Spectrophotometric Determination of Mefenamic Acid Using The Oxidation Reduction Reaction of Iodide and Iodate Ions

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Abstract

A sensitive indirect spectrophotometric method is proposed for determining mefenamic acid in pure form and in its pharmaceutical preparations. The method is based on the reaction of mefenamic acid with potassium iodate and potassium iodide to liberate iodine , which is immediately converted to triiodid ion complex in presence of an excess of potassium iodide solution to form yellow dye ,which exhibits maximum absorption at 347 nm. Beer's law is obeyed over the range 10 to 1200 µg of mefenamic acid in final volume 25 ml, i.e.,0.4- 48 ppm with a molar absorptivity of 0.904×10^4l. $mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.0266 $\mu g.cm^{-2}$, a relative error of -0.625 to 1.470 % and a relative standard deviation of ±0.336 to ±1.764 % depending on the concentration level . The proposed method has been applied successfully to determine mefenamic acid in pharmaceutical preparations.

التقدير الطيفي لحامض الميفيناميك باستخدام تفاعل الاكسده والاختزال لايونات اليوديد واليودات

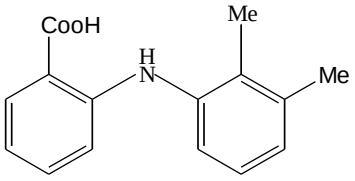
الخلاصة

تم اقتراح طريقة طيفية غير مباشرة وحساسة لتقدير حامض الميفيناميك بشكله النقي وفي مستحضراته الصيدلانية. اعتمدت الطريقة على تفاعل حامض الميفيناميك مع يودات البوتاسيوم ويوديد البوتاسيوم حيث يتحرر اليود الذي يتحول بسرعة الى الايون المعقد ثلاثي اليوديد عند وجود زيادة من يوديد البوتاسيوم ليكون صبغة صفراء تقاس اعلى شدة امتصاص لها عند الطول الموجي ٣٤٧ نانوميتر وكانت حدود قانون بير في مدى التركيز من 10 إلى ١٢٠٠ مايكروغرام من حامض الميفيناميك في حجم نهائي ٢٥ مللتر أي من ٠,٤ إلى ٤٨ جزء/مليون وكانت الامتصاصية المولارية للصبغة الناتجة ٠,٩٠٤×١٠^{٤} لتر.مول$^{-١}$.سم$^{-١}$ ودلالة ساندل

للحساسية ٠,٠٢٦٦ مايكروغرام.سم$^{-٢}$و الخطأ النسبي من ٠,٦٢٥– إلى ١,٤٧٠+% والانحراف القياسي النسبي من ٠,٣٣6± إلى ١,٧٦٤±% اعتماداً على مستوى التركيز وتم تطبيق الطريقة المقترحة بنجاح لتقدير حامض الميفيناميك في المستحضرات الصيدلانية .

Introduction

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



M.wt =241.3 g /mol.

Different of techniques have been described for the determination of mefenamic acid as pure and in dosages forms. These techniques include titrimetric[(2,3)], chromatographic[(4-6)], luminescence [(7)], flow injection[(8,9)], electrometric[(10)], spectroflurometric [(11,12)],and spectrophotometric methods[(13-19)].Also several spectro-photometric methods have been described for the simultaneous determination of mefenamic acid in the mixture with other active drugs in the same pharmaceutical preparations[(20-21)].

However some of these procedures suffer from one or another disadvantage such as extraction to organic solvent [(13)], require non-aqueous medium [(17)] and other need control of temperature [(16,18)]. The objective of investigation reported in this paper is to evaluate a simple, sensitive and accurate method for the assay of mefenamic acid(in an aqueous medium) , either in pure form or in pharmaceutical preparations .The method based on oxidation reduction reaction of iodide and iodate ion in acidic medium (mefenamic acid) to produce yellow dye which its intensity proportional to mefenamic acid present in solution (indirect method).

Experimental

Apparatus

All spectrophotometric measurements are performed on Shimadzu UV-visible recording spectrophotometer UV-160 using 1-cm silica cells.

pH meter type Philips PW 9420 is used for pH reading.

Reagents

All chemicals used are highest purity available.

Standard mefenamic acid solution, 100μg.ml^{-1}. This solution is prepared by dissolving 0.01 g of mefenamic acid (SDI- Iraq) in ethanol and the volume is diluted to 100 ml with ethanol in a volumetric flask.

