A comparative study of the determination of Metoclopramide.HCL in pharmaceutical formulations by Flow injection Spectrophotometric and Reverse phase-High performance liquid chromatography RP-HPLC methods .

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Abstract

This study involves development of a new flow injection spectrophotometric method and a reverse phase-high performance liquid chromatography RP-HPLC method for the determination of Metoclopramide.HCl in aqueous solution and in pharmaceutical preparations.

FIA method was based on the oxidative coupling reaction of Metoclopramide.HCl with phenothiazine in the presence of ferric nitrate in neutral medium to form a green water soluble dye that is stable and has a maximum absorbance at λ_{max} 600 nm. A linear calibration graph was in the range of 2.5-100 $\mu g.ml^{-1}$, with a correlation coefficient of 0.9998, detection limit of 1.2 $\mu g.ml^{-1}$ and a relative standard deviation of 0.62%. The nature of the coloured produced dye was determined and found to be 1:1. The method was applied successfully for the determination of Metoclopramide.HCl in pharmaceutical preparations (mean recovery 99.85-101.00%).

In HPLC method, the drug was analyzed using RP-HPLC method with a Supelco BDS-C_{18} -DS (25 cm x 46 mm i.d) analytical column (5 μm particle size) and isocratic elution with a mobile phase containing 50% acetonitrile in 0.05 M KH_2PO_4 buffer (pH 5.5), at a flow rate of 1 $ml.min^{-1}$, 20 μL sample loop, and the UV detector was set at λ_{max} 273 nm. Calibration graph was in the range of 0.05–0.75 $\mu g.ml^{-1}$ with a correlation coefficient of 0.9998, and a relative standard deviation of

2.37%. The method was applied successfully for the determination of Metoclopramide.HCl in pharmaceutical preparations with a recovery of 98.78% - 99.35%.

االخلاصة

تضمنت هذه الدراسة على جزئين أساسيين ضم الجزء الأول تطوير طريقة طيفية باستخدام تقنية الحقن الجرياني، لتقدير دواء الميتوكلوبرامايد هيدروكلورايد تستند على تفاعل الازدواج التأكسدي للعقار المذكور مع كاشف الفيناثيازين بوجود نترات الحديديك المائية كمادة مؤكسدة. إذ تم الحصول على صيغة خضراء ذائبة في الماء أعطت أعلى امتصاص لها عند الطول الموجي 600 نانوميتر. وكان مدى الخطية بين 25-100 مايكروغرام/ مل ومعامل الارتباط 0.9998. وحد كشف مقداره 1.2 مايكروغرام/ مل. كذلك تم دراسة طبيعة الصيغة الناتجة بطريقة النسبة المولية ووجد ان نسبة الارتباط كانت 1:1 ميتوكلوبرامايد:فينوثيازين أما قيم الانحراف القياسي النسبي المئوي فكان 0.62%. طبقت الطريقة بنجاح على المستحضرات الصيدلانية الحاوية على مادة ميتاكلوبرومايد. هيدروكلوريد وبلغ الاسترداد النسبي 99.89 – 101.00%.

أما في الجزء الثاني فتم تطوير طريقة كروماتوغرافية جديدة لتقدير العقار المذكور أعلاه باستخدام طريقة كروماتوغرافيا السائل العالي الأداء الطور العكوس RP-HPLC باستعمال عمود جديد لم يسبق استخدامه وهو من نوع Spleco (25 cm x 46 nm) BDS-C_{18} محشو بدقائق ذات أقطار 5 مايكروميتر بازاحة ايزوكراتيكية Isocratic elution وبطور متحرك مكون من مزيج يحتوي على 50% أسيتونتريل ومحلول بفر بتركيز 0.05 مولاري من KH_2PO_4 ذو دالة حامضية=5.5 وبسرعة جريان قدرها 1 مل/ دقيقة وحجم مادة محقنة 20 مايكروليتر وبدرجة حرارة 33°م وباستخدام مكشاف المنطقة فوق البنفسجية والمرئية عند الطول الموجي 273 نانوميتر. وكانت الحدود الخطية للطريقة تتراوح بين 0.05–0.75 مايكروغرام/مل وبمعامل ارتباط 0.9998 وانحراف قياسي نسبي مئوي مقداره 2.37% وحد كشف 0.7 نانوغرام/مل. طبقت الطريقة بنجاح على مستحضرات صيدلانية متوفرة تجارياً حاوية على الميتوكلوبرامايد هيدروكلورايد وكان الاسترداد المئوي 98.78 – 99.3%.

Introduction

Metoclopramide.HCl.(l).4-amino-5-chloro-N-[(2-diethylamino)ethyl]-2-methoxy benzamide.hydrochloride[1].is A white crystalline powder, very soluble in water and freely soluble in alcohol, it melts at about 183°C [2]. Many methods have been developed for the determination of Metoclopramide.HCl in various matrices such as pharmaceutical formulation, blood, urine and aqueous solutions It has been colorimetrically determined in tablets, injection and syrups through its reaction with HNO_3 to form a yellow nitroso compound that has a max absorption at 375 nm[3]. Atomic absorption and UV-VIS spectrophotometer have been used for the determination of (I) by forming a stable ion pair with ammonium

thiocyanatocobaltate which had an absorption max at 625 nm, the extracted product was employed for indirect determination of (I) by estimating cobalt in the organic phase using flameless AAS [4]. Emmanuel and Yegyanayanan[5] determined (I) colorimetrically through its reaction with citric acid acetic anhydride to form a blue complex which shows max absorption at 600 nm. Bhatkar and Etal[6]. determined (I) by precipitation with ammonium reineckate, the ppt, was dissolved in acetone

Forming a dye product that has a max absorption at 526 nm. Verbiese[7] and Hanoca employed HPLC technique for the determination and identification of (I) in pharmaceutical preparations. Riley[8]. used HPLC for the determination of (I) in human plasmas by reversed phase ion-pair. Albani etal [9], determined (I) using liquid chromatography with fluorescence detector in cirrhotioc patients. Also ion selective electrodes has been used for the determination of (I) in the presence of large organic and inorganic materials [10-11].

Oxidative coupling organic reaction have recently been used for the determination of many drugs such as L. DOPA[12], 4-Amino antipyrine[13],, folic acid[14] and etc. The present paper describe a flow injection Spectrophotometric method for the determination of Metoclopramide.HCl in pharmaceutical preparations based on the oxidative coupling reaction with phenothiazine in the presence of ferric nitrate as oxidizing agent to form a green-water-soluble dye which can be measured at 600 nm. The proposed method was applied successfully to pharmaceutical preparations.The proposed FIA-spectrophotometric method was compared succssfuly with newRP-HPLC method and were applied for the analysis of pharmaceutical preparations samples containing metoclopramide.HCl.

