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Spectrophotometric determination of Mefenamic acid viadiazo coupling reaction using different reagents in dosage forms

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Abstract

Three simple, accurate and precise spectrophotometric methods were proposed for determining of mefenamic acid in pure form as well as in dosage forms. The proposed methods were based on the coupling of mefenamic acid with diazotized 4-aminohippuric acid, 4-nitroaniline and 4-aminoantipyrine in alkaline medium for methods A, B and C, respectively. The colored azo dyes were quantitated photometrically at 432, 453, and 406 nm for methods A, B and C, respectively. Different variables affecting the reactions were optimized. Beer's law was obeyed over the concentration ranges of 0.5-20, 1.0-16 and 0.2-14 µg/ml for methods A, B, and C, respectively. The colored azo dyes remained stable for more than 3 hours in the three methods. The proposed methods were applied successfully to determine mefenamic acid in dosage forms.

Introduction

Mefenamic acid [2-(2,3-dimethyl phenyl)amino] benzoic acid (Figure 1)is a non-steroidal drug which has analgesic, anti-inflammatory and antipyretic actions and it is used specially in the treatment of rheumatoid arthritis and osteoarthritis and other muscular-skeletal diseases[1].

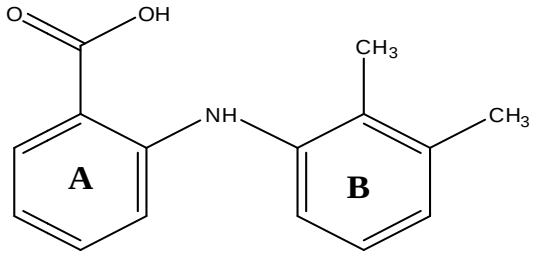


Figure-1: Chemical structure of mefenamic acid

Various methods have been reported for the determination of mefenamic acid as pure form and in dosage forms. These methods include titrimetric [2,3], chromatographic [4-6], luminescence [7] flow injection [8,9], electrochemical analysis [10-13], spectrofluorimetry[14,15] spectrophotometry[16-21], and atomic absorption spectrometry [22].The standard method for the assay of the pure drug is titrimetry, using sodium hydroxide as a titrant and phenol red as indicator [23].

The purpose of the present study was to evaluate sensitive and accurate spectrophotometric methods for the determination of mefenamic acid based on the coupling of mefenamic acid with diazotized 4-aminohippuric acid, 4-nitroaniline and 4-aminoantipyrine in alkaline medium to form colored azo dyes.

Experimental

- ***Apparatus***

A (Cecil CE3021-England) UV-VIS spectrophotometer was used for all spectral and absorbance measurements with matched 1.0 cm quartz cells.

- ***Reagents***

All chemicals used were of analytical grade reagents and deionized water was used to prepare all solutions.

A pure mefenamic acid (provided from Awamedica Company for Drug Industries and Medical Applications Awa, Erbil, Iraq). A solution of (100 µg/ml) was prepared by dissolving 0.01g of mefenamic acid in a solution of sodium hydroxide (0.1M) containing 5 ml ethanol and the volume was diluted to 100 ml with sodium hydroxide (0.1M) in a volumetric flask [24]. Serial dilutions with deionized water were made to cover the working range of the calibration graph. Sodium hydroxide (2M), sodium nitrite solution (1% w/v) and different interferences solution (1000 µg/ ml) were prepared by dissolving the proper amounts in deionized water.

- ***Preparation of diazotized 4-aminohippuric acid solution (2.5 ×10^{-3} M).***

A 0.0485g of 4-aminohippuric acid (BDH) was dissolved in 10 ml deionized water, and 3 ml of concentrated hydrochloric acid was added then the mixture was transferred to a 100 ml volumetric flask and cooled at 0-5 ^{o}C in an ice-bath. A 3.5 ml of 1% of sodium nitrite solution was added and the mixture was stirred vigorously. After 5 min., the solution was made up to 100 ml with cold deionized water. The solution was kept in a brown bottle in refrigerator and was stable for at least one week [25].

- ***Preparation of diazotized 4-nitroaniline solution (2.5 × 10^{-3} M).***

A 0.0345g of 4-nitroaniline (Fluka) was dissolved in 10 ml of deionized water and then 3 ml of concentrated hydrochloric acid was added, the clear mixture was then transferred to a 100 ml volumetric flask and cooled at 0-5 ^{o}C in an ice-bath. A 3.5 ml of 1% of sodium nitrite solution was added and the mixture was stirred vigorously. After 5 min., the solution was made up to 100 ml with cold deionized water. The solution was kept in a brown bottle in refrigerator and it was stable for at least five days [26].