Potassium iodide solution, 0.015 M. This solution is prepared by dissolving 0.2490 ***g*** of potassium iodide (Fluka) in 100 ml distilled water in a volumetric flask.

Potassium iodate solution, 0.01 M. This solution is prepared by dissolving 0.2140 g of potassium iodate (Fluka) in 100 ml distilled water in a volumetric flask.

Mefenamic acid capsule solution, 100μg.ml^{-1}. Weight and mix the contents of five capsule (each one contain 250 mg mefenamic acid), an accurately weighed amount of powder equivalent to 0.01g mefenamic acid is dissolved in 75ml ethanol , after filtration of the solution the volume is completed to 100 ml with ethanol in a volumetric flask.

Mefenamic acid suspension solution, 100μg.ml^{-1}. The content of the container (100 ml, each 5ml contain 50 mg mefenamic acid) is mixed with 400 ml of ethanol then the solution is warmed , then filter and the volume is completed to 500 ml with ethanol, 5 ml which equivalent to 0.01 g mefenamic acid is transferred in to a 100 ml calibrated flask and the volume is completed with ethanol.

General Procedure and calibration graph

To series of 25 ml calibrated flasks, which contain increasing volume(0.1-16) ml of mefenamic acid 100μg /ml , 3 ml of potassium iodate (0.01M) and 6 ml of pot-assium iodide solution (0.015M) are added then the flask stand for 15 minutes, the absorbances of the yellow coloured products are measured immediately after dilution at 347 nm against the reagent blank, a liner calibration graph is obtained over the concentration range of 10-1200 μg mefenamic acid / 25 ml concentration above 1200 μg mefenamic acid / 25 ml give negative

deviation from Beer's law (Fig. 1). The apparent molar absorptivity, referred to mefenamic acid, has been found to be 0.904×10^{4}l.mol^{-1}.cm^{-1}