2. Experimental

FIA Apparatus

Shimadzu 120 UV-VIS spectrophotometer equipped with a (cecil) 50 μL flow cell was used. A Shimadzu 1650 PC UV-VIS double beam spectrophotometer was used for $\lambda_{max.}$ determination. A two-channel manifold (Fig. 1) was employed for the FIA Spectrophotometric determination of Metoclopramide.HCl. A peristaltic pump (Gilsason minipuls (2)) was used to transport the carrier solution equipped with flexible polyvinyl chloride tubes of 0.8 mm internal

diameter. Injection valve (Rheodyne-USA) was employed to provide appropriate injection volumes of standard solutions and samples. Channel A in the Manifold was used to transport phenothiazine while channel B to transport ferric nitrate solution served as oxidizing agent. The sample was injected into the ferric nitrate stream. The reaction coil of 150 cm length with 0.5 mm internal diameter was used in the flow injection to ensure a good mixing between the drug and oxidant.

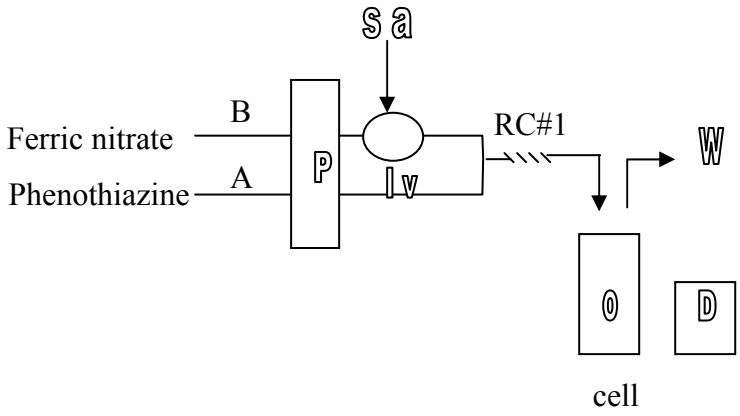


Fig (1): Manifold employed for FI Spectrophotometric determination of Metoclopramide with phenothiazine

IV. Injection value.
P: peristaltic pump
R: Reaction coil
Sx : sample
D: detector
W: west
C: Flow cell

HPLC Apparatus

The analysis was performed on a Shimadzu HPLC (Tokyo-Japan). Two solvent reservoirs of about 500 ml capacity round bottle were used. Two groups Model – (LC-6A Shimadzu), high performance pumps (pressure range 0 – 500 $kg.cm^{-1}$), which delivered the mobile-phase (A) and (B) from solvent reservoirs to the mixing cell. (Rcrheodyne 7125 USA) injection valve fitted with 20 μL sample loop. Separation of drugs were carried out on a (25 cm x 46 mm i.d) stainless steel, (5 μm particle size) BDS-C_{18}-DB, reversed-phase deactivated base column provided from Supelco

Reagent

Analytical reagent grade and deionized water were used throughout the work. The Metoclopramide.HCl stock solution (1000 μg/ml) was prepared by dissolving 0.100 g Metoclopramide.HCl in 100 ml of deionized water. The phenothiazine standard solution (5 x 10^{-4} M) was prepared by dissolving (0.0996 g) in one liter of ethanol. Stock solution of (0.01 M) ferric nitrate was prepared by dissolving 0.4040gm of hydrous ferric nitrate in deionized water containing 2.5 ml of 1 M Nitric acid and the solution was made up to 250 ml with deionized water. Potassium dihydrogen phosphate solution (50 mM) for HPLC analysis using potassium dihydrogen phosphate which was prepared by dissolving 3.400 g of pure material in 500 ml of deionized water. Ethanol, C_2H_6O, 96-98%, Fluka . Methanol, CH_3OH, HPLC grade, Analar.Acetonitrile, CH_3CN, HPLC grade.

Pharmaceutical preparation

Mecloden tablets: provided from (SDI) Samara – Iraq**.**

Ten tablets were grinded well and a certain portion of the final fine powder was accurately weighed to give an equivalent to about 10 mg of Metoclopramide.HCl. And was dissolved in deionized water The solution was filtered by using a Whatmann filter paper No. 42 to avoid any suspended particles. The prepared solution was transfer to a 100 ml volumetric flask and made up to the mark with deionized water forming a solution of 100 $\mu g.ml^{-1}$ concentrations.

Voperan Syrup: provided from Al-Razi Lab. Syria. Each 100 ml contains 100 mg of Metoclopramide.HCl. A solution of drug was prepared by diluting 10 ml of the syrup to 100 ml with deionized water to obtain 100 $\mu g.ml^{-1}$.

General procedure for FIA

100 μl sample of Metoclopramide.HCl was injected into a stream of 1x10-^{2}M ferric nitrate and allowed to react with a stream of 5x10^{-4}M phenothiazine at a PTFE -T-piece. The reaction was carried out by passing the mixture through a 150cm reaction coil. The absorbance of the resulting product was measured at λ_{max} 600 nm .All stream are pumped at an individual flow rate of 1.6 $ml.min^{-1}$.

Results and discussion

PART(1): FIA Method for determination of Metoclopramide in pharmaceutical formulations

It was found that the reaction of Metoclopramide.HCl with phenothiazine in the presence of ferric nitrite produced a highly coloured green water soluble dye[15] that has a maximum absorption at λ_{max} 600 nm (Fig. 2). The absorbance is directly related with the concentration of Metoclopramide.HCl and can be utilized for its determination usig FIA-spectrophotmetric system. . Initial studies were started with initial parameters given in Table (1).

Table(1): Initial Experimental Chemical and Physical Conditions

	Preliminary parameter	Value
1.	Conc. of phenothiazine	1×10^{-4} M
2.	Conc. of ferric nitrate	1×10^{-2} M
3.	Flow rate of phenothiazine	1 $ml.min^{-1}$
4.	Flow rate of ferric nitrate	1 $ml.min^{-1}$
5.	Reaction coil length	100 cm
6.	Injected sample volume	50 µL
7.	Conc. of sample injected	50 $\mu g.ml^{-1}$
8.	Temperature	Room temp. 25°C

* All reagents must be degassed before use.

