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Spectrophotometric Determination of Mefenamic Acid in Pharmaceutical Preparations

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Three simple, rapid and accurate spectrophotometric methods were developed for the determination of mefenamic acid. The first method (Method **I**) is based on the reaction of mefenamic acid as N-donor with *p*-chloranilic acid as a π-acceptor. A red colour product shows peak at 520 nm and its absorbance is linear with concentration over the range 10-300 µg/mL with correlation coefficient (n = 12) of 0.9997. The second method (method **II**) involves oxidation of mefenamic acid with N-bromosuccinamide. A yellow colour product shows peak at 362 nm and its absorbance is linear with concentration over the range 5-70 µg/mL with correlation coefficient (n = 8) of 0.9999. The third method (method **III**) is based on the formation of an oxidative coupling product by the reaction of mefenamic acid with 3-methylbenzo-thiazolin-2-one hydrazone as a chromogenic reagent in presence of ferric chloride solution. A green colour product shows peak at 602 nm and its absorbance is linear with concentration over the range 1-6 µg/mL with correlation coefficient (n = 6) of 0.9999. The different parameters affecting the reaction pathway were thoroughly studied and optimized. The developed methods could be successfully applied to the determination of mefenamic acid in either pure form and in pharmaceutical formulations. The results obtained were in good agreement with those obtained using official methods.

Key Words: Mefenamic acid, N- Bromosuccinamide, Chloranilic acid, 3-Methylbenzo-thiazolin-2-one hydrazone/ Ferric chloride system, Pharmaceutical formulations, Spectrophotometry.

INTRODUCTION

Mefenamic acid (MFA) (Fig. 1) N-[(2,3-dimethyl phenyl)amino]benzoic acid, is a non-steroidal antiinflammatory drug which is a derivative of N-phenylanthranilic acid. It is used as potent analgesic and antiinflamanatory agent in the treatment of osteorthritis, rheumatoid arthritis and other painful musculosketal illnesses[1].

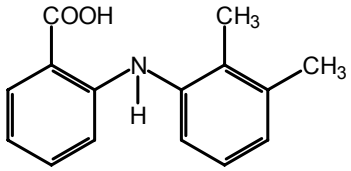


Fig. 1. Chemical structure of mefenamic acid

Numerous analytical methods have been developed for the determination of mefenamic acid in pure from, dosage forms and biological fluids. mefenamic acid is official in both United States Pharmacopoeia[2] and British Pharmacopoeia[3]. The USP recommends a high performance liquid chromatographic (HPLC) method for the determination of mefenamic acid both in raw materials and dosage forms while the BP recommends an acid-base titration method for the analysis of mefenamic acid in raw materials and its dosage forms. The therapeutic importance of mefenamic acid initiated several reports on its determination both in formulations and in biological fluids, *viz.*, titrimetry[4], conductometric titrimetry[5], spectrophotometry[6-11], spectrofluorometry[6,12-14], luminescence[15,16], chemiluminescence[17], atomic spectroscopy[18], proton NMR[19], potentiometry[20], voltammetry[21], polarography[22], high performance liquid chromatography[23-26], high performance liquid chromatography/mass spectrometry[27], liquid chromatography/mass spectrometry[28], gas chromatography[29], gas chromatography/high performance liquid chromatography[30], gas chromatography/mass spectrometry[31-34], high performance thin layer chromatography[35], ion- pair partition chromatography[36], capillary electrophoresis[37], capillary electrophoresis/mass spectrometry[38,39], capillary isotachophoresis[40].

This led us to study the reaction of mefenamic acid with the reagents mentioned above in an attempt to develop simple, sensitive and reliable methods for its determination in bulk and dosage forms. The results obtained were satisfactorily accurate and precise.

EXPERIMENTAL

An ultrospec 2100 pro/80-2112-21/Amersham Bioscience Spectrometer was used equipped with 1 cm quartz cuvettes for the λ_{max} determination and all absorbance measurements.

