------Jou. Raf. Sci., Vol. 20, No.3 pp 39- 47, 2009------

Indirect Spectrophotometric Method for the Determination of Mefenamic Acid in Pharmaceutical Formulations*

Nief R. Ahmed **Zainab M. Al-mufty**
The State Company for Drug Industries and Medical Appliances
Mosul , Iraq

(Received 10 / 11/ 2008 ; Accepted 4 / 5 / 2009)

ABSTRACT

A simple, sensitive and accurate spectrophotometric method for the determination of mefenamic acid in pure form and in some pharmaceutical formulations was developed. The method is based on the oxidation of mefenamic acid by iron(III), and subsequent complexation of iron(II) with o-phenanthroline, forming a red-colored complex (ferroin) having the maximum absorbance at 510 nm. Beer's law is obeyed in the concentration range of 0.4-2.0 µg\ml, the molar absorptivity and Sandell's sensitivity are 2.9×10^4 $L.mol^{-1}.cm^{-1}$ and 8.3 $ng.cm^{-2}$, respectively and the relative standard deviation (RSD) is less than 2.0 (n=10). The limits of detection and quantitation are 0.065 and 0.195 $\mu g.ml^{-1}$,respectively. The method is applied successfully to determination of mefenamic acid in some pharmaceutical formulations (capsules and tablets). The common excipients do not interfere with the proposed method. A statistical comparison of these results with those of official method using (t and F) values at 95% confidence level shows good agreement and indicates no significant difference in the precision and the present method has good validity.

Keyword: Mefenamic acid, Spectrophotometry; Iron (ш); phenanthroline

---

طريقة طيفية غير مباشرة لتقدير حامض الميفيناميك في حالته النقية ومن بعض مستحضراته الدوائية

الملخص

تم وصف طريقة طيفية بسيطة وحساسة وذات دقة عالية لتقدير حامض الميفينامك في حالته النقية وفي بعض مستحضراته الدوائية. تعتمد الطريقة على أكسدة الحامض بواسطة الحديد الثلاثي حيث يكـون نـاتج التفاعل الحديد الثنائي والذي بدوره يكون معقد احمر اللون مع كاشف اورثو فينانثرولين (الفروين) والذي له أقصى امتصاص عند الطول الموجي 510 نانوميتر وقد لوحظ ان قانون بير يسري علـى الكميـات التـي تتراوح بين (0.4-2) جزء بالمليون وبامتصاصية مولارية2.9×10^4 لتر. مول$^{-1}$.سم$^{-1}$وبدلالة ساندل(دلالـة الحساسية) 8.3 نانو غرام.سم$^{-2}$. إن الانحراف القياسي النسبي للطريقة اقل من 2%.

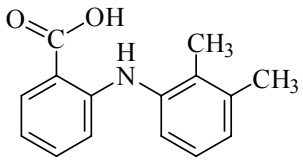
---

*تم إلقاءه في المؤتمر العلمي الأول للكيمياء المنعقد بتاريخ 22-23 نيسان 2008 فى قسم الكيمياء / كلية العلوم/ جامعة الموصل

وان حد الكشف والحد الكمي كانا 0.065 و0.195 مايكرو غرام.مل$^{-1}$ على التوالي. وطبقت الطريقة بنجاح لتقدير حامض الميفينامك في بعض مستحضراته الدوائية (حبوب و كبسول). كما وجد أن لا تأثير للمضافات الدوائية على نسبة استعادة حامض الميفيناميك باستخدام الطريقة المقترحة. واختبر نجاح الطريقة بمقارنتها مع الطريقة القياسية الدستورية المعتمدة باستخدام اختباري (t و F) وعند حدود ثقة 95% مما يدل على صلاحية التطبيق التحليلي للطريقة.

INTRODUCTION

Mefenamic acid: N-2, 3-xylylanthranilic acid, is an analgesic, antipyretic with minor anti-inflammatory properties (The pharmaceutical codex, 1979).



