TWO SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF GRISEOFULVIN IN TABLETS

EMAN Y.Z. FRAG*, AHMED M.K. MOHAMED, GEHAD G. MOHAMED, EBTESAM E. ALRAHMONY

Chemistry Department, Faculty of Science, Cairo University, Giza, 12613, Egypt.
Corresponding author: Eman Y.Z. Frag
(E-mail: e_uossry@yahoo.com, Tel: +2 0235676896; Fax: +20235728843)

Abstract

Two simple and rapid spectrophotometric methods have been described for the assay of griseofulvin (GRF) either in pure form or in pharmaceutical formulations. The first method (A) is based on charge transfer complex reaction using 7,7,8,8-tetracyanoquinodimethane (TCNQ). The complex showed absorbance maximum at 842 nm. Beer's law is obeyed over the concentration range of 5–200 μg mL^{-1}. The second method (B) is based on the formation of coloured ethylenechloride extractable ion-association complex of GRF with Mo(V)-thiocyanate with absorption maximum at 470 nm. Beer's law is obeyed in the concentration range of 2-150 μg mL^{-1}. The proposed methods were applied successfully to the determination of the examined drug either in a pure or pharmaceutical dosage forms. No interference was observed from common excipients present in pharmaceutical formulations.

Key words: griseofulvin, TCNQ, ion-pair formation reaction, tablets.

Introduction

Griseofulvin (GRF) is an antifungal drug that is administered orally. It is used both in animals and in humans, to treat fungal infections of the skin (commonly known as ringworm) and nails. It is derived from the mold Penicillium griseofulvin [1]. It has the IUPAC name (2S,6'R)-7-chloro-2',4,6-trimethoxy-6'-methyl-3H,4'H-spiro [1-benzofuran-2,1'-cyclohex[2]ene]-3,4'-dione. Its formula is $C_{17}H_{17}ClO_6$ and its molecular mass: 352.766 g/mol. It has the structure shown in Figure (1).

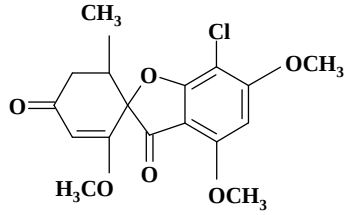


Figure (1). Structural formula of GRF drug.

Various analytical techniques for the determination of griseofulvin in pure form, in different chemical mixtures, in biological fluids and in fermentation samples were described including the use of paper chromatography, thin layer chromatography, gas chromatography, high performance liquid chromatography [2-8], electrometric method [9], and spectrophotometry, spectrofluorimetry [10-16] microbiological assay for the estimation of griseofulvin.

This paper describes two spectrophotometric methods for the determination of GRF. The first method is based on the reaction of the GRF drug with reagents such as Mo(V)-thiocyanate in hydrochloric acid medium. The second method is based on charge transfer complex formation between GRF (electron donor) and TCNQ (π-acceptor reagent). The proposed methods were applied successfully to the determination of GRF in pure or in tablets with good accuracy and precision. The results were compared well with those were given by the official method.

Experimental:

Reagents and materials

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. They included GRF that provided by DELTA Pharma Company, Egypt and reagents used included 7,7,8,8-tetracyanoquinodimethane (TCNQ) was supplied from Aldrich.

Nitric, sulphuric, hydrochloric and glacial acetic acids were supplied from Merck. Absolute ethanol and sodium hydroxide were supplied from Adwic, while n-propanol and acetonitrile (AR) were supplied from Aldrich. Carbon tetrachloride, chloroform, methanol, acetone, ethylene chloride, 1,4–dioxane, n-butanol, methylene chloride, dimethyl formamide, tetrahydrofuran, ascorbic acid, ammonium thiocyanate and ammonium molybdate were supplied from El-Nasr Company.

The GRF pharmaceutical preparations were purchased from ultragriseofulvin (griseofulvin ultramicronised) and labelled 125 mg tablet **(**KAHIRA Pharm. Chem. Ind. Co., Egypt).

2.8×10^{-3} mol L^{-1} of GRF drug was prepared by dissolving the accurate weighed amount in a definite volume of acetonitrile, to get the required concentration. Dilute solutions were prepared by

accurate dilution from the stock solution to get the desired concentration.

0.02% (w/v) of 7,7,8,8-tetracyanoquinodimethane (TCNQ) reagent was prepared by dissolving 20 mg of TCNQ in 100 mL acetonitrile.

10% (w/v) solutions of each of ascorbic acid and ammonium thiocyanate were prepared by dissolving the accurate weight (10 g) of each substance in 100 mL bidistilled water. Stock solution of ammonium molybdate (0.02% w/v) was prepared by dissolving the accurately weighed 0.02 g of ammonium molybdate in 100 mL bidistilled water.