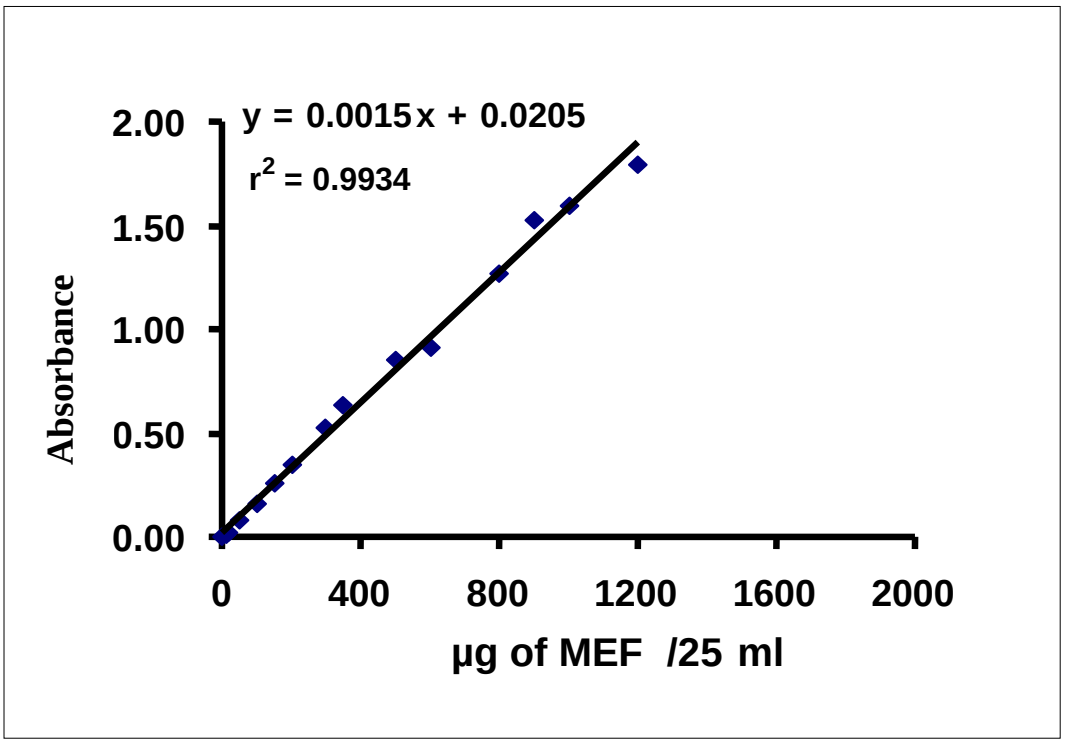


Fig 1 Calibrated graph determination of mefenamic acid

Results and Discussion

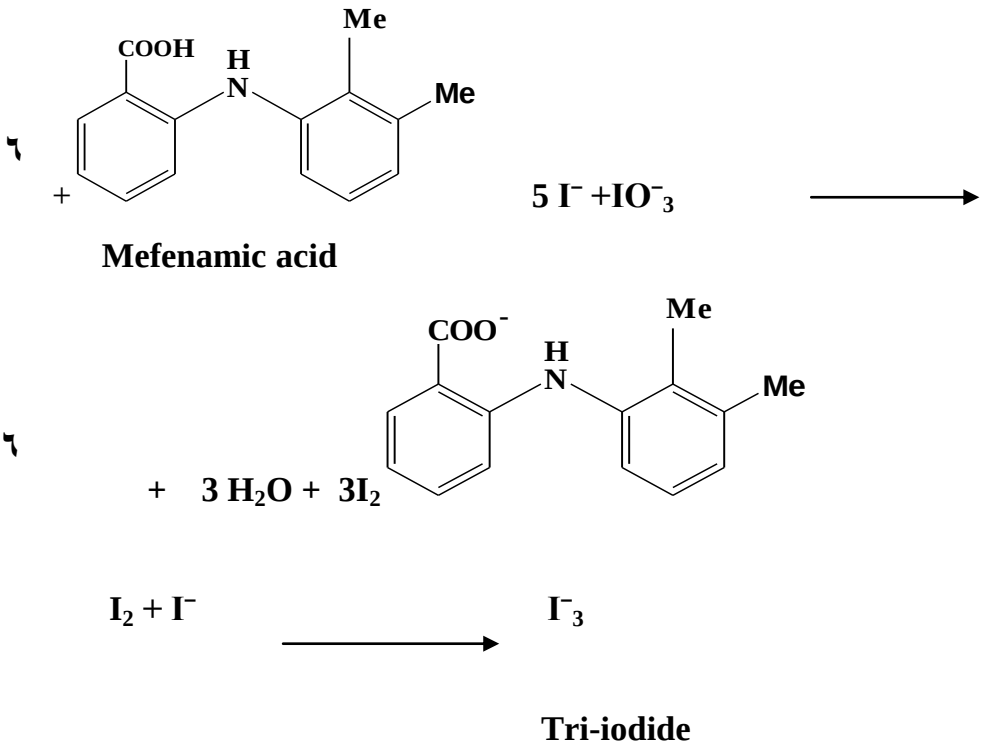
Principle of reaction

It has been suggested that water-soluble acidic compound liberate iodine from solution containing both IO_3^- and I^- ions as shown in the reaction below (22)

$$5I^- + IO_3^- + 6\,H^+ \longrightarrow I_2 + 3H_2O$$

The yellow colour of solution is due to the immediately converted of I_2 to tri-iodide ion in presence of excess iodide ion.

Mefenamic acid contain acidic group and can undergoes similar reaction with iodide-iodate ions.



Method optimization

The effect of various parameters on colour development is tested to establish the optimum conditions for reaction of mefenamic acid with iodate and iodide ions.

For the subsequent experiments, 100 µg of mefenamic acid is taken in 25 ml final volumes .

Effect of iodate amount

Various volume of potassium iodate solution(0.01M) are tested , the final results indicated that 3 ml of potassium iodate solution is more suitable amount which is gives the highest value of intensity to the colour of the product formed (Table1).

Table 1. Effect of potassium iodate amount on absorbance

ml of KIO_3 (0.01M)	Absorbance/µg of MEF present							B*	r^{2**}
	٥٠	١٠٠	١٥٠	٢٠٠	٣٠٠	٦٠٠	١٢٠٠		
١	٠,٠٦٦	٠,١٤٥	٠,٢٠٨	٠,٢٥٨	٠,٣٦٥	0.495	١,٠١١	٠,٠١٢	٠,٩٨٥٤
٢	٠,٠٥٩	٠,١٢٦	٠,٢١٠	٠,٢٦٦	٠,٣٦٧	٠,٥٢٢	١,١٠٩	٠,٠١٧	٠,٩٨٧٤
٣	٠,٠٦٢	٠,١٣٤	٠,٢١٦	٠,٢٨٥	٠,٣٩٥	٠,٥٧٥	١,١٤٠	٠,٠١٣	٠,٩٨٨٦
٤	٠,٠٥٦	٠,١٢٩	٠,٢١٣	٠,٢٨٥	٠,٤٠٦	٠,٥٨٠	١,١٥١	٠,٠١٢	٠,٩٨٦٨
٥	٠,٠٥٧	٠,١٣٠	٠,٢١٤	٠,٢٨٨	٠,٣٩٣	٠,٥٨٣	١,١٤٥	٠,٠١٠	٠,٩٨٨٦