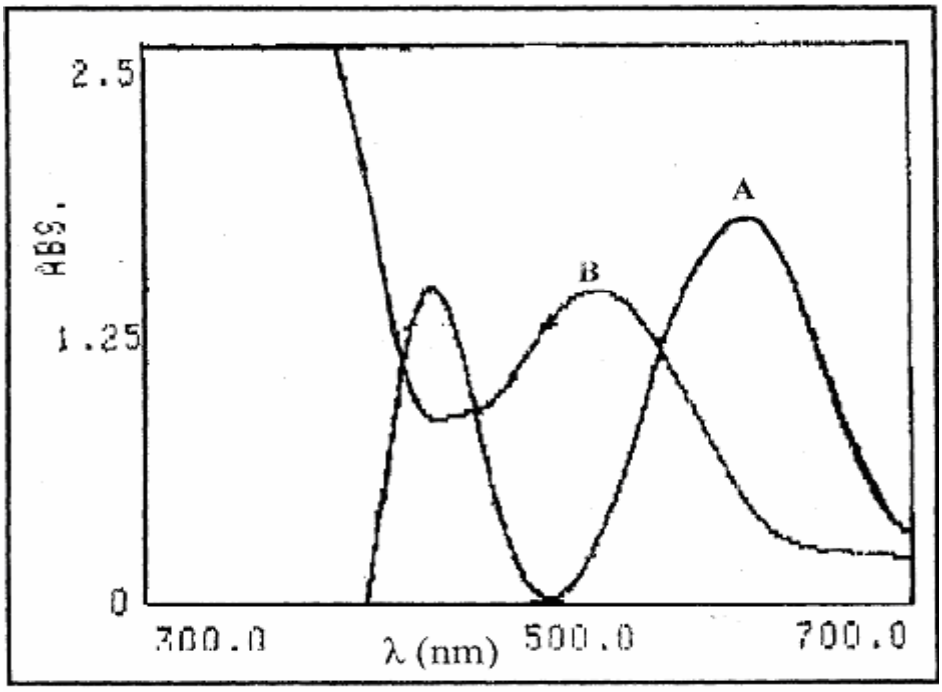


Fig. (2): Absorption spectra of A (40 µg.ml^{-1}) of Metoclopramide.HCl treated as described under procedure and measured against a reagent blank and B the reagent blank measured against distilled water.

The development of the color product depends on the reaction conditions and were optimized as follows:

Effect of the phenothiazine concentration:

The effect of various concentrations of phenothiazine was investigated. A concentration of $5x10^{-4}$ M gave the highest absorbance and was chosen for further use. Results obtained are shown in Fig. (3).

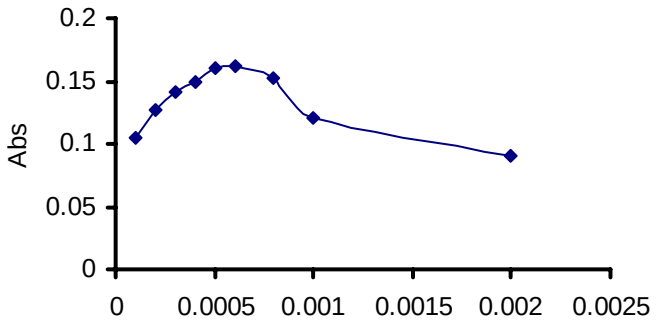


fig (3): effect of phenothiazine concentration on the coloured reaction product

Effect of the oxidizing agent concentration

The reaction between Metoclopramide.HCl and phenothiazine depends on the oxidation process with ferric nitrate. The effect of different concentration of ferric nitrate was investigated. A concentration of $1x10^{-2}$M gave the highest absorbance and was chosen for further use as shown in Fig. (4).

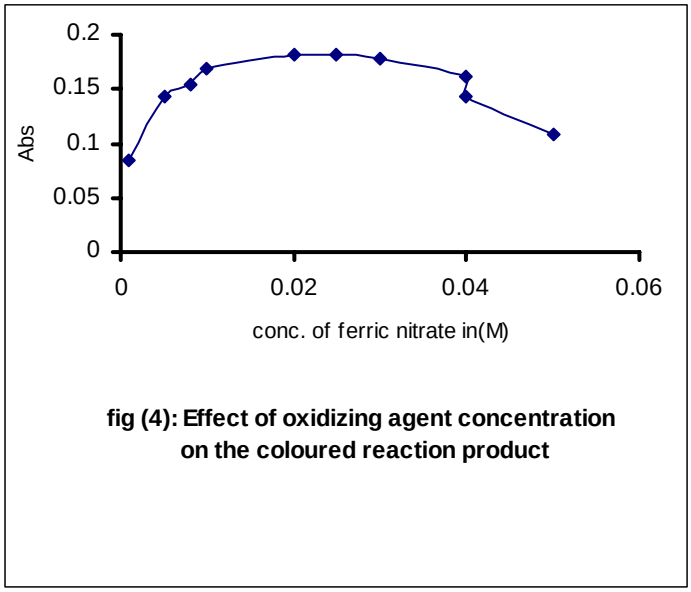
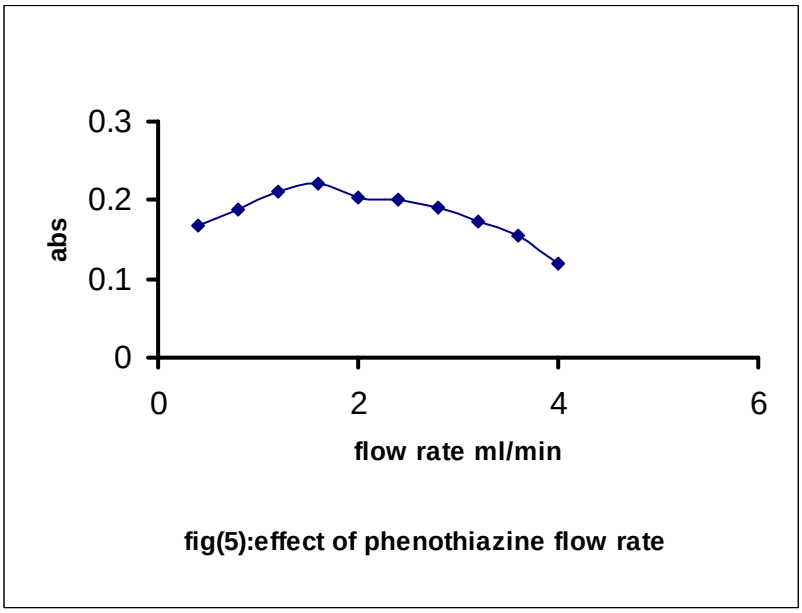


fig (4): Effect of oxidizing agent concentration on the coloured reaction product

Effect of phenothiazine flow rate:

Flow rate is an essential parameter and can be controlled by peristaltic pump. The effect of phenothiazine solution flow rate on the sensitivity of color product was studied keeping all other conditions constant. The result obtained showed that a flow rate of 1.6 ml.min^{-1} gave the highest absorbance as shown in Fig. (5).



fig(5):effect of phenothiazine flow rate

Effect of the oxidizing agent flow rate:

The effect of ferric nitrate flow rate on the sensitivity of the colored reaction product was similarly studied in the range of (0.4 – 4.0) ml.min^{-1}. The result obtained showed that a flow rate of 1.6 ml.min^{-1} gave the best result as shown in Fig. (6).