A reference standard sample of mefenamic acid was obtained from Nile Co. for Pharmaceutical Industries (Cairo, Egypt). Commercial dosage forms containing the studied drug were obtained form the local market. All the reagents used were of analytical reagent grade, solvents were of spectroscopic grade. *p*-Chloranilic acid, 0.014 mol/L (MERCK) in acetone (BDH), aqueous sodium hydroxide, 0.1 mol/L (MERCK), N-bromosuccinamide, 0.05 mol/L (Riedel-de Haen) in methanol (BDH), 3-methylbenzothiazoline-

2-one hydrazone hydrochloride, 0.015 mol/L (WAKO) in methanol, iron(III) chloride-6-hydrate, 0.15 mol/L aqueous solution (Surechem Products LTD) and dimethylformamide (WINLAB).

Standard solutions: Standard stock solutions of pure mefenamic acid must be freshly prepared for each method.

For method (I): (1 mg/mL) was prepared by dissolving 25 mg of pure mefenamic acid in 5 mL dimethylformamide in a 25 mL volumetric flask, a drop of phenolphthalein indicator was added followed by the addition of 0.1 mol/L NaOH until the appearance of a pink colour and then diluted with acetone to the mark.

For methods (II) and (III): (0.5 mg/mL) was prepared by dissolving 12.5 mg of pure mefenamic acid in methanol in a 25 mL volumetric flask and was further diluted with the same solvent as appropriate.

Construction of calibration curves: Calibration curves were constructed according to the optimum conditions in Table-1.

TABLE-1
ANALYTICAL DATA FOR THE DETERMINATION OF MEFENAMIC ACID WITH DIFFERENT REAGENTS

Parameter	Proposed methods using		
	p-CA	NBS	MBTH
λ_{max} (nm)	520	362	602
Concentration range (μg mL^{-1})	10-300 (n = 12)	5-70 (n = 8)	1-6 (n = 6)
Regression equation:			
Intercept (a)	-0.0128	0.0069	-0.0845
S_a	0.0060	0.0040	0.0060
Slope (b)	0.0054	0.0126	0.2101
S_b	0.0000	0.0000	0.0010
Correlation coefficient (r)	0.9997	0.9999	0.9999
LOD (μg mL^{-1})	2.50	0.51	0.06
LOQ (μg mL^{-1})	8.43	1.75	0.20
% RSD (n = 10)	0.56 (10 μg mL^{-1})	1.103 (10 μg mL^{-1})	1.575 (1 μg mL^{-1})

S_a = Standard deviation of the intercept; S_b = Standard deviation of the slope.

***p*-Chloroanilic acid (*p*-CA) method:** Accurately measured aliquots from stock solution of mefenamic acid equivalent to 10-300 μg/mL were transferred into a series of 10 mL volumetric flasks. 3mL of *p*-CA reagent were added to each flask and the volumes were completed to the mark with acetone. The absorbances were measured at 520 nm against a reagent blank. The calibration curve was obtained by plotting the absorbances *vs*. the final concentrations. Alternatively, the corresponding regression equation was derived.

N-Bromosuccinamide (NBS) method: Different aliquots of mefenamic acid stock solution equivalent to 5-70 µg/mL were transferred into a series of 10 mL volumetric flasks. 2 mL of NBS reagent were added to each flask and volumes were completed to the mark with methanol. The absorbances were measured at 362 nm against a reagent blank and the calibration curve was obtained as described above.

3-Methylbenzo-thiazolin-2-one hydrazone (MBTH) method: In a series of 10 mL volumetric flasks, different aliquots of mefenamic acid stock solution equivalent to 1-6 µg/mL were transferred. 4 mL of $FeCl_3$ aqueous solution were added followed by 2 mL of MBTH reagent. The volumes were completed with methanol and the absorbance was measured to each solution at 602 nm against a reagent blank. The calibration curve was constructed as described above.

Procedure for dosage forms

For *p*-CA method: Weigh accurately a quantity of the mixed contents of 10 pulverized tablets equivalent to 25 mg of the drug. Transfer into a small conical flask, add 5 mL of dimethylformamide, shake and add one drop of phenolphthalein indicator. Add 0.1 mol/L NaOH until appearance of a pink colour. Filter in a 25 mL volumetric flask, complete to volume with acetone and proceed as described for method(I).