(Mefenamic acid)
(N-2, 3-xylylanthranilic acid)

Mefenamic acid is used in musculoskeletal and joint disorder such as rheumatoid arthritis, osteoarthritis (Martindale, 1999) and primary dysmenorrheal (Zhang and Li, 1998 and Remington,1995). Different methods for the determination of mefenamic acid have been described, such as titrimetry (official method) for the assay of pure form and pharmaceutical preparation using sodium hydroxide as titrant and phenol red as indicator (British pharmacopeia, 2005), HPLC (Chao *et al.,* 1997 and Hirai *et al.,* 2002) gas chromatography (Chao, 1997) and cloud point extraction with spectrofluorimetry and spectrophotometry. (Tabrizi, 2006).

The most widely used methods for the determination of mefenamic acid are spectrophotometric methods (Idown *et al.,* 2002; Sastry and Rao,1989; Sastry and Rao, 1987, Dinc *et al.,* 2002; Gangwal *et al.,*1996; Sastry and Rao,1989; Aman *et al.,* 2005 , Zommer *et al.,*1986 and Alarfaj *et al.,* 2009). However, all of these methods suffer from one or more disadvantage such as insufficient sensitivity, selectivity, tedious and use of complex solvent extraction procedures. Therefore, a simple method for assay of mefenamic acid is necessary for routine analysis and quality evaluation.

EXPERIMENTAL

Apparatus

Spectro-scan 50 UV- visible (double beam) spectrophotometer with 1.0 cm quartz cells was used for absorption measurements, and Jenway 3310 pH meter was used for pH measurements.

Reagents

All chemicals used were of analytical or pharmaceutical grade and distilled water was used throughout.

Mefenamic acid stock solution:(100 ppm).

This solution was prepared by dissolving 0.01 g of mefenamic acid in 100 ml 0.01 N NaOH solution.

Mefenamic acid standard solution (10ppm) (4.1×10^{-5} M)

This solution was prepared by diluting 10 ml of stock solution to 100 ml with distilled water in a volumetric flask.

Ferric chloride solution 0.1% in 0.01 N HCl

This solution was prepared by dissolving 0.1 g of ferric chloride in 100 ml of distilled water containing 2 ml of concentrated HCl

Buffer solution(pH 3)

This solution is prepared by mixing 16.2 ml of 0.1 M chloroacetic acid with 8.8 ml of 0.1M KOH, then the volume is completed to 100 ml with distilled water in a volumetric flask (Zommer and Bojarowicz,1986).

1,10-phenanthroline solution: 1% in 0.01 N HCl

This solution was prepared by dissolving 1 g of 1,10 phenanthroline in 100 ml 0.01 N HCl solution.

Recommended procedure

A known volume of sample solution containing 10-50 μg of mefenamic acid was transferred into a 25- ml calibrated flask followed by 3 ml of ferric chloride solution then 2 ml of 1,10-phenanthroline solution and 2 ml of buffer solution pH 3.0 were added, diluting the solution to the mark with distilled water, the flask was placed in a water bath maintained at 70 $\pm 3^0$C for 15 min. The absorbance of the red-colored product was measured at 510 nm against a reagent blank.

Analysis of pharmaceutical preparations

Tablets

Weigh and powder 10 tablets. Dissolve a quantity of the powdered tablets containing 0.01 g of mefenamic acid in about 100 ml of 0.01 N sodium hydroxide. It was shaken thoroughly for about 10-15 min, and filtered. The filtrate was made up to 1 L with distilled water. Treate 3 ml of this solution as mentioned under recommended procedure.

Capsules

Dissolve a quantity of the mixed contents of 10 capsules containing 0.01 g of mefenamic acid in 100 ml of 0.01 N sodium hydroxide and mixed for 10-15 mint and then filtered. The filtrate was made up to 1L with distilled water. Treate 3 ml of this solution as described under recommended procedure.

RESULTS AND DISCUSSION

The method is based on the oxidation of mefenamic acid by iron (III) in the presence of o-phenanthroline in acidic medium. The iron (II) formed was quantitively and rapidly converted to the stable tris (o-phenanthroline) iron (II) complex (ferroin) having an absorption maximum at 510 nm (Marczenko, 1976)as shown below (Fig. 1) thus permitting the indirect estimation of mefenamic acid. The reaction variables were optimized by varying each variable while keeping others constant for obtaining maximum absorbance.