*Absorbance of blank versus distilled water at 347 nm.
** Determination coefficient

Effect of iodide amount

The effect of the amount of potassi-um iodide solution (0.015M) on maximum absorbance of the dye formed has been investigated and the result are illustrated in Table 2

Table 2. Effect of potassium iodide amount on absorbance

ml of 0.01M KIO_3	Absorbance/µg of MEF present							B	r^2
	٥٠	١٠٠	١٥٠	٢٠٠	٣٠٠	٦٠٠	١٢٠٠		
٣	٠,٠٥٨	٠,١٣٥	٠,٢١٨	٠,٢٨٨	٠,٤٠١	٠,٥٨١	١,١٣١	٠,٠٠٩	٠,٩٨٧٠
٤	٠,٠٦٣	٠,١٤٧	٠,٢٣٨	٠,٣١٣	٠,٤٧٠	٠,٦٥٥	١,٤٦٧	٠,٠١١	٠,٩٨٧٠
٥	٠,٠٥٦	٠,١٥١	٠,٢٤٩	٠,٣٣٣	٠,٤٩٢	٠,٧٢٠	١,٦٨٤	٠,٠١٤	٠,٩٨٨٠
٦	٠,٠٧١	٠,١٦٣	٠,٢٦٣	٠,٣٥١	٠,٥٢٠	٠,٩٠٨	١,٧٩٩	٠,٠١٩	٠,٩٩٨٠
٧	٠,٠٦٧	٠,١٦٠	٠,٢٦٢	٠,٣٥٩	٠,٥١٥	٠,٩١٥	١,٨١١	٠,٠٢٠	٠,٩٩٨٠
٨	٠,٠٦٥	٠,١٦١	٠,٢٥٩	٠,٣٦٣	٠,٣٤٥	٠,٩١١	١,٨٠٦	٠,٠٢١	٠,٩٩٦٤

From the result in Table 2, 6 ml of potassium iodide has been recommended for the subsequent experiments.

Effect of time on oxidation reaction

The oxidation reaction time is determined by following the colour development at room temperature (23±1C). It is observed that the absorbance reached maximum after 15 minute, this time (15 minute) is chosen for subsequent experiments.

Table 3. Effect of oxidation time

Time*(min)	٠	٥	١٠	١٥	٢٠	٢٥	٣٠	٤٠
Absorbance	٠,٠٨١	٠,١٤١	٠,١٤٩	٠,١٥٧	٠,١٥٦	٠,١٥٧	٠,١٥٧	٠,١٥٦

***Before dilution with distilled wate**

Effect of surfactant

The effect of several types of surfactants on intensity of the dye has been investigated.(Table 4).

Table 4. Effect of surfactant

Surfactant Solution	Absorbance/ Order* of addition					
	I		II		III	
	A	Δλ	A	Δλ	A	Δλ
SDS (1×10^{-3} M)	٠,١٣٦	٩٤	٠,١٤٧	٩٦	٠,١٥٦	٩٤
CPC (1×10^{-3} M)	٠,١٦٢	٩٥	٠,١٢٨	٩٦	٠,١٥٤	٩٦
TritonX-100 1%	٠,٠٧٤	٨٢	٠,٠٠٧	٦١	٠,١١٤	٦٣

* I -MEF +Surfactant(S)+ Potassium iodate ($IO3^-$) +Potassium iodide(I^-) -
IIMEF+ $IO3^-$+S+ I^-
III - MEF + $IO3^-$ + I^- +S
Not. Absorbance without surfactant=0.161 and Δλ = 96

The results in Table 4 indicate that addition of surfactants give no useful effect [an increase in the intensity or an improve in the colour contrast (Δλ)],therefore it has been recommended to eliminate the use of surfactants in the subsequent experiments.