- ***Preparation of diazotized 4-aminoantipyrine solution (2.5 × 10^{-3} M).***

A 0.051g of 4-aminoantipyrine (Fluka) was dissolved in 60 ml deionized water and then 3 ml of concentrated hydrochloric acid was added followed by heating. Finally the mixture was transferred to a 100 ml volumetric flask and cooled at 0-5 ^{o}C in an ice-bath. A 3.5 ml of 1% of sodium nitrite solution was added and the mixture was stirred vigorously. After 5 min., the solution was made up to 100 ml with cold deionized water and was kept in a brown bottle in refrigerator for 1 hour before using, and it was stable for at least five days [27].

- ***Determination of mefenamic acid in tablets and capsules.***

A quantity of the powdered mixed contents of 10 (tablets or capsules) containing 0.01 g of mefenamic acid was dissolved in 100 ml of 0.1 M sodium hydroxide and mixed for 10 min. and then filtered. The filtrate was completed to 100 ml with deionized water. A suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

- ***General procedure***

Method A:

Aliquots of aqueous solution containing (5-200) µg of mefenamic acidwere transferred into a series of 10 ml volumetric flasks. To each flask 1.0 ml of diazotized 4-aminohippuric acid (D-4AHA) and 1.0 ml of 2M sodium hydroxide solutions were added. The contents were diluted to the mark with deionized water and mixed well. The absorbance of the yellow colored azo dye was measured at 432 nm against the reagent blank.

Method B:

Aliquots of aqueous solution containing (10-160) µg of mefenamic acid were transferred into a series of 10 ml volumetric flasks. To each flask 1.0 ml of diazotized 4-nitroaniline (D-4NA) and 1.5 ml of 2M sodium hydroxide solutions were added. The contents were diluted to the mark with deionized water and mixed well. The absorbance of the yellow colored azo dye was measured at 453 nm against the reagent blank.

Method C:

Aliquots of aqueous solution containing (2-140) µg of mefenamic acidwere transferred into a series of 10 ml volumetric flasks. To each flask 1.0 ml of diazotized 4-aminoantipyrine (D-4AAP) and 1.5 ml of 2M sodium hydroxide solutions were added. The contents were diluted to the mark with deionized water and mixed well. The absorbance of the yellow colored azo dye was measured at 406 nm against the reagent blank.

Results and Discussion

The proposed methods were based on the coupling of mefenamic acid with (D-4AHA) (method A), (D-4NA) (method B) and (D-4AAP) (method C) in alkaline medium. The absorption spectra of the azo dyes formed of mefenamic acid with (D-4AHA), (D-4NA)and (D-4AAP) showed absorption maxima at 432, 453, and 406 nm,respectively (Figure 2). The chemical structure of mefenamic acid consist of two aromatic rings (A and B) joined by an amino bridge (Figure 1). The *para*position to the amino group is favored for coupling on both rings, considering the combined directing influence of the ring substituents toward electrophilic aromatic substitution reaction. However, the *para*position on ring B is more stereocally hindered thus making ring A the most favor point of attack, also the ring A has a substituents of the carboxyl group on the *ortho*position; so the fast coupling reaction observed was on the *para*position to amino group as well as meta position tocarboxyl group.Both substituents favor the same point of attack. The suggested mechanism of the proposed methods A, B and C were shown in Scheme 1.