For NBS and MBTH methods: Weigh accurately a quantity of the mixed contents of 10 pulverized tablets equivalent to 12.5 mg of the drug. Transfer into a small conical flask and add 5 mL of methanol. Filter in a 25 mL volumetric flask, complete to volume with methanol and proceed as described for methods (**II**) and (**III**). Determine the nominal content from the corresponding calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

***p*-CA method:** π-Acceptors react with basic nitrogenous or carboxylic group compounds as N-donors to form charge transfer complexes or radical anions according to the polarity of the solvent used. Mefenamic acid has two centers which can act as N-electron donors (the carboxylic and the amino groups) and are responsible for the formation of charge transfer complexes with *p*-CA as π-electron acceptor.

Mefenamic acid reacts with *p*-CA in acetone forming a red-orange coloured product which exhibits absorption maxima at 520 nm (Fig. 2).

Since a polar solvent is used (acetone), this band may be attributed to the formation of *p*-CA radical anions.The reaction may be represented by the following equation:

$$\underset{\substack{\text{Donor}\\(\text{mefenamic acid})}}{D^{\bullet\bullet}} + \underset{\text{Acceptor}}{A} \longrightarrow \underset{\text{Complex}}{[D : A]} \xrightarrow{\text{Polar solvent}} \underset{\text{Radical ions}}{D^{+} + A^{\bullet -}}$$

(*p*-CA)

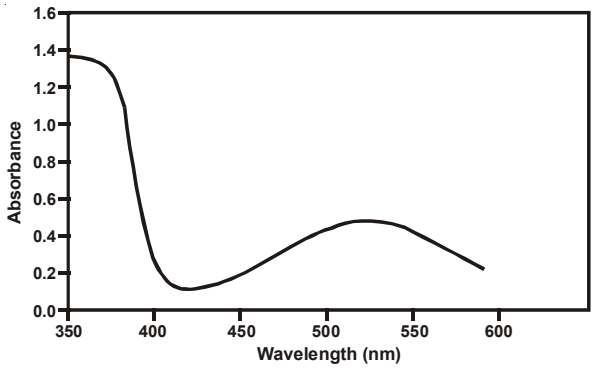


Fig. 2. Absorption spectrum of mefenamic acid (100 µg/mL)/*p*-CA (0.042 M) complex in acetone

The reaction conditions were optimized, the concentration of the reagent and the effect of time. Acetone was found to be the solvent of choice for mefenamic acid to affect its dissolution. Maximum absorption at the relevant maxima was obtained upon using 3 mL of 0.014 M *p*-CA in acetone. Higher reagent concentrations did not affect the colour intensity. Maximum colour intensity at ambient temperature was obtained immediately with *p*-CA and remained stable for up to 0.5 h.

NBS method: N-Bromosuccinamide, (NBS) as an oxidant reacts with mefenamic acid in methanol to form a dark yellow oxidation product which exhibit absorption maxima at 362 nm (Fig. 3).

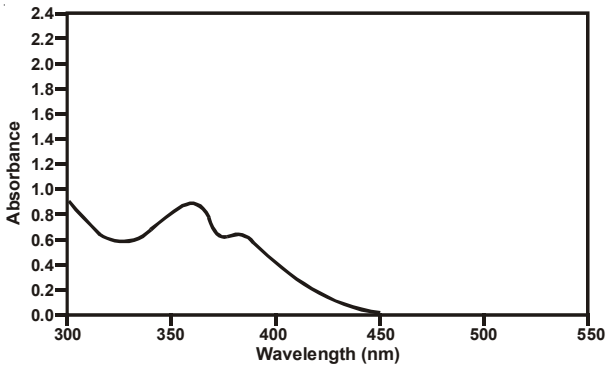
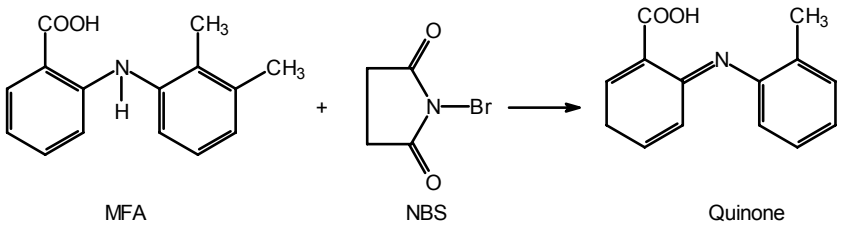


Fig. 3. Absorption spectrum of mefenamic acid (70 µg/mL)/NBS (0.01 M) product

This may be attributed to the oxidation of the secondary amino group in mefenamic acid by NBS and quinone is produced as an oxidation product. The reaction may be represented as in **Scheme-I**.