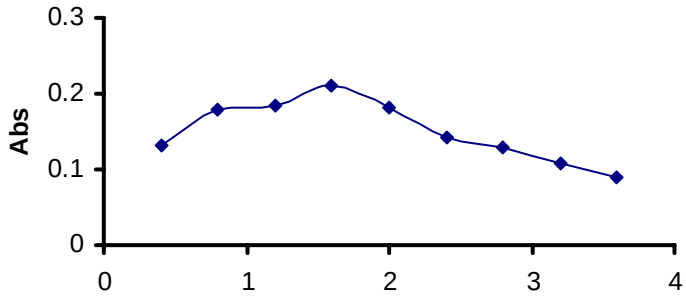


fig (6): Effect of oxidizing agent flow rate on the sensitivity of the coloured reaction product

Effect of the reaction coil length

Coil length is also another important parameter for the reaction. Various reaction coil lengths (25, 50, 75, 100, 125, 150, 175, 200, 225, 250 cm) were investigated. A length of 150 cm gave the highest absorbance as shown in Fig. (7).

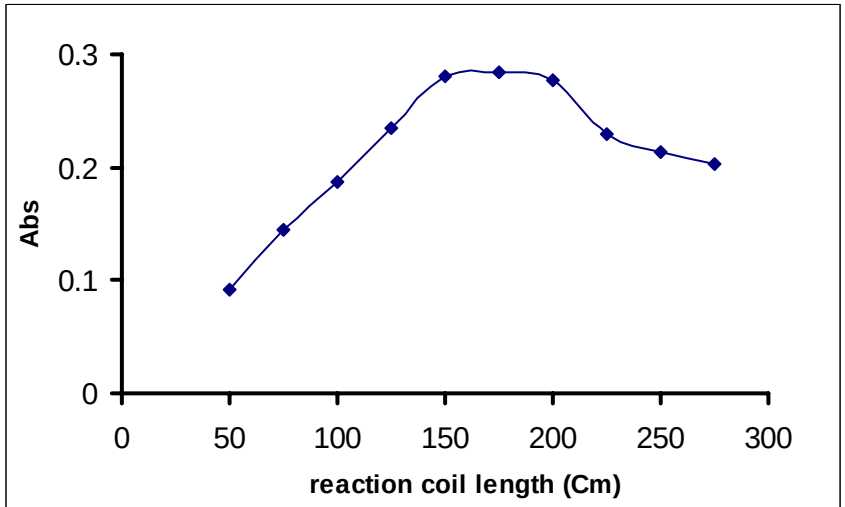
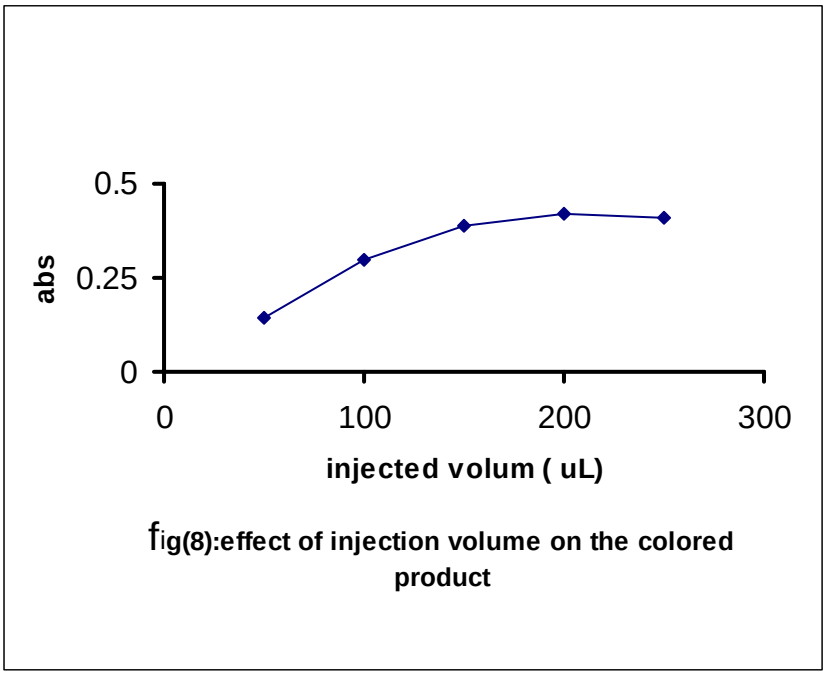


Fig. (7): Effect of reaction coil length on the absorbance of coloured reaction product

Effect of injected sample volume

A 50, 100, 150, 200 µl sample loops were tested. Fig. (8) represents the result obtained which indicate that a sample loop of 100 µl volume gave the best results,



fig(8):effect of injection volume on the colored product

Effect of temperature

Under the optimum conditions obtained, a sample of metoclopromide.HCl 25 µg.ml^{-} was injected in the FIA manifold [Fig. (1)] and the absorption was measured at different temperature. The results obtained indicated that measurments at room temperature gave the best sensitivity and minimum blank value (Table 2).

Table (2): Effect of temperature on the sensitivity of the coloured product

Temp. ^{o}C	Absorption *
Zero	0.141
Room temp. 25	0.189
45	0.151

* Each result is the average of three readings.