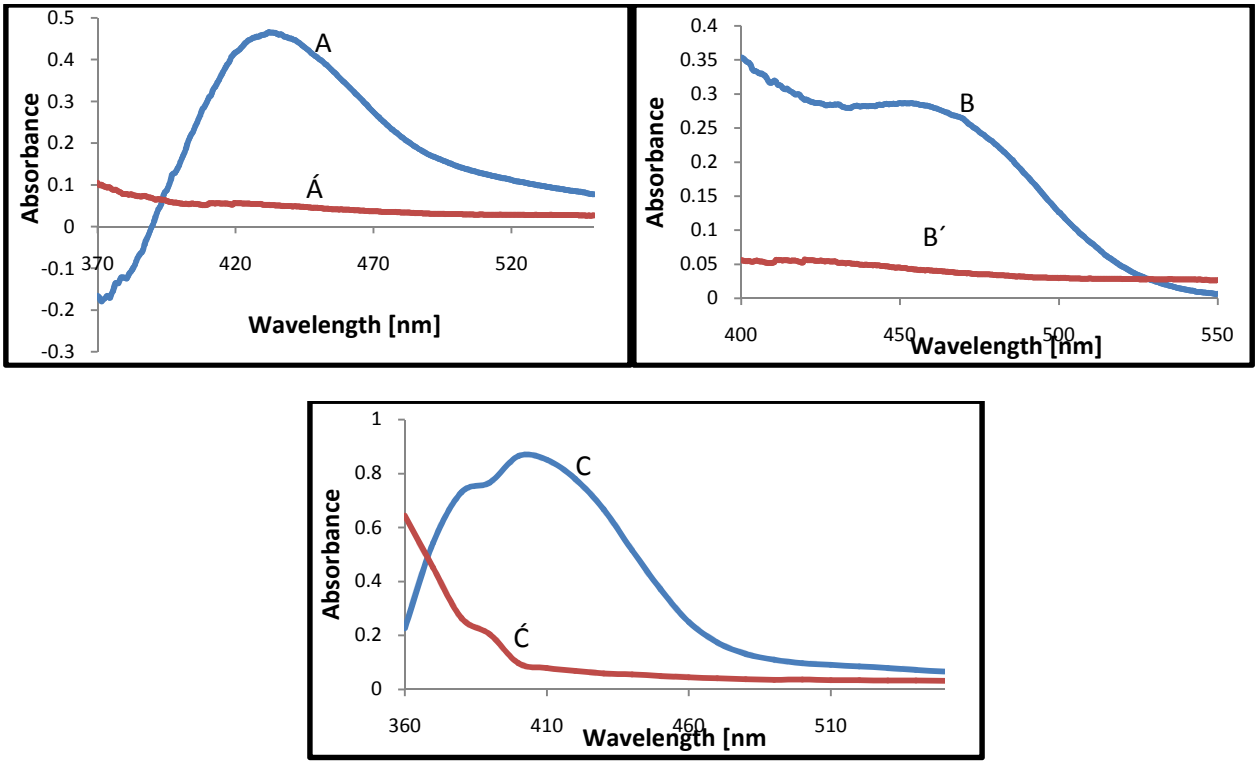
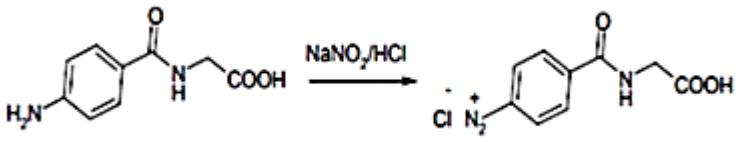
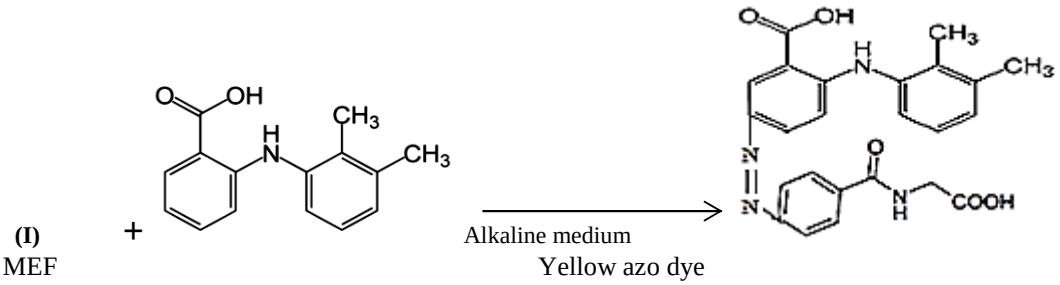


Figure-2: A, B and C are absorption spectra of reaction products of (10 µg/ml) MEF against reagent blanks for methods A, B and C, respectively. Á, B´ and Ć are absorption spectra of reagent blanks against water for methods A, B and C, respectively.

