Identification and Estimation of Metoclopramide in Rat Blood by High Performance Liquid Chromatography

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ABSTRACT

Objectives: to evaluate a rapid and sensitive high performance liquid chromatographic method for the determination of metoclopramide in rat serum.

Methods: The assay was performed after liquid-liquid extraction with sodium hydroxide and dichloromethane.

Results and conclusion: Chromatographic separations were performed on $C_{١٨}$ stationary phase with a mobile phase composed of acetonitrile : ١% triethyleamine (٥٠:٥٠,v/v) at pH (٦.٨). Analytes were detected at wave length of ٢٧٠nm. This method was validated for specificity and linearity with a correlation coefficient, r=٠.٩٤.

Key words: metoclopramide, chromatographic method, HPLC, rat serum.

الخلاصة

الهدف : يوضح البحث سرعة و حساسية طريقة الاستشراب السائل عالي الاداء المنجزة لقياس تركيز الـ Metoclopramide في مصل الدم للجرذان.

الطريقة : التجربة أنجزت بعد عملية الاستخلاص بواسطة محلول مادتي هيدروكسيد الصوديوم و دايكلوروميثان للدواء من مصل الدم.

النتائج : تم الفصل بواسطة إستخدام الطور الثابت من نوع C١٨ و الطور المتحرك المتكون من المواد التالية أسيتونايتريل :1% تراي إثايل أمين بنسبة 50:50% بمعيارية مقدارها 6,8 ، وقد قيست العينات على الطول الموجي 270 نانوميتر لتحديد تركيزها. هذه الطريقة أثبتت دقتها و استقامة قياساتها بمعامل ارتباط مقداره 0,94.

وصف في هذا البحث طريقة سريعة و حساسة وهي طريقة الاستشراب السائل عالي الأداء لقياس تركيز دواء الـ Metoclopramide في مصل الدم للجرذان في المختبر.

Metoclopramide is an antiemetic and gastroprokinetic agent [١,٢]. It is commonly used to treat nausea and vomiting, to facilitate gastric emptying in people with gastroparesis, and as a treatment for the gastric stasis often associated with migraine headaches [٣]. It is also used as a preventive medicine for cancer chemotherapy -induced emesis at higher doses [٤,٥]. In animals is commonly used to prevent vomiting and also used as a gut stimulant [٦].

Due to the applications of metoclopramide in clinical and experimental medicines, many methods are available for its determination in biological fluids and dosage forms [٧]. Both the United States Pharmacopoeia (USP, ٢٠٠٠) [٨] and the British Pharmacopoeia (BP, ١٩٩٨) [٩] recommend a non aqueous acid-base titration with potentiometric detection of the end-point for the evaluation of the raw material of metoclopramide from its dosage forms. USP recommends HPLC method and the BP describes spectrophotometric method of analysis [٥].

Normal-phase HPLC using a silica column and reversed-phase (RP) HPLC or ion-pair HPLC methods on octadecyl column were described for the analysis of metoclopramide in biological fluids [١٠]. However, to reduce consumption of more organic solvents, and reducing long run time, a modified HPLC technique with manipulation of the conditions was applied.

The present study describes a sensitive, specific and rapid sample

preparation assay with short run time of ٦ minutes for determining metoclopramide in rat serum by using HPLC with no interference from it's metabolites.

Materials and methods

Chemicals and reagents

Purified free base of metoclopramide for research purposes was provided by NDI/Nenava Drug Industry/Iraq . All solvents used were HPLC grade, and all chemicals were analytical grade: HPLC-grade acetonitrile Scharlau/Spain, HPLC-grade triethylamine Scharlau/Spain and deionised water NDI/Iraq. Analytical grade sodium hydroxide and dichloromethane were from GCC company/UK.

Instrumentation

The analyses were carried out using a chromatographic system from Shimadzu Corporation (Japan). This instrument consisted of a pump, a UV-visible detector, a system controller, and a manual injector. Software was used to control the LC system and data acquisition.

Analyses of metoclopramide were performed at room temperature on a (GL Sciences Inc.) C١٨ column (٤.٦mm × ٢٥٠mm I.D.,٥µm particle size) under isocratic conditions using acetonitrile : ١% triethyleamine (٥٠:٥٠,v/v) as mobile phase at pH (٦.٨) and flow rate of ١.٢ mL/min. UV detector was operating at ٢٧٠nm. The mobile phase was filtered through a Millipore membrane filter (٠.٤٥µm)(Steril-R/USA) and degassed ultrasonically prior to use.