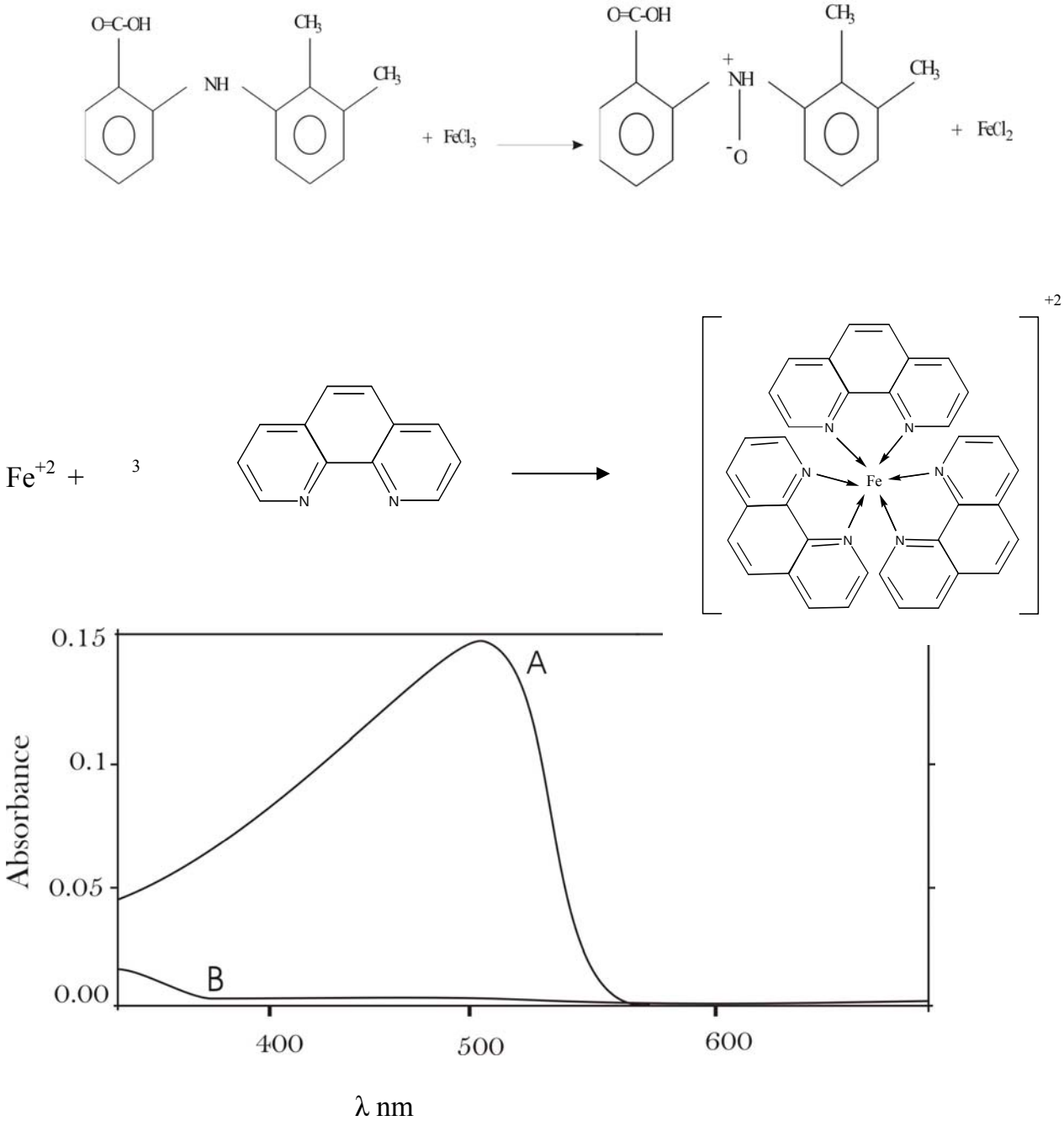


Fig 1: Absorption spectra of A :30 µg/25 ml of mefenamic acid with $FeCl_3$-phenanthroline against reagent blank. B:blank against distilled water.