Recommended Analytical Conditions

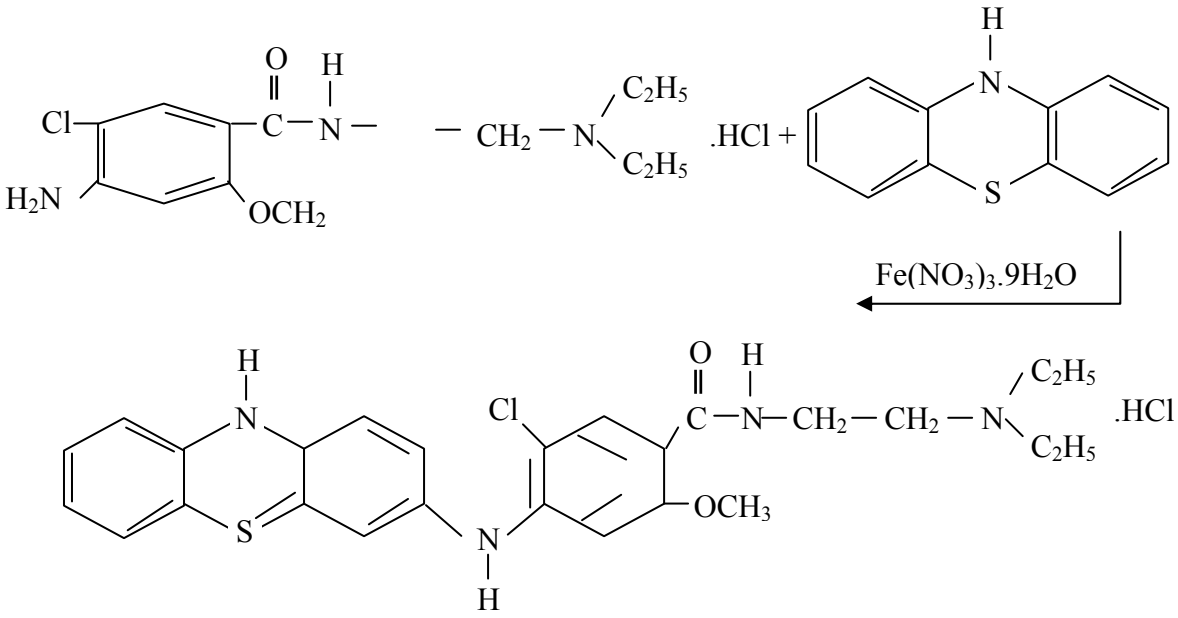
According to the results obtained previously the optimum conditions for the determination of Metoclopramide.HCl using FIA spectrophotometric method are given in Table (3).

Table (3): Optimum conditions for the determination of Metoclopramide.HCl \using FIA system

Parameters	Value
Conc. of phenothiazine	5×10^{-4} M
Conc. of ferric	1×10^{-2} M
Total flow rate	3.2 $ml.min^{-1}$
Reaction coil length	150 cm
Injected volume	100 µl
Temperature	25 C
Wave length	600 nm
Reaction tubing diameter	0.5 mm i.d.

Nature of the dye product

The stoichiometry of the reaction was investigated under the optimized conditions using a molar ratio method[16]. The results obtained Fig. (9) shows that a 1:1 drug to reagent was formed. The formation of the dye may probably occurs as:



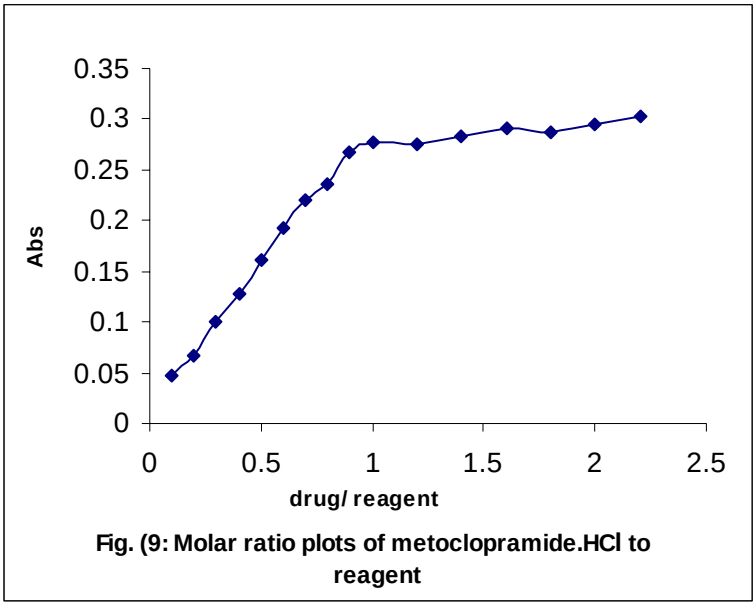


Fig. (9: Molar ratio plots of metoclopramide.HCl to reagent

Interference

Metoclopramide.HCl is usually formulated in a tablet, syrup and injection forms. Therefore, the effects of some common excipients usually present in pharmaceutical preparation were investigated. The presence of 100 $\mu g.ml^{-1}$ of starch, glucose, lactose and sucrose gave no significant interfering effect on the absorption of 10 $\mu g.ml^{-1}$. Metoclopramide.HCl.

Calibration data

A linear calibration graph for Metoclopramide.HCl Fig. (10) under the optimized conditions was obtained. Beer's Law is obeyed over the concentration range of 2.5-100ug.ml^{-1} with a correlation coefficient of 0.9997 and intercept of 0.0021. The relative standard deviation of the method was 0.62% for 100µg.ml^{-1} Metoclopramide.HCl, based on 10 replicate determinations. Table (4) shows the Analytical data for the determination of Metoclopramide.HCl using FIA system

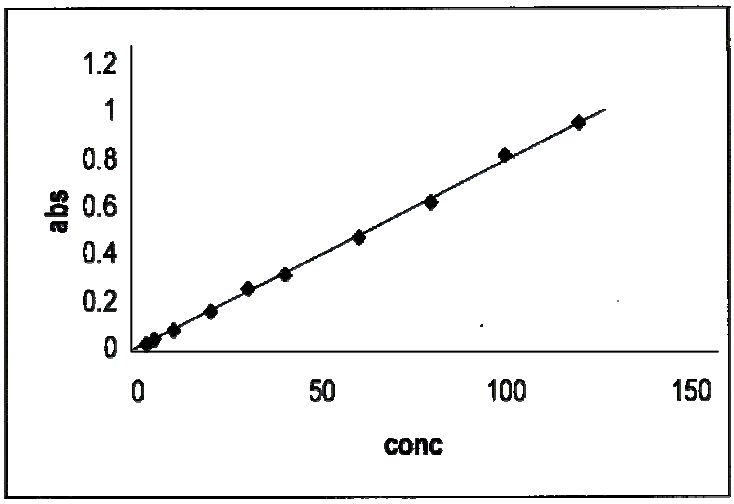


Fig. (10) Calibration graph for Metoclopramide.HCl

Y=0.0079X + 0.0021

R^2 =99966

Table (4): Analytical data for the determination of Metoclopramide.HCl using FIA system

Analytical data	Value
Detection limit (D.L.)	1.2 µg.ml^{-1}
Correlation coefficient (r)	0.9998
Linear range	2.5 – 100 µg.ml^{-1}
RSD%	0.62%
$\in_{max}$	1.64 x 10^4 L.mol^{-1}.cm^{-1}

Pharmaceutical applications

The proposed method was applied for the determination of Metoclopramide.HCl in tablets and syrup. Good precision and recovery were obtained. The method was successfully comparable with the British Pharmacopoeia standard

method and (U.S.P standard method). The results obtained are summarized in Table (5).