Order of addition

To obtain optimum results the order of addition of reagents has been studied (Table 5).

Table 5. The order of addition

Reaction component	Order number	Absorbance
MEF+ KIO_3 +KI	I	0.160
MEF+ KI+ KIO_3	II	0.150
+ MEF+ KI KIO_3	III	0.158
+KI +MEF KIO_3	IV	0.152
KIO_3+ MEF + KI	V	0.151

The result indicate that the order I(give maximum absorbance) should be followed as give under the general procedure.

Effect of Time

The effect of time on the development and stability of the coloured complex for different amounts of mefenamic acid is investigated under the optimum experimental conditions established. The low concentration of mefenamic acid produce a stable coloured species which is stable for at least one hour, while concentrations from 75-100µg /25 ml give stability period from 0-20 minutes and concentrations ≥200µg mefenamic acid / 25 ml give unstable products (Table 6).

Table 6. Effect of time on the absorbance of complex

Time /minute	Absorbance / µg of Mefenamic acid in 25 ml					
	٢٥	٥٠	٧٥	١٠٠	٢٠٠	٨٠٠
٠	٠,٠٣٠	٠,٠٦٣	٠,١٣٠	٠,١٥٩	٠,٣٢٥	١,٢٦٥
٥	٠,٠٣٠	0.063	٠,١٢٩	٠,١٥٨	٠,٣١١	١,٣١٧
١٠	٠,٠٢٩	٠,٠٦٤	٠,١٢٩	٠,١٥٦	٠,٢٩٦	١,٣٤٨
١٥	٠,٠٣٠	٠,٠٦٤	٠,١٢٧	٠,١٥٥	٠,٢٧٧	١,٣٦٦
٢٠	٠,٠٣٠	٠,٠٦٤	٠,١٢٤	٠,١٥١	٠,٢٦١	١,٣٨٧
٢٥	٠,٠٣٠	٠,٠٦٣	٠,١٢٣	٠,١٥٠	٠,٢٤٠	١,٤٠٥
٣٠	٠,٠٢٩	٠,٠٦٤	٠,١٢٢	٠,١٤٨	٠,٢٢٠	١,٤٢٥
٣٥	٠,٠٣٠	٠,٠٦٢	٠,١٢٠	٠,١٤٦	٠,٢٠١	١,٤٤٠
٤٠	٠,٠٣٠	٠,٠٦٢	٠,١١٨	٠,١٤٤	٠,١٨٣	١,٤٥١
٤٥	٠,٠٣٠	٠,٠٦٢	٠,١١٧	٠,١٤٤	٠,١٦٩	١,٤٦٩
٥٠	٠,٠٣٠	٠,٠٦٢	٠,١١٧	٠,١٤٢	٠,١٥٦	١,٤٧٨
٥٥	٠,٠٢٩	٠,٠٦٠	٠,١١٥	٠,١٤٠	٠,١٤١	١,٤٩٢
٦٠	٠,٠٢٩	٠,٠٥٩	٠,١١٤	٠,١٣٨	٠,١٢٧	١,٥٠٣

Effect of interference

The effect of the presence of some common pharmaceutical additive on the efficiency of suggested method has been studied. The result in (Table7) indicate that there is no significance interference produced by these foreign substances on suggested method except starch in high concentration.

Table 7. The effect of foreign compounds on assaying mefenamic acid

Foreign compound	Recovery(%) of MEF per µg foreign added		
	١٠٠	٥٠٠	١٠٠٠
Glucose	٩٧,٩٣	٩٨,٦٢	١٠١,٣٧
Lactose	٩٦,٥٥	١٠٠,٠٠	٩٧,٩٣
Starch	٩٧,٩٣	٩٥,٨٦	90.34
Gum Arabic	٩٧,٢٦	١٠١,٠٩	١٠٢,٧٣
Glycerin	٩٩,٣١	١٠٢,٧٥	٩٦,٥٥

Final absorption spectra

Potassium iodate and potassium iodide undergo oxidation reduction reaction in acidic medium (mefenamic acide) as listed in the recommended procedure to produce a yellow colour production .The absorption spectrum (Fig 2) shows a maximum absorption at 347 nm against the reagent blank which give maximum absorption at 251 nm.