The oxidation reaction was found to be quantitative in acidic medium, the pH 2.0-4.0 is considered optimum. Then, a pH of 3.0 is selected for the subsequent investigation. Four types of buffer solution pH3 of different compositions (Perrin and Dempsey, 1974) have been tested for this purpose. Table (1) shows the results of this investigation.

Table 1: Effect of different buffers on the absorbance

Ml of buffer solution	Absorbance			
	B1	B2	B3	B4
0.5	0.142	0.097	0.062	0.095
1	0.147	0.116	0.081	0.099
2	0.147	0.116	0.093	0.108
3	0.147	0.115	0.095	0.108
4	0.145	0.109	0.096	0.112
5	0.142	0.110	0.095	0.108

B1: Buffer of 0.1M chloroacetic acid-0.1M KOH
B2: Buffer of 0.1M glycine -0.2M HCl
B3: Buffer of 0.2M KCL-0.2M HCl
B4: Buffer of 0.1M citric acid-0.2M Na_2HPO_4

It was found that 1-3 ml of buffer B1 gives high sensitivity and 2.0 ml has been used for subsequent experiments.

The effect of the amount of $FeCl_3$ and o-phenanthroline amounts on the absorbance was investigated. A maximum and constant absorbance was found with 3.0 ml $FeCl_3$ and 2.0 ml of 1% o-phenanthroline, which were therefore adopted as being optimal.

The color reaction occurred even at room temperature (25^0C), though at higher temperature the color developed more rapidly. The maximum absorbance was observed after 15 min of heating at 70^0C. A temperature of 70^0C and a reaction time of 15 min were selected for reproducible results.

Under the experimental conditions described, Beer's law is obeyed over the concentration range 0.4-2.0 µg/ml Fig[2]. Linear regression equation: A=0.0021+0.048C (r = 0.9993 n = 8).

Where A is the absorbance and C is the concentration in µg\ml

The apparent molar absorptivity was 2.9×10^4 $L.mol^{-1}.cm^{-1}$ and Sandell's sensitivity was 8.3 $ng.cm^{-2}$. The limit of detection and quantification were evaluated as (Bassavaiah and Somashekar, 2007):

$$LOD = 3.3\frac{So}{b} \qquad \text{LOQ} = 3\ \text{LOD}$$

Where b is the slope and So is the standard deviation of the regression line. The limit of detection was 0.065 µg.ml $^{-1}$ and the limit of quantification as the lowest standard

concentration which could be determined with acceptable accuracy, and precision was 0.195 µg. ml $^{-1}$