Scheme-I: Reaction of mefenamic acid and N-bromosuccinamide

Methanol is a good solvent for NBS and mefenamic acid and it affords maximum colour intensity. Maximum absorption at the relevant maxima was obtained upon using 2 mL of 0.05 M NBS, where higher reagent concentrations did not affect the colour intensity.

Maximum colour intensity at ambient temperature was attained immediately with NBS and remained stable for up to 0.5 h.

MBTH method: Mefenamic acid reacts with the reagent MBTH in the presence of ferric chloride solution in methanol medium and a green colored product that absorbed at 602 nm is obtained (Fig. 4).

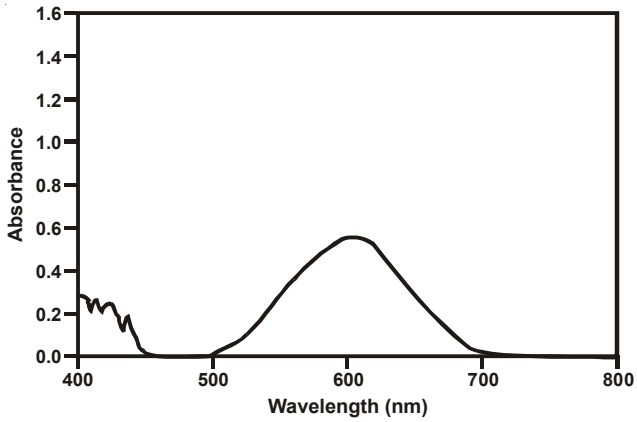
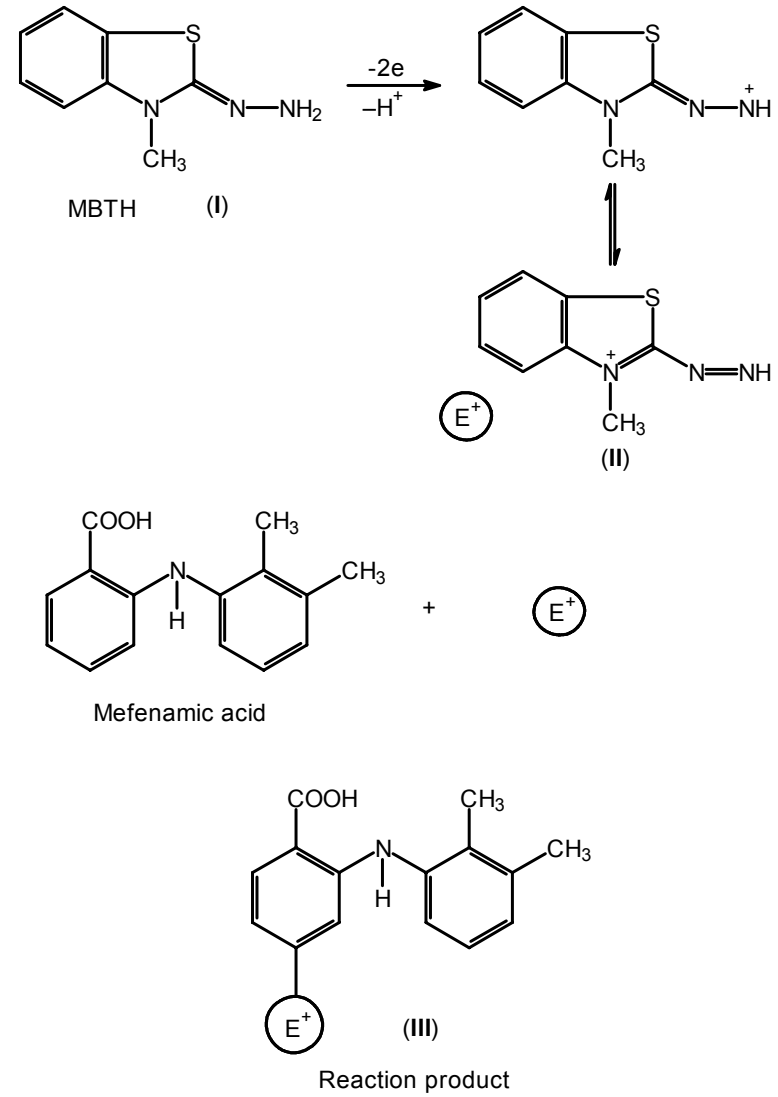