Preparation of stock solution and working standards

Stock solution of metoclopramide was freshly prepared in mobile phase solution at the concentration of (١٠mg/١٠mL). Working standards of metoclopramide were freshly prepared in the concentrations of (٠.١٢٥, ٠.٢٥, ٠.٥, ١, ٢µg/mL) and made by the dilution of the stock solution with mobile phase.

Animals and Sample preparation

Adult albino rats were used in this work that have been taken from animal house of the College of Veterinary Medicine, University of Mosul. This study was carried out on ٥ animals (male and female), their weights were between ٢٥٠-٣٥٠ g. The work was done at laboratory of the College of Veterinary Medicine, University of Mosul. One ml of blood samples were collected from healthy adult rats, not taking any kind of drug, in non heparinized glass tubes, then blood samples were collected from each animal after (١٥, ٣٠, ٦٠min) of administration of therapeutic dose of metoclopramide (٥mg/kg) was given by i.p. route to each animal [١,٦]. The blood samples were centrifuged at ٣٠٠٠rpm for ١٥min and the serum was frozen and stored at -٢٠°C, no longer than ٧٢h.

Extraction of the samples (liquid-liquid extraction) LLE

Serum (٢٠٠µL) was mixed with, sodium hydroxide ١M (٥٠µL) in a ١٠mL stoppered test tube and the tube was vortexed for aboute ٢٠ seconds. The solution was mixed with dichloromethane (٢mL), vortexed at high speed for ١min. The resultant mixture was centrifuged at ٤٠٠٠rpm for ٥min. The aqueous layer was aspirated to a waste and the organic layer was transferred to a clean tube. The tube containing the organic layer was placed in a water bath (٥٠°C) and evaporated to dryness. The residue was reconstituted in (١٠٠µL) of mobile phase prior to injection into the chromatograph for analysis [٦].

Chromatographic conditions

Several chromatographic conditions, such as mobile phase, type of column and its length, mobile phase pH, flow

rate, temperature and volume of injection were studied to obtain a satisfactory chromatographic separation (good resolution and efficacy) for the compound. In addition, the total time required for the analysis was also an important factor because the analysis could be unfeasible since interfering compounds could elute close to the metoclopramide, modifications were performed in order to reduce the analysis time.

Various solvents or mixture of solvents at different compositions were used to extract the metoclopramide from rat serum using LLE.

To optimize the HPLC parameters, one mobile phase composition was tried :
Acetonitrile : ١% Triethylamine (٥٠:٥٠,v/v) [٢].

The buffer solution : about ١٠mL of triethylamine was diluted to ١L with deionised water to obtaine ١% triethylamine buffer solution.

A mixture of Acetonitrile and ١% triethylamine in the ratio of ٥٠:٥٠%,v/v was prepared at pH (١١.٣). The peak of triethylamine interfered with the peak of metoclopramide and changing the flow rate did not separate between the two peaks but, adjusting the pH to ٦.٨ resulted in a typical peak for metoclopramide with a retention time ٢.٥min without interfering with any other compounds .

Results

A satisfactory separation and good peak symmetry was found in a mixture of acetonitrile : ١% triethylamine in the ratio of ٥٠:٥٠%,v/v at a pH (٦.٨) and flow rate of ١.٢mL/min. The optimum wavelength for detection was set at ٢٧٠nm at which much better detector response for drug was obtained. The retention time was ٢.٥min for metoclopramide and no interferences were observed in formulation sample, also with a better reproducibility.

Quantification was achieved with UV detection at ٢٧٠nm based on the peak area. Better resolution of the peaks with clear base line separation is found as shown in Table.١,Fig.١ and Fig.٢.

Table. ١ Optimized chromatographic conditions for estimation of metoclopramide

Mobile phase	Acetonitrile : ١% triethylamine ٥٠:٥٠%,v/v
Mobile phase pH	٦.٨
Pump mode	Isocratic
Diluent	Mobile phase
Column	GL Sciences Inc. C١٨ column(٤.٦ × ٢٥٠mm, ٥µm)
Column temp	Ambient
Wavelength	٢٧٠nm
Injection volume	٢٠ µL
Flow rate	١.٢mL/min
Run time	٦min
Retention time	٢.٥min