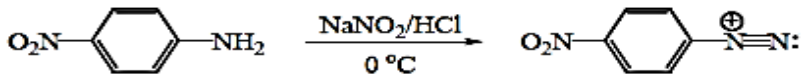
1-Principle of the method A



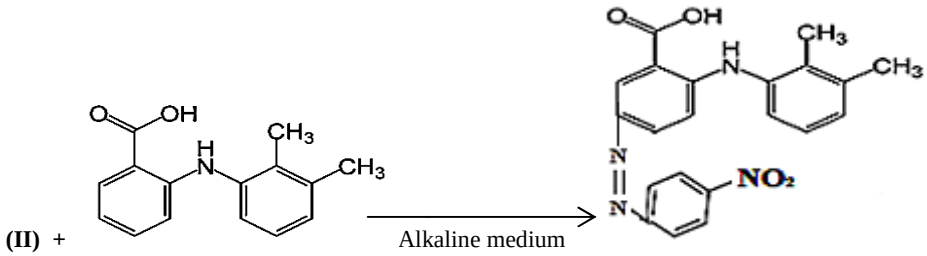
4-Aminohippuric acid Diazotized 4-aminohippuric acid (D-4AHA) (I)



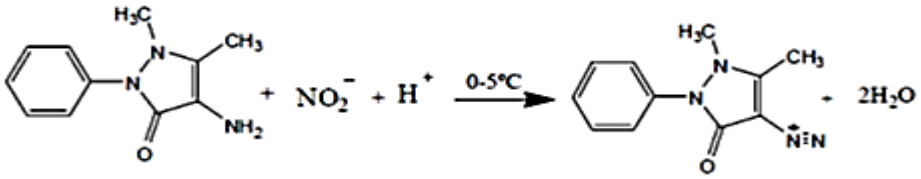
2- Principle of the method B



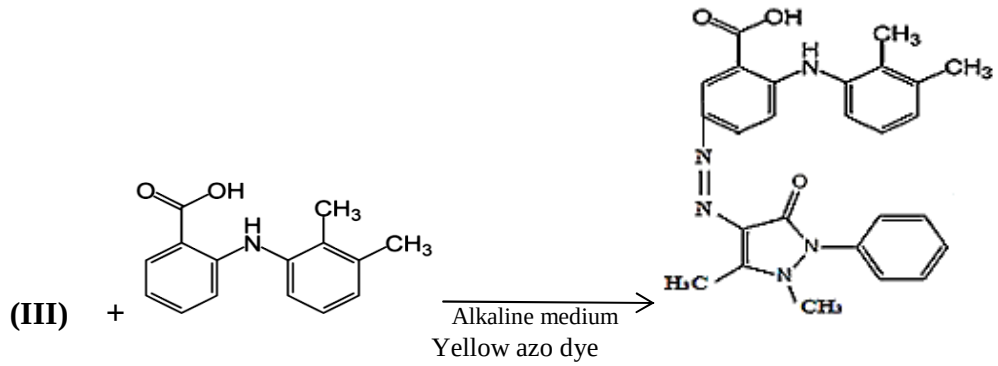
4-Nitroaniline (4NA) Diazotized 4-nitroaniline (D-4NA) (II)



3- Principle of the method C



4-AminoantipyrineDiazotized 4-aminoantipyrine (D-4AAP) (III)



Scheme-1:The suggestedmechanismreaction for method A, B and C.

Optimum reaction conditions

The optimum conditions for the color development of methods (A,B and C) were established by varying one parameters at a time keeping the others fixed and observing the effect produced on the absorbance of the colored species.

- ***Effect of diazotized reagents concentration***

The effect of concentration of (D-4AHA), (D-4NA) and (D-4AAP) were investigated in the proposed methods by measuring the absorbance at specified wavelengths in the recommended procedure for solutions containing a fixed concentration of MEF and varying amounts of diazotized reagents. The results indicated that the maximum absorbance was obtained with 1 ml of reagent in all methods for determination of MEF. The higher concentration of reagents didn't affect the sensitivity for all methods (Figure 2).

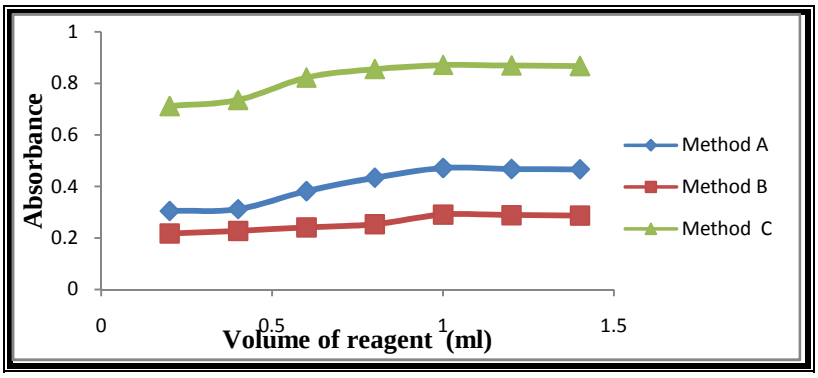


Figure 3:Effect of concentration of diazotized reagents on the reaction product of 10 (µg/ml) MEF for methods A, B and C.

