfaj, N.A; Altamimi, S.A; almarshady, I.Z, Spectrophotometric determination of mefenamic acid in pharmaceutical preparation . Asian J chem. 2009. 21. 217-26.
13. Padmalatha ,H.; Vidyasagar, G., Validated RP - HPLC Method for the Estimation of Mefenamic acid in Formulation. Int.J.Res. Pharm.&Biomed .Sci. (2011).2.(3).1261-1265.
14. Sanjay ,D.; Bari, B.; Peasad Prakash.; Suvarna Bhoir.; Ashok Mukund Bhagwat., High performance liquid chromatographic estimation of drotaverine hydrochloride and mefenamic acid in human plasma. int.j.pharm .res. (2009).8.(9).209-215
15. Madhukar Badgujar, A.; kiran Mangaonkar,V., Simultaneous determination of paracetamol and mefenamic acid in tablet dosage form by high performance liquid chromatography. j.chem.pharm.

res. (2011).3.(4).893-898.

16. Wahab,S.U.; Mohamed, P.U.; Firthouse.; Mohamed.Halith, S.; Chandran ,M.; Sirajudeen .Kadher,S., Development of RP-HPLC Method for the Simultaneous Determination of Mefenamic Acid and Drotaverine HCl Combined Tablet Dosage Form. Int.J. Pharm.Sci. (2011).3. (1).115-117.

17. Jinging ,Yu.; Jangwen ,Li.; Fagiong, Z., Charcteriztion of carbon-gold nanoparticals Composite film modified electron and voltametric determination of mefenamic acid . J. Braz. Chem. Soc. (2008). 19. (5). 849-855.

18. Santni, A. O.; Pezza ,H.R.; Pezza, L., Development of apotentionmetric mefenamate ion senser for the determination of mefenamic acid in pharmaceutical and human blood serum . Sensor and Actuators B: Chem. (2007) .128. (1).117-123.

19. Liu, L.; Song, J., Voltammetric determination of mefenamic acid Lanthanumhydroxide nanowiremodified carbon paste electrode . Anal Biochem. (2006).354, 22-27.

20. Loannou,P. C.; Andrikopoulou, D. A.; Glynon, K. M.; Rusakova, N. V., Spectrofluorometric determination of anthranilic acid derivatives based on terbium sensitized fluorescence. The Analyst. (1998) . 123. 2839-2843.

21. Arnaud, N.; Georges, J., Investigation of luminescent properties of terbium- anthranilate complexes and application to the determination of anthranilic acid derivatives in aqueous solution . Anal . Chim. Acta. (2003). 476.(1).157-194.

22. Albero, M.; Garcia, M., Flow injection spectrofluorimetric determination of flufenamic acid . Journal of pharmaceutical and biomedical analysis. (1995) . 13(9). 1113- 1117.

23. Narayana, B.; Divya, N.S., A New method for spectrophotometric determination of thiocolchicoside. Jsir. (2010). 69.368-372.

24. Krishina, M.V.; Sankar, D.G., Extractive spectrophotometric methods for the determination of rosuvastatin calcium in pure form and in pharmaceutical formulation by using safranin o and methylene blue . coden ecjhao . (2007). 4.(1). 46-49.

25. Shetty ,D.N.; Narayana, B.; Ismaail, N.B.S., Simple and Sensitive method for the spectrophotometric determination of perfloxacin. Der Pharmacia Lettre. (2016) .8.(11).230-235.

26. Madhuri, L.; Shireesha, M.; Tulieran, G., Spectrophotometric determination of valsartan and benazepril hydrochloride in pure and pharmaceutical formulation. latr .j.Chem. Tech. Res. (2011).3.1830-1834.

27. Krishna M.V.; Sankar, D.G., Spectrophotometric determinatin of gamifloxacin mesylate in pharmaceutical formation through ion pair complex formation . E-J.Chem. (2008). 5.515-520.

28. Acharyulu, M.L.N.; Murthy,K.V.R.; Babu, K.; Reddy,T.S., Analytical application of safranin O Innovare . J.Sci. (2013). 1.25-27.

29. Rani, A.B.; Nagalashhmi, C.; Bhawni, S.; Bhawni , C.; Selcaran, B., Determination of perindopril erbumine in tablet by spectrophotometry . J. Chem. (2012). 7.413-426.

30. Sreevidya ,T. V.; Narayana, B., A simple and rapid spectrophotometric method for determination of artesunate in pharmaceutical. Eurasian J. Anal. Chem. (2009).63.(7). 637-642.

31. Roopa, K.P.; Basavaiah, K.; Jayanna, B.K., Optimization and Validation of the Spectrophotometric Methods for the Assay of Dexmedetomidine Hydrochloride in Pure and Dosage Forms. J Appl Spectrosc. (2019). 86. 740–747 https://doi.org/10.1007/s10812-019-00888-0).