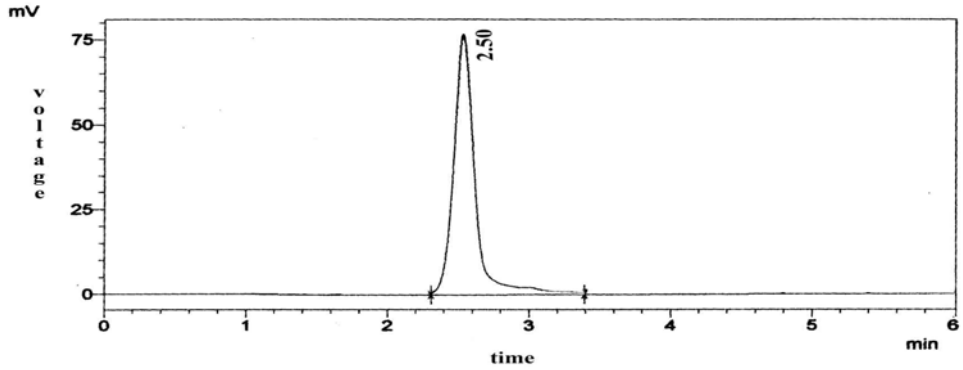

Figure.١ Chromatograms of validation of the method

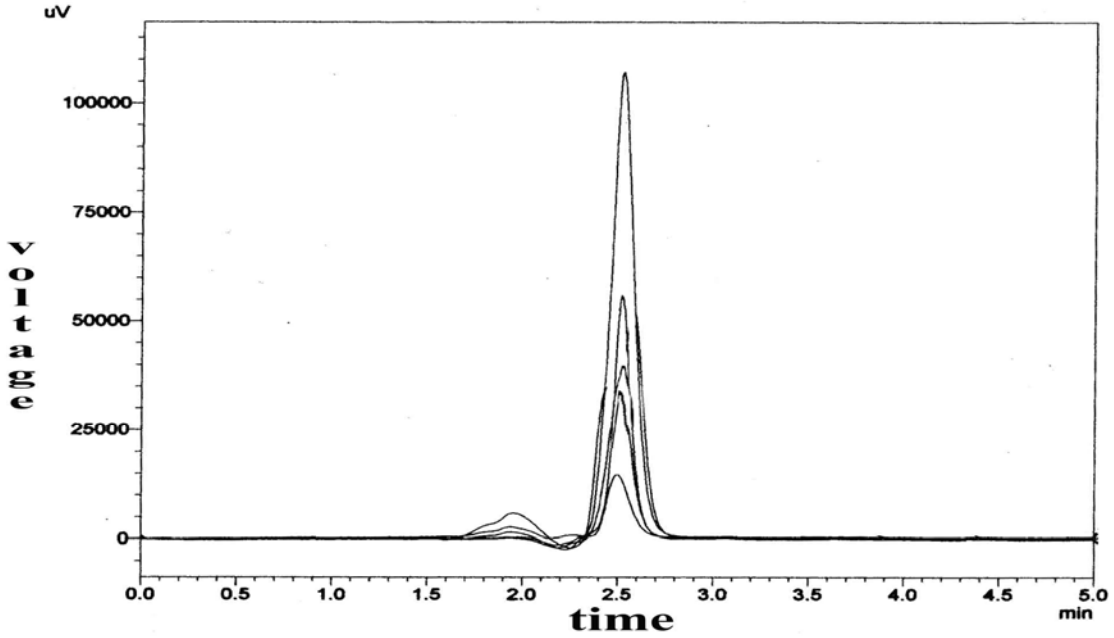

Figure.٢ Chromatograms of standards solution with different concentrations

Specificity

The specificity of method was performed by comparing the chromatograms of blank, standard and sample. It was found that there was no endogenous interference and also found good correlation (r=٠.٩٤) between the retention times of standard and sample are shown in Table.٢, Fig.٣, Fig.٤ and Fig.٥.