Table (5): Application of the proposed method for the determination of Metoclopramide.HCl in Pharmaceutical preparations.

Sample	Recovery		RSD% *
	proposed method	Standard method	
Pure Metoclopramide	99.85	99.2	0.59
Mecloden tablets	100.50	100.3	0.62
Voperan syrup	101.00	101.7	0.63

* Each result is the average of three determinations

PART(2): Determination of Metoclopramide.HCl Using a New RP-HPLC-Method

The determination of the Metoclopramide.HCl by RP HPLC is based on the isocratic elution (i.e. separation in which the mobile phase composition remains unchanged) of the species on a BDS-RP-DS column.

Optimization of Experimental Conditions

Effect of Different Percentage of Organic Modifier in the Mobile Phase Generally, polar eluents or mixtures of polar solvents were used as a mobile phase in RP-HPLC. Two organic modifiers are commonly used in RP-HPLC, these are MeOH and MeCN.. In this work a mixture with different percentage of acetonitrile (MeCN) [10,25,50,75,90]% and a potassium dihydrogen phosphate (KH_2PO_4) buffer (0.05 M) of pH 5.5, were used as a mobile phases. A best sensitivity, high peak symmetry and reasonable analysis time were obtained at 50% of acetonitrile Fig. (11) show the results obtained and 50% MeCN was chosen for subsequent work.

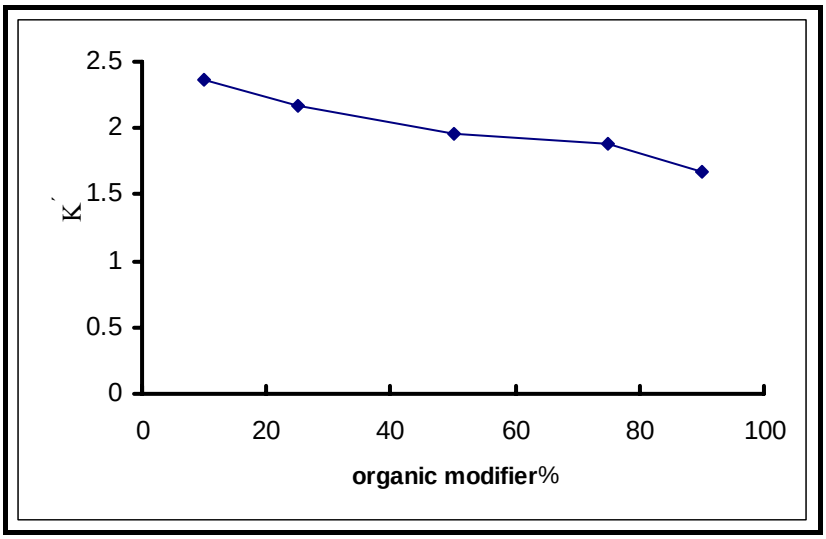


Fig. (11): Plots of capacity factor K' of drug versus different percentage of organic modifier mixed with 0.05 M KH_2PO_4 buffer.

Effect of pH using 0.05M Potassium Dihydrogen Phoasphate Buffer

In general the t_R value of each species can be correlated with the values of pKa of the solute molecule. To investigate the effect of the pH of the mobile phase on the elution of the studied compound, the pH of the mobile phase was varied from 4.0 to 7.0 with interval of 0.5. Fig. (12) show that there were no significant difference in values of t_R & K' observed, but the peak symmetry and the peak height were the best at pH range between 4.0–5.5. A pH value of 5.5 was chosen for subsequent work, since at this value no further adjustment of the pH of the mobile phase was needed during preparation.

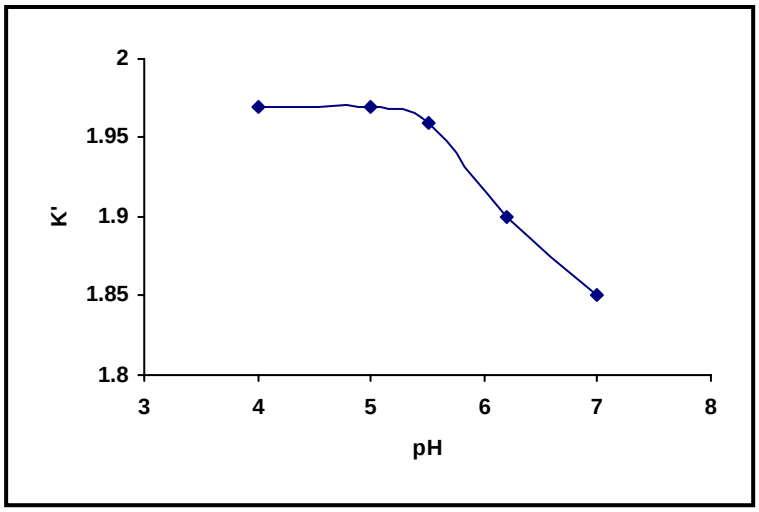


Fig. (12) Plots of capacity factor K' versus pH using 0.05M KH_2PO_4 buffer.

Effect of Flow rate of the mobile phase

The aim of choosing optimum flow rate is to perform the analysis in a short time with a reasonable sensitivity and peak symmetry and preventing any band diffusion which finally leads to high column efficiency. [162]. A change in mobile phase flow rate from 0.6 to 2.0 ml.min^{-1} caused a decrease in analysis time from 6.2 minutes to 1.72 minutes. Fig. (13) show the effect of flow rate on the retention time.

The result obtained indicated that the suitable flow rate of the mobile phase for separation of the studied drugs is 1.0 ml.min^{-1}.