- ***Effect of base type and its amount***

The formation of azo dye depends upon the nature of reaction medium. Various bases have been used and their optimum concentration to be used. Sodium hydroxide was found to be more suitable for the reaction compared to the other bases due to the stable and high intense color of the azo dye formed. The results in Table 1 indicated that 1 ml of sodium hydroxide (2M) was optimum for method A and 1.5 ml of sodium hydroxide (2M) was optimum for method B and C, therefore these volumes have been recommended for the subsequent experiments.

Table-1: Effect of base type and its amount.

2 M of base	**Absorbance / volume of base used (ml) for method** A				**Absorbance /volume of base used (ml) for method** B				**Absorbance / volume of base used (ml) for method** C			
	0.5	**1.0**	**1.5**	**2.0**	**0.5**	**1.0**	**1.5**	**2.0**	**0.5**	**1.0**	**1.5**	**2.0**
NaOH	0.122	**0.475**	0.430	0.408	0.165	0.291	**0.438**	0.413	0.541	0.871	**0.892**	0.824
KOH	0.126	0.321	0.301	0.276	0.132	0.241	0.361	0.328	0.519	0.829	0.845	0.802
Na_2CO_3	0.095	0.185	0.095	0.062	0.082	0.165	0.227	0.210	0.321	0.447	0.489	0.421

- ***Effect of solvent***

The choice of solvent for the reaction mixture has been studied. Water, methanol, ethanol, and acetone were tested as diluting solvents. Water was the best diluent for methods A, B and C.

- ***Effect of time and stability of the formed azo dye***

The effect of time on the development and stability of the dye obtained of MEF has been investigated under the optimum experimental conditions described above. The formation of colored dye was completed immediately at room temperature (20ºC) and provided satisfactory results. The absorbance of the colored species remained stable for more than 3 hours in the three methods. The results were shown in Table 2.

Table-2: Effect of time and stability of the formed azo dye.

Absorbance for (10 µg/ml)MEF	**Time (min.)**									
	0	**10**	**20**	**30**	**50**	**60**	**80**	**100**	**120**	**180**
(method A)	0.475	0.475	0.474	0.474	0.473	0.472	0.472	0.471	0.470	0.469
(method B)	0.439	0.439	0.439	0.438	0.437	0.437	0.436	0.435	0.436	0.434
(method C)	0.891	0.891	0.890	0.889	0.888	0.888	0.887	0.886	0.886	0.884

- ***Effect of order of reactants addition.***

The order of addition of reagent (R) and corresponding volume of NaOH (B) to the sample solution (10 µg/ml of MEF) had been examined (Table 3). The results indicated that order (I) of addition was the optimum order for all methods due to the high intensity of the formed azo dye.

Table-3: Effect of order of reactants addition.

Reaction component	Order number	Absorbance of method A	Absorbance of method B	Absorbance of method C
MEF + R+ B	I	0.476	0.441	0.892
MEF + B + R	II	0.315	0.309	0.654
R + B + MEF	III	0.460	0.411	0.819

Analytical characteristics

Analytical characteristics such as regression equation, linear range, relative standard deviation, recovery, molar absorptivity and Sandell's sensitivity values of each method were determined under the optimized conditions as shown in Table 4. The limits of detection (LOD) and quantitation (LOQ) were calculated according to the International Union of Pure and Applied Chemistry (IUPAC) definition *[28]* using the formula:

LOD = 3S/b and LOQ = 10S/b

Where:

S is the standard deviation of blank absorbance and b is the slope of the calibration plot.

Table-4: Analytical characteristics of proposed methods.