Table ٢:specificity study

Name of the solution	Retention time in Min
Blank	No peak
standard	٢.٥
sample	٢.٥

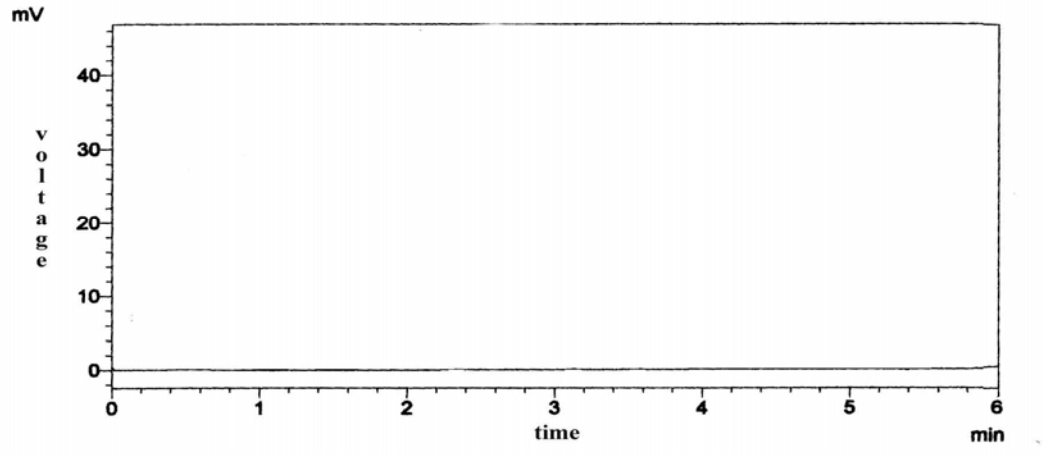

Figure.٣ Chromatogram of blank

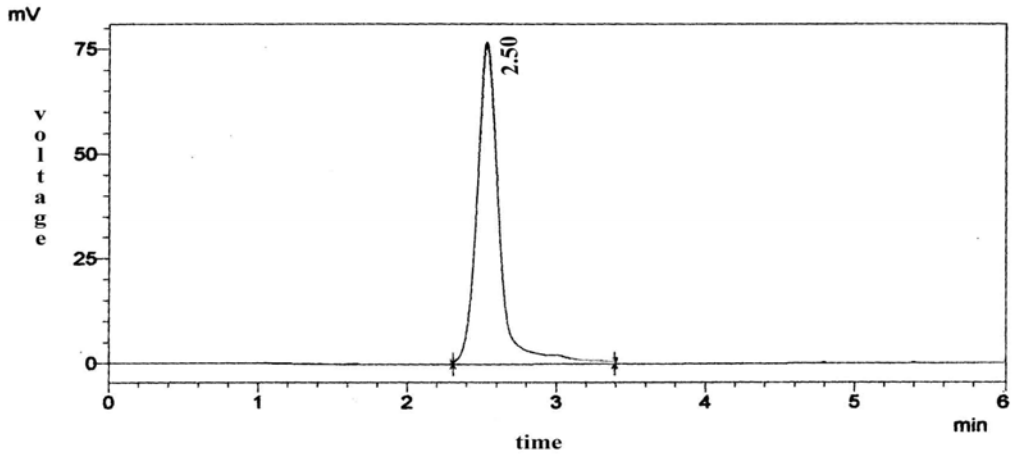

Figure.٤ Chromatogram of standard metoclopramide

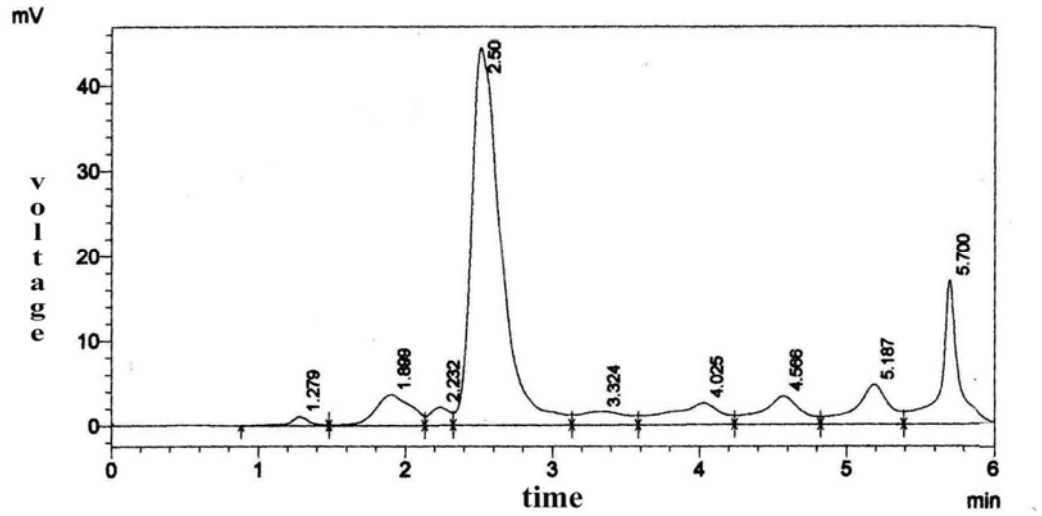

Figure.٥ Chromatogram of sample

Linearity

Linearity was performed by preparing standard solutions of metoclopramide at different concentration levels including working concentration mentioned above. Twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at ٢٧٠nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plot of concentrations over the mean peak areas were constructed. The regression of the plot was computed by least square regression method. Linearity results were presented in (Table.٣), calibration plot was shown in (Fig.٦) and calibration plots of the samples was shown in (Fig.٧).