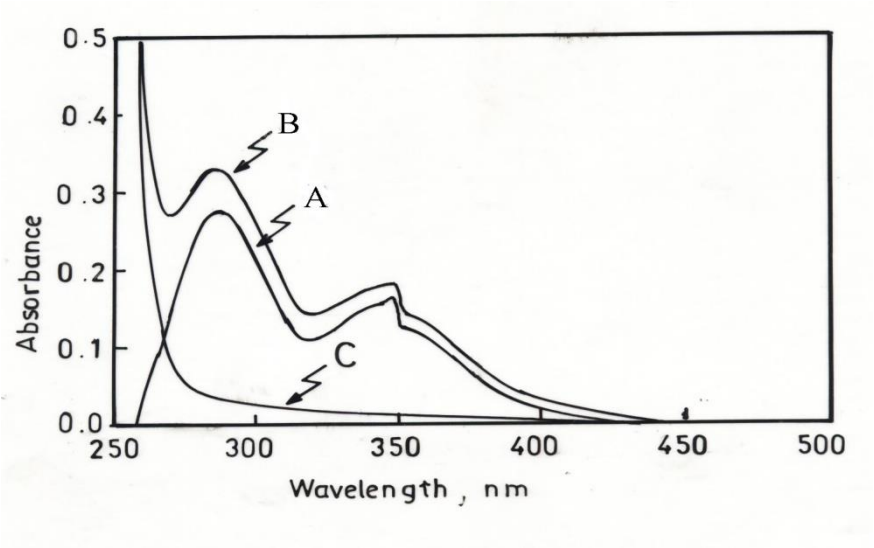


Fig (2): Absorption spectra of (A)the complex against blank ,(B) complex against distilled water and(C) blank against distilled water

Accuracy and precision

To check the accuracy and precision of the calibration graph three different concentration within linearity range are selected (50 ,100 ,200,800) and determined. The results are shown in Table 8 which indicate that proposed method is satisfactory.

Table 8. Accuracy and precision

Amount of MEF µg ، taken	Relative error %*	Relative standard deviation %*
٥٠	١,٤٧٠	±1.764
١٠٠	- 0.625	±1.623
٢٠٠	-٠,٥٧٩	٠,٨٨١±
٨٠٠	-0.168	٠,٣٣٦±

*Average of five determinations

Analytical applications

The proposed method was applied to determine mefenamic acid in different pharmaceutical prepar-ations(the pH of solutions should be the same of standard mefenamic acid solution), included capsule and suspension . On applying proposed procedure, good recoveries are obtained as shown in Table 9

Table 9. Analytical application of proposed method

Pharmaceutical preparation	µg mefenamic acid present/25ml	µg mefenamic acid measured/25ml	Recovery* (%)
Ponstidin capsule(250mg) N.D.I-Iraq	25	24.99	99.96
	50	48.80	97.60
	100	99.80	99.80
Ponstidin capsule(250mg) GMBH,Germany	25	24.75	99.00
	50	51.40	102.80
	100	96.87	96.87
Mefaman(50mg/5ml) AL-mansour Pharma-Ind. (Baghdad-Iraq)	25	24.77	99.08
	50	49.80	99.60
	100	100.96	100.96

*Average of three determinations

Evaluation of the proposed method

The performance of the proposed method is assessed by calculating the student's t– test compared with the standard method (British Pharmacopeia , 2000). At the 95% confidence limit for four degree of freedom , the calculated t - values do not exceed the theoretical value (2.776). The results in Table 10 indicate that there is no significant difference between the proposed method and the standard method.

Table 10.Analysis of mefenamic acid in pharmaceuticals by proposed and official method

Drug	Recovery%*		t-exp
	Present method	Official method[2]	
Ponstidin capsule (250mg) N.D.I-Iraq	٩٩,٥٢	١٠٠	0.181
Ponstidin capsule (250mg) GMBH, Germany	٩٨,٥٧	٩٩,٤٥	٠,٧٠٣
Mefaman suspension (50mg/5ml AL-Mansour Baghdad-Iraq	١٠٠,٨١	١٠١,١٩	٠,١٦٠

Conclusion

Accurate and sensitive spectrophotometric method for the determination of trace amount of mefenamic acid in aqueous solution based on reaction of iodide and iodate ions with mefenamic acid(as an acid of reaction).The proposed method has been successfully applied to assay of mefenamic acid in various pharmaceutical preparations.The t-value indicate that there is no significant difference between the proposed method and the standard method .However, the proposed method need neither temperature control nor extraction step.

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