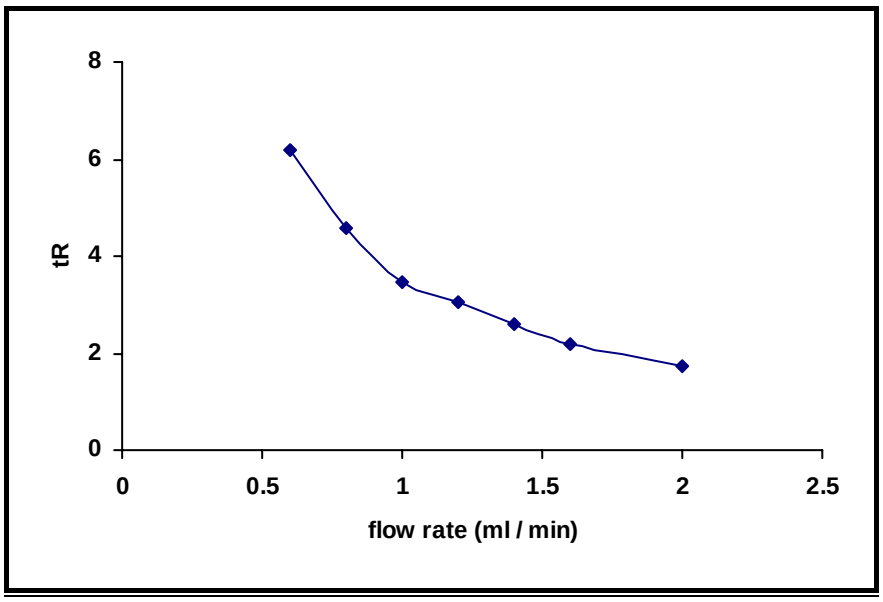


Fig. (13): Plot of t_R of drug against flow rate (0.6–2.0 ml.min^{-1}) of the mobile phase.

Effect of Column Temperature

Generally, increasing column temperature in RP-Chromatography decrease the retention time of separation bands and increasing column efficiency by decreasing mobile phase viscosity, which is in turn lowering the column head pressure. The effect of column temperature in the range of 25 to 53^{o}C on the retention time, asymmetry of the peak and the sensitivity of the drug under study was investigated.The relation between lnK′ and 1/T was plotted, and the plot obtained Fig. (14) shows that K' of drug under study decrease with increasing column

temperature and the optimum column temperature chosen to be 33°C.

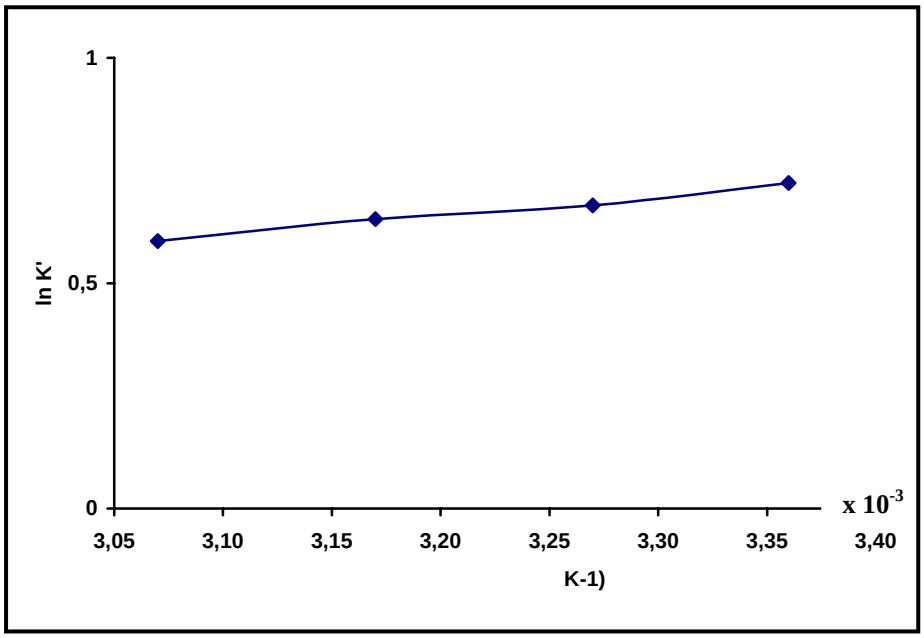


Fig. (14): Plot of ln K' of drug against 1/T

Recommended Analytical Conditions

According to the results obtained previously the optimum experimental conditions established for the reversed-phase HPLC determination of Metoclopramide-HCl in pharmaceutical preparations are given and summarized in Table (6), Figure (15) illustrates the shape and retention time of the separated signal of Metoclopramide-HCl.

Table (6) The recommended analytical conditions for the determination of Metoclopramide.HCl using RP-HPLC system.

Parameter	Recommended value
Column	BDS-RP-DS (25 cm x 46 mm i.d)
Organic modifier	50% Acetonitrile
Injected sample volume	20 µl
Buffer	0.05 M $KHPO_4$
pH	5.5
Flow rate	1.0 $ml.min^{-1}$
Column temperature	33°C
Detector	U.V detector at 273 nm

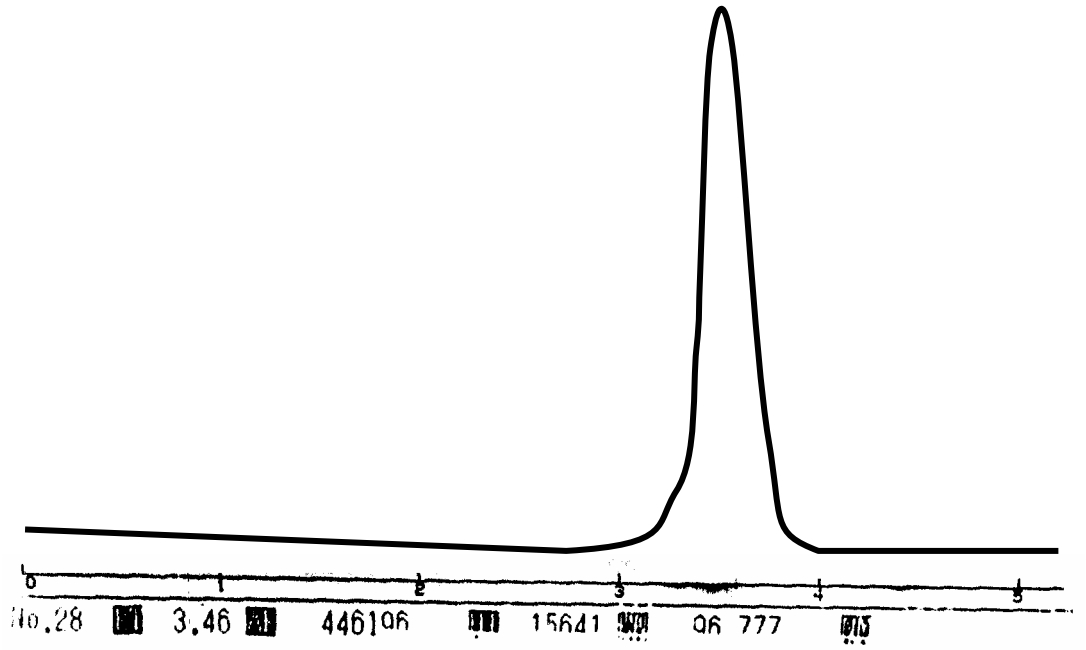


Fig (15): shape and retention time of the separated signal of Metoclopramide.HCl

Calibration Graph

The recommended analytical conditions [Table (6)] were used to construct of Metoclopramide.HCl calibration graph by plotting the concentration ($\mu g.ml^{-1}$) of drug against the peak area. A linear calibration graph for the determination of Metoclopramide-HCl was obtained in the range of 0.05–1.0 $\mu g.ml^{-1}$ Fig. (16). The analytical data obtained from the calibration graph are summarized in Table (7). The linear regression equation for the range of 0.05–1.0 $\mu g.ml^{-1}$ of Metoclopramide-HCl is: $Y = 387700\ X - 2.5$