Table.٣ Linearity Results

Level	Concentration of metoclopramide in (µg/mL)	Mean peak area (mV)
Level-١	٠.١٢٥	١٣٠٦٥٥.٥
Level-٢	٠.٢٥	٤٠٧٧٥٥
Level-٣	٠.٥	٤٣٣٣٠٠
Level-٤	١	٥٨٠٦٢٨.٥
Level-٥	٢	١٠٩٢٧٨١
Range:٠.١٢٥ to ٢	Slope Intercept Correlation coefficient	٢٤٩٠٦٧.٣ ٤٠٤٨٥١.٩ ٠.٩٤٤٧

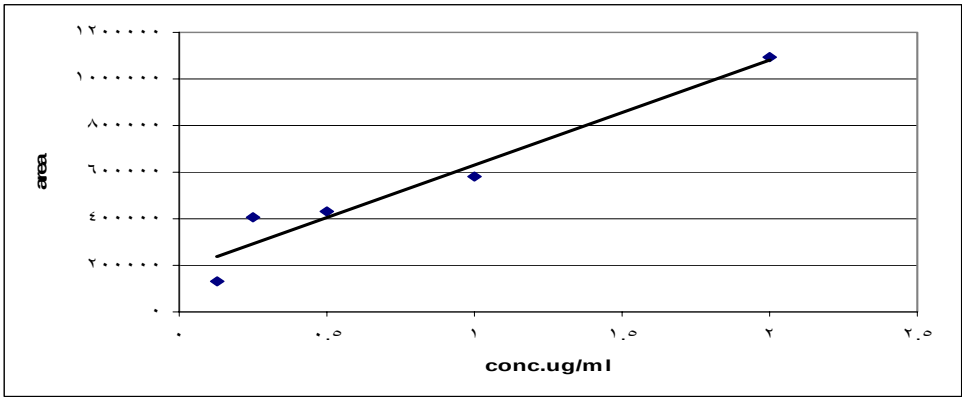

Figure. ٦ Calibration plot for metoclopramide on X axis concentration of sample (µg/mL) , on Y axis peak area response (mV)