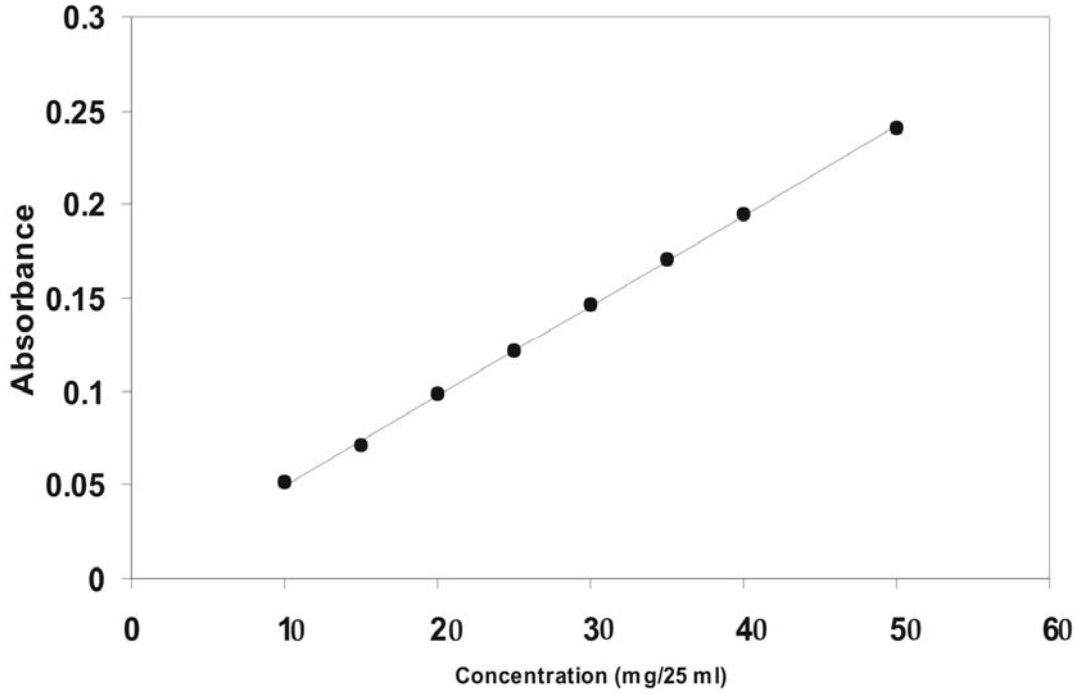


Fig 2: Calibration graph of mefenamic acid

ccuracy and precision

The accuracy and precision of the method were established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy is 100± 0.92% revealing high accuracy of the method. The relative standard deviation (RSD), which is an indicator of precision, is better than ± 2.0% the results are complied in Table(2).

Table 2: Optical characteristics and statistical data for regression equation of the proposed method

Parameters	**Value**
λmax (nm)	510
pH	3.0
Beer's law limits, (µg.ml^{-1})	0.4-2.0
Molar absorptivity, (l.mol^{-1}.cm^{-1})	2.9×10^4
Limit of detection, (µg\ml)	0.065
Limit of quantification, (µg\ml)	0.195
Sandell's sensitivity, (ng\cm^2)	8.3
Correlation coefficient (r)	0.9993
Regression equation (A= γ + bx)	A=0.0021+0.048C
Intercept (γ)	0.0021
Slope (b)	0.048
Recovery, (%)	100 ± 0.92
Relative standard deviation, (%)	< 2.0

INTERFERENCES

To study the potential interference from the commonly used excipients and other additives such as povidone, starch, talc, microcrystalline cellulose, magnesium stearate and mannitol, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (30 µg\25 ml), excipients in different concentrations were added and analyzed. Results of the recovery analysis are presented in Table (3). Excipients at the concentration shown in Table (3) don't interfere with the assay. In addition, recoveries in most cases were around 100 %.

Table 3: Determination of mefenamic acid in presence of excipients

Excipients	Amount taken (µg\ml)	Average recovery* %
Talc	300 1000	99.5 100.05
Mannitol	300 1000	100.08 99.91
Mg-stearate	300 1000	100.05 99.93
Povidine (polyvinyl pyrrolidone)	300 1000	100.0 99.96
Starch	300 1000	100.08 100.05
Microcrystalline cellulose	300 1000	99.83 99.94

* Average of 6 replicate analyses

APPLICATION OF THE PROPOSED METHOD

The proposed method was successfully applied to the analysis of mefenamic acid in capsules and tablets. The results of analysis for pharmaceutical formulations Table (4) were compared statistically by student t-test and by the variance ratio F-test with those obtained by official method at 95% confidence level. The calculated t -and F- values did not exceed the theoretical values indicating that there was no significant difference between the precision of the proposed and official methods.

Table 4: Assay of mefenamic acid in pharmaceutical formulations.

Pharmaceutical formulations supplied by NDI	**Amount of mefenamic acid***		**t Value**	**F Value**
Ponstadin capsules (250 mg/capsule)	250.83	249.23	1.52	1.31
Ponstadin tablets (500 mg/tablets)	501.01	498.83	1.99	1.18

* Mean of ten determinations.
T values (n = 10, at 95% confidence level tabulated value 2.262)
F values (n_1 and n_2 = 10, at 95% confidence level tabulated value 3.18)

CONCLUSION

The spectrophotometric method proposed is simple, sensitive, rapid, low-cost, does not involve solvent extraction steps and gives precise and accurate results. The proposed method was successfully applied to analysis of mefenamic acid in tablets and capsules.