Table (7): Analytical data for the determination of Metoclopramide-HCl

Analytical data	Value
Detection limit (D.L)	0.7 $ng.ml^{-1}$
Correlation coefficient (r)	0.9998
Linear range	0.05 – 1 $\mu g.ml^{-1}$
Average RSD %	2.37%

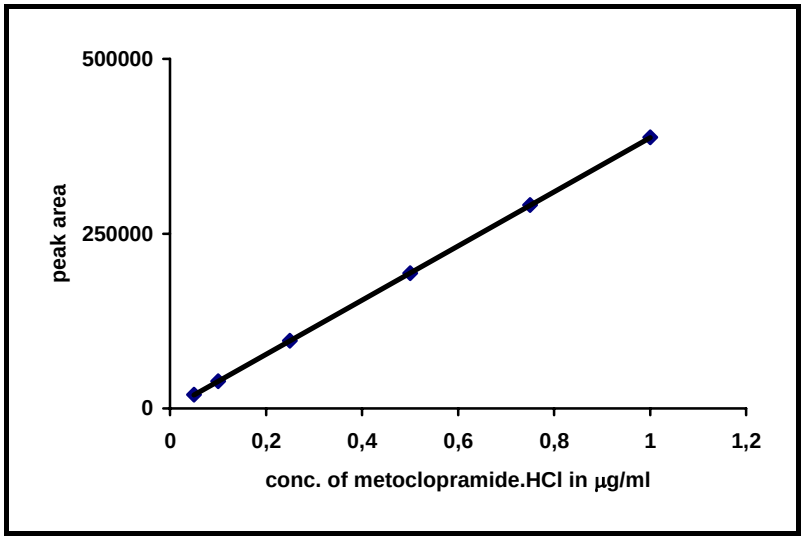


Fig. (16): Calibration graph for the determined of MetoclopramideHCl

Application of the Developed RP-HPLC Method for the Determination of Metoclopramide.HCl in Some Pharmaceutical Preparations

The analysis of drug in pharmaceutical preparations become one the most important applications of modern RP-HPLC, moreover, the combination of HPLC with UV-Visible detection provides an accurate, precise and robust method for quantitative analysis of pharmaceutical product.

Two types of Tablets containing Metoclopramide.HCl, were analyzed using the developed method and the results compared with the British Pharmacopoeia standard method. The results obtained are shown in Table (8).

Table (8): Application of the proposed method for the determination of Metoclopramide-HCl in pharmaceutical preparations

Sample	Recovery %		RSD %
	Proposed	Standard	
Pure Metoclopramide.HCl	99.2	99.2	2.37
Mecloden Tablets	98.78	100.3	2.34
Meclopam Tablets	99.35	99.5	2.18

Comparison the two methods

The two proposed methods was compared as shows in table9.

Table(9):The statistical comparison of results in the FI spectrophotometric and PLC method

The method	Regression equation	Linearty (ug/ml)	Correlation coefficient (r)	Intercept	Recovery%	RSD%
FIspectrophotometric	Y=0.0079X+0.0021	2.5-100	0.9998	0.0021	99.85-101	0.62
HPLC	Y=387700X-2.5	0.05-1.0	0.9998	2.5	99.2-100.3	2.37

Conclusion

In part(1) A simple, accurate and sensitive FI Spectrophotometric method for the determination of Metoclopramide.HCl in pharmaceutical preparation has been developed. The proposed method can be carried out at room temperature with no need for solvent extraction step or pH control.

Part(2) include a simple and sensitive high pressure liquid chromatographic (HPLC) method for the determination of metoclopramide.HCl in pharmaceutical preparations.

References

1. " British Pharmacopoeia on CD-ROM", 3rd Ed., Copyright by System Simulation Ltd., The Stationary Office, London, (2000).
2. " The Merck Index on CD-ROM", 12th ED., Copyright by Merck Co., Inc., Witheeho, (2000).
3. Shingbal. D. and Naik, S.D. ***Indian Drugx***, 1981, **18 (19)**, 441.
4. Park, Manki; Lim, Byung Ryun; YU, Kynrg Soo; and Yong, Kunko, ***Yakhan Heo Chi***, 1978, **22 (1)**, 27.
5. Emmanuel, J. and Yegyanaranan, T.V., ***East, Pharm.***, 1981, **24 (258)**, 133.
6. Bhatkar., and Chadankar, S.K. ***East. Phar.***, 1981, **24 (279)**, 125.
7. VEVBIESE. N, Hanoca. M., ***International J. Pharm***, 1979, **2**, 155.
8. Riley. C.M, ***J. Pharm. Biomed, Anal***.; 1984, **2 (1)**, 81.
9. Guyon, F. Delfour. C, Delattre. C, ***Clin. Chem.***, 1987, **33(1)**, 190.
10. Albani, F., Riva, R., Contin, M; ***Biomed. Chromatography***, 1987, **2 (3)**, 135.
11. Budawy. S.S; Shoukry. A.F; Issa, Y.M; ***Analyst*** (London)., 1986, **111 (12)**, 1363.
12. BERWEEN A. HASAN, KARIM. D KHALAF, and MIGUEL DE LA GUARDIA, ***Tallanta***, 1995, **42(4)**, 627.
13. SADEEM, S. ABED; (1999). M.Sc Thesis. Baghdad Univ; College of Science.
14. Mouayed Q. Al-Abachi and Ragad. S; ***National J. Chemistry***; 2002, **7**, 363.

15. Mouayed Q. Al-Abachi., and Al-Ward.Hind, ***National Journal of Chemistry***, 2002, **7**, 363.
16. Calatayud J.M., P.C. Falco and M.C.P. Marti, ***Analyst***, 1986, **111**, 1317.