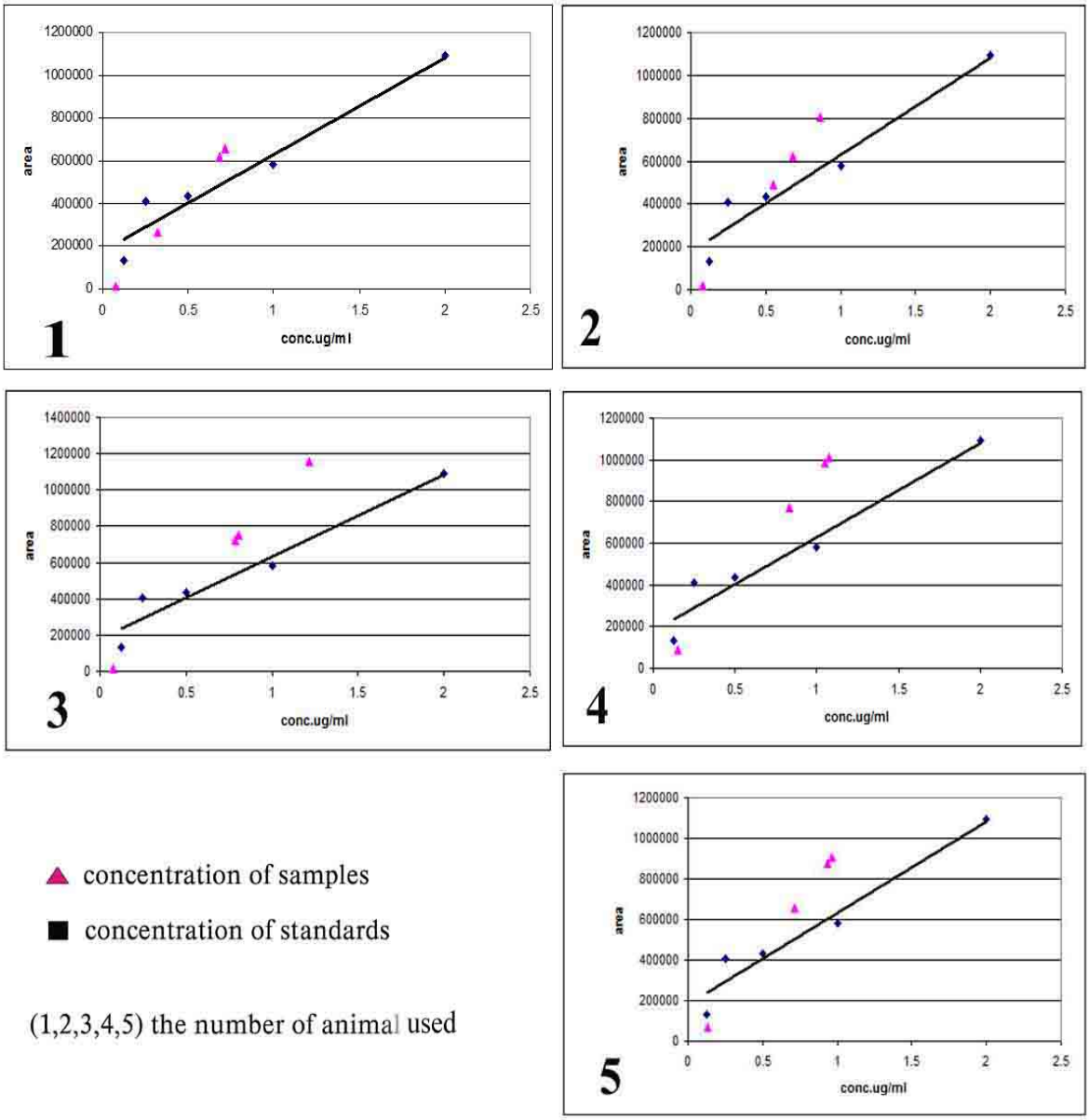

Figure.٧

- Calibration curves were found to be linear with correlation coefficient (٠.٩٤٤٧), the intercept and the slope values were found to be (٤٠٤٨٥١.٩) and (٢٤٩٠٦٧.٣) respectively.

- Calibration plots for metoclopramide plasma concentration-area at (٠,١٥,٣٠,٦٠min) after i.p. administration of the drug in ٥ animals.

Discussion

Many analytical methods reported for the quantification of Metoclopramide in dosage forms and in biological fluids are various chromatographic procedures, gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) methods with UV, fluorescence or electrochemical detection have been reported [١١,١٢,٥].

The performance of (HPLC) instruments has been remarkably progressed, and plays a conspicuous role in analysis of medicaments in formulations and biological samples. It's sensitivity ranging from microgram to picogram level. Hence, current HPLC methods for determination of medicament in biological sample are described[١٣].

The chromatographic mobile phase composition is a critical factor for the separation of monitored compound and impurities of biological fluids. The reported mobile phase (acetonitrile : ١% triethyleamine buffer pH ٦.٨; ٥٠:٥٠ v/v) in our work was optimized primarily for rapid and interference-free chromatograms of serum extracts. In (Fig.٣) shows representative chromatogram of extracted drug-free serum, and (fig.٥) serum sample taken from rat after i.p. administration of ٥mg/kg metoclopramide. A comparison of figures (٣) and (٥) indicates that metoclopramide peaks are free from matrix interference. Using the chromatographic conditions described, metoclopramide was well resolved with mean retention time of ٢.٥ min.

Least-squares regression calibration curve was found to be linear at serum concentrations between (٠.١٢٥ to ٢ µg/mL) of metoclopramide. The mean linear regression equation of the peak area ratios (y) *vs*. drug concentrations (x) of metoclopramide was typically of the from y =٤٥٢١٠٤x+١٧٨٦٤٣. the mean correlation coefficient, r was generally > ٠.٩٤٤٧.

Several workers [٢,٦,١٤] have described methods for analysis of metoclopramide in pharmaceutical dosage form and its use in pharmacokinetic studies.

In this work the described reversed-phase high-performance chromatographic method is sensitive, accurate and rapid and could be reliable alternative to other separation methods for the analysis of metoclopramide. The procedure has been successfully applied to quantitative determination of metoclopramide in rat serum, and suitable for our laboratory conditions.

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