REFERENCES

Alafraj, N. A.; Altamimi, S. A. ; Almarshady, L. Z. (2009). Spectrophotometric determination of mefenamic acid in pharmaceutical preparations, *Asian J. Chem.*, **21, 217-226**.

Aman, T.; Kazi, A. ; Mateen, B.(2005). p- (dimethylamino) benzaldelyde as a new chromogenic reagent for the determination of nonsteroidal anti-inflammatory drug by first-order derivative spectrophotometery. *Anal. Lett.*, **38,1899-1912.**

Bassavaiah, K. ; Somashekar, B.C.(2007). Quantitation of rantidine in pharmaceuticals by titrimetry and spectrophotometry using dichromate as the oxidimetric reagent. *J. Iranian Chem. Soc.*, **4, 78-88**

British Pharmacopeia(.2005). 1001p.

Chao, T.C.; Yao.Ong., Y. S. E ; Koh, T. (1997). Acid and neutral drug screen in blood with quantitation using microbore high performance liquid chromatography- diod array detection and capillary gas chromatography-flame ionization detection. *For. Sci. Intern.*, **90, 205-214.**

Dinc-E; Yucesoy, C. ; Onnr, F.(2002). Simultaneous spectrophotometric determination of mefenamic acid and paracetamol in pharmaceutical preparation using ratio spectra derivative spectrophotometric and chemometric methods. *J. Pharm. and Biomed. Anal.*, **28,1091-1100**.

Gangwal, S. ; Sharma, A. (1996). Simultaneous spectrophotometric determination of mefenamic acid and paracetamol in combined pharmaceutical dosage forms. *Indian J.. Pharm. Sci*. **58, 216-218.**

Hirai, T.; Metsumoto, S. ; Kishi, I.(1997). Simultaneos analysis of several non-steroidal anti-inflammatory drugs in human urine by hplc with normal solid-phase extraction. *J. Chromatogr. B*, ,**692, 375-388.**

Idown, S.; Tambo, S.; Adegoke, A. ; Olaniyi, A.(2002). Novel colorimetric assay of mefenamic acid using 4-amino-3,5-dinitrobenzoic acid. *Trop. J. Pharm. Res.*, **1, 15-22.**

Marczenko, Z. (1976). Spectrophotometric Determination of Elements. *Ellis worwood limited, Chicheaster***, 309-312.**

Martindale, (1999)." The Complete Drug Reference". The Pharmaceutical Press, London, 51 p.

Perrin, D. D. ; Dempsey, B.(1974). "Buffer for pH and Metal Ion Control". Champan and Hall Ltd, London. pp. 132-136.

Remington, (1995). "The Science and Practice of Pharmacy", Mack Publishing Company. Easton Peunsylvania, Vol. 2, 1215 p.

Sastry, C. ; Rao, A., (1995). Spectrophotometric method for the determination of pentazocine and mefenamic acid. *Indian J. .Pharm. Sci.*, **1987, 49, 95-96.**

Sastry, P., Prasad, R. ; Suryanarayana, V. (1989). Extractive spectrophotometric determination of some anti-inflammatory agents with methylene blue. *Analyst*, **114, 513-515**.

Sastry, S.P. ; Rao, A. R. (1989). Spectrophtometric determination of some analgesic and anti-inflammatory agents with 3-methyl-2-benzothiazone hydrazone hydrochloride. *Microchim. Acta* **, 97, 237-244**.

Tabrizi, A. B.(2006). Determination of mefenamic acid in human urin by means of two spectroscopic methods by using cloud point extraction methodology as a tool for treatment of samples. *Bull Korean Chem*. **soc. 27, 1780-1784**.

"The Pharmaceutical Codex", London, (1979). 520 p.

Zhang, W.Y. ; Wan, L.1, (1998). Efficacy of minor analgesics in primary dysmenorrhea. asystematic review. *Brit. J. Obst. and Gynaec.*, **105,780-789.**

Zommer, S. ; Bojarowicz, H. (1986). Spectrophotometric investigations on protolytic equilibria of mefenamic acid and determinations by means of fe (iii) in methanol-aqueous media. *J. Pharm. Biomed. Anal.*, **4, 475-481.**