Parameter	Method A	Method B	Method C
λ_{max}, nm	432	453	406
Beer's law range (µg /ml)	0.5-20	1.0-16	0.2-14
LOD (µg / ml)	0.11	0.36	0.05
LOQ (µg/ml)	0.32	1.05	0.14
ε (L/mole. cm)	0.9699×10^4	0.9386×10^4	0.2053×10^5
Sandell's sensitivity (µg/cm^2)	0.0249	0.0257	0.011
Regression equation	y = 0.0238x + 0.059	y = 0.0389x + 0.0443	y = 0.0851x + 0.0739
Intercept (a)	0.059	0.0443	0.0739
Slope (b)	0.0238	0.0389	0.0851
Determination coefficient(R^2)	0.9982	0.9984	0.9984
RSD, %	0.186	0.311	0.127
Recovery, %	98.65	101.22	100.81

Interference studies.

In order to assess the possible analytical applications of the proposed analytical methods which described above to the assay of commercial mefenamic acid formulations, the effect of some foreign compounds used in pharmaceutical preparations were investigated by adding different amount of foreign compounds to 100 µg of mefenamic acid in a final volume 10 ml (Table 5). No interference was observed from the presence of starch, glucose and lactose in the ratios commonly used in pharmaceutical preparations.

Table-5: Effect of foreign compounds for determination of mefenamic acid.

Foreign compounds	10 µg MEF/ µg foreign compound	Recovery% (n=3) for method A	Recovery% (n=3) for method B	Recovery% (n=3) for method C
Starch	100	103.8	101.5	102.6
	50	101.1	100.7	100.7
	10	100.3	99.8	99.8
Glucose	100	97.1	96.8	102.9
	50	98.7	97.9	101.4
	10	99.4	99.1	98.9
Lactose	100	96.8	102.4	103.6
	50	99.0	101.8	101.2
	10	99.9	100.5	100.8

Analytical applications

The proposed methods were successfully applied to determine MEF in pharmaceutical preparations. The obtained results were compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision [29], with the standard method [23] at the 95 % confidence level with five degrees of freedom, as cited in (Table 6). The results showed that the experimental t-test and F-test were less than the theoretical value (t=2.776, F=6.39), indicating that there was no significant difference between the proposed methods and standard method, suggesting that the proposedmethods were comparable to the reference method relation to accuracy and precision.

Table-6: Analytical applications of proposed methods.

Pharmaceutical preparation	% Recovery reference method [23]	% Recovery* Method A	% Recovery* Method B	% Recovery* Method C
Ponstidin capsule(250 mg)GMBH-Germany	101.35	98.38 *t =1.28* *F =3.80*	101.44 *t =0.69* *F =2.55*	100.76 *t =1.02* *F =2.91*
Ponstadintablets(500 mg) S.D.I- Iraq	99.16	99.25 *t =1.17* *F =3.62*	102.09 *t =0.25* *F =2.77*	100.43 *t =0.57* *F =2.83*

* Average of six determinations.

Comparison of the methods

Comparison of analytical variables between the present methods with other in literature spectrophotometric methods. The results shown in Table 7 indicated that the proposed methods were more sensitive and needs neither temperature control nor extraction step, also the present methods have a good value oflimit of detection.As the three methods compared to each other; it has been found that the method C was more sensitive than methods A and B.

Table-7: Comparison of the proposed methods with literature method for the determination of MEF.

Analytical parameters	Present methodA	Present methodB	Present methodC	Literature method[16]
Color of the dye	Yellow	Yellow	Yellow	Reddish-pink
Media	Aqueous (Alkaline)	Aqueous (Alkaline)	Aqueous (Alkaline)	Non- aqueous
Type of reaction	Diazo-coupling	Diazo-coupling	Diazo-coupling	Diazo-coupling
Reagent	4-Aminohippuric acid	4-Nitroaniline	4-Aminoantipyrine	4-Amino-3,5-dinitrobenzoic acid
Temperature (°C)	Room temperature	Room temperature	Room temperature	30
λ_{max} , nm	432	453	406	490
Beer's range(µg/ml)	0.5-20	1.0-16	0.2-14	1.0-6.0
Determination coefficient (R^2)	0.9982	0.9984	0.9984	0.9875
LOD (µg/ ml)	0.11	0.36	0.05	0.96

Conclusions

The proposed methods for the determination of mefenamic acid in dosage forms were sensitive, simple, don't involve solvent extraction steps and less time consuming. The colored complexes formed were fairly soluble in aqueous solution and remained stable for more than 3 hours. The specificity of the three methods was investigated by notice that no interference was observed from common tablet and capsule excipients. The simplicity of the proposed methods and the stability of the formed azo dyes permitted the determination of mefenamic acid in commercial tabletsand capsules.

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