Fig. 4. Absorption spectrum of mefenamic acid (3 µg/mL)/MBTH (0.003 M) + $FeCl_3 \cdot 6H_2O$ (0.06 M) product in methanol

The reaction of MBTH with mefenamic acid in the presence of an oxidant, proceeds *via* oxidative coupling. MBTH (I) loses two electrons and one proton on oxidation with oxidizing agent ferric chloride hexahydrate, forming the electrophilic intermediate (II), which is the active coupling species[41,42]. The reagent would be expected to attack carbon atom with maximum electron density as in mefenamic acid to form the colored product (III), according to **Scheme-II**.



Scheme-II: Mechanism of the reaction of mefenamic acid and MBTH

The optimum conditions for the reaction were carefully studied. Maximum absorption at 602 nm was obtained immediately upon using 4 mL of 0.15 M $FeCl_3 \cdot 6H_2O$ and 2 mL of 0.015 M MBTH at ambient temperature and the product remained stable for up to 35 min.

The methods were tested for linearity, specificity, precision and reproducibility. With the above spectrophotometeric methods, linear regression equations were obtained. The regression plots showed that there was a

linear dependence of the relative absorbance intensity on the concentratios of the studied drug in the ranges listed in Table-1. Statistical evalution of the experimental data regarding standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) were calculated (Table-1). The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficients (close to 1 in all cases).

The validity of the methods could be proved by analyzing authentic samples of the drug. The results obtained (Table-2) are in good agreement with those given by the comparison method[3].

TABLE-2
DETERMINATION OF MEFENAMIC ACID IN PURE FORM AND TABLETS BY THE PROPOSED AND OFFICIAL METHODS [Ref. 3]

Preparation	% Recovery ± SD			
	p-CA	NBS	MBTH	Official method
Pure mefenanic acid	100.73±0.681[a]	100.01±1.251[a]	99.91 ± 0.728[a]	100.1 ± 1.21[a]
	n = 12	n = 11	n = 6	n = 3
t-value	1.278 (2.16)	0.1157 (2.179)	0.3164 (2.365)	
F-value	3.155 (3.98)	1.0682 (19.4)	2.760 (5.79)	
Ponstan Fort tablets[b]	99.25 ± 1.251	100.04 ± 1.466	99.29 ± 0.832	100.2 ± 0.4
(500 mg/tablet)	n = 6	n = 5	n = 5	n = 3
t-value	1.258 (2.365)	0.1814 (2.447)	1.784 (2.447)	
F-value	9.781 (19.3)	13.423 (19.2)	4.328 (19.2)	

[a]% Found ± SD; [b]Product of Godecke Co. Germany; Figures in parentheses are the theoretical t and F values at p = 0.05 confidence limit.

The specificity of the methods were investigated by observing that no interference was encountered from common tablet excipients. The simplicity of the methods and the stability of the reaction products permitted the determination of mefenamic acid in commercial tablets. The results obtained (Table-2) were statistically comparable with those given using the previously mentioned comparison method[3].

In conclusion, the proposed procedures have the advantages of being simple, accurate, time saving, inexpensive, sensitive, and requires minimum equipments and chemicals. The MBTH method was more sensitive than the *p*-CA and NBS methods.

These methods can be used as general methods for spectrophotometric determination of mefenamic acid in bulk powder and in dosage forms. The proposed methods are suitable for routine quality control.

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