4 mol L^{-1} Acid solutions (HCl, H_2SO_4 and HNO_3) were prepared by accurate dilution with bidistilled water from concentrated solutions.

Apparatus

The spectrophotometric measurements were carried out using the manual Unico 1200 spectrometer (United Products and Instruments, Inc.) in the wavelength range from 325-1000 nm and quarts cell of 1cm optical length was used. Small volumes were taken using automatic pipettes Socorex Swiss (50-200 μL).

Assay procedure for pure drug

For the first method (A): in calibrated 5 mL volumetric flask, 0.5 mL of 2.8 x 10^{-3} mol L^{-1} of GRF was added to 1 mL of 0.02% (w/v) TCNQ solution. The volume was completed to the mark with acetonitrile. The absorption spectra of the resulted CT complex product was scanned in the wavelength range from λ = 600-900 nm from which the best wavelength for drug was selected.

For the second method (B): to 2 mL of 0.02% (w/v) of Mo(VI) solution was added 2 mL of 4 mol L^{-1} HCl, 2 mL of ammonium thiocyanate (10% (w/v)) and 2 mL of ascorbic acid (10% (w/v)) solutions. They were mixed well in 100 mL capacity separating funnel. After 15 minutes [17], 0.5 mL of drug solution was added. The mixture was diluted with bidistilled water to 10 mL, and left for 10 minutes. The ion pairs were extracted with dichloroethane twice with 5 mL portions after shaking for one minute. The ion pair was collected in 5 mL measuring flask and the absorption spectra of the resulted solution was scanned in the wavelength range from 350-600 nm from which the best wavelength for drug was selected.

Assay procedure for tablets

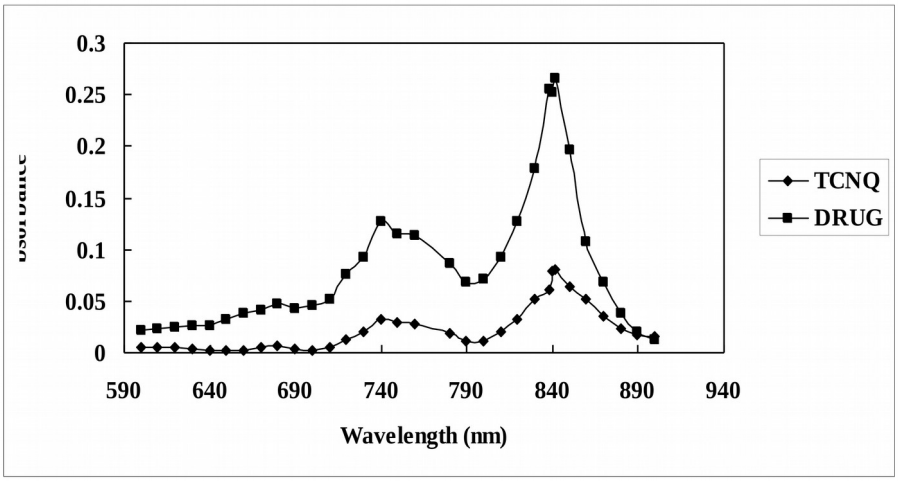
For the method (A): a portion of tablets powder or solution equivalent to 100 mg of GRF drug was prepared in 100 mL acetonitrile, for first the method, to different concentrations of GRF base was added 1 mL of 0.02% (w/v) TCNQ reagent. The volumes were made up to the mark with acetonitrile in 5 mL calibrated measuring flask. The absorbance was measured at λ_{max} = 842 nm for GRF using TCNQ reagent, against reagent blank.

For the second method (B): in a 100 mL separating funnel, 2 mL Mo(VI) (0.02% (w/v)) was mixed well with 2 mL of 4 mol L^{-1} HCl, 2 mL of ammonium thiocyanate solution (10% (w/v)) and 1.5 mL of ascorbic acid solution (10% (w/v)), and the resulting solutions were left for 15 minutes. Then different concentrations of GRF solutions were added, mixed well and after10 minutes the ion-pairs were extracted as explained before.

Results and discussion

Determination of the suitable wavelength (λ_{max}):

The absorption spectra of TCNQ reagent with GRF drug in acetonitrile solvent is shown in Figure (2). Solution gives an intense greenish colour which has characteristic long-wavelength absorption bands, frequently with two maximum at λ = 740 and 842 nm. The selected wavelength that has a maximum absorbance value is 842 nm and it gives reproducible results.



.Figure (2). Absorption spectra of TCNQ with acetonitrile and GRF CT complex

The absorbtion spectrum of the extracted Mo(V)-thiocyanate–GRF ion-pair in dichloroethane is scanned against blank reagent from 350-600 nm and the result obtained is given in Figure (3). This Figure shows that the ion-pair attain maximum absorption at 470 nm for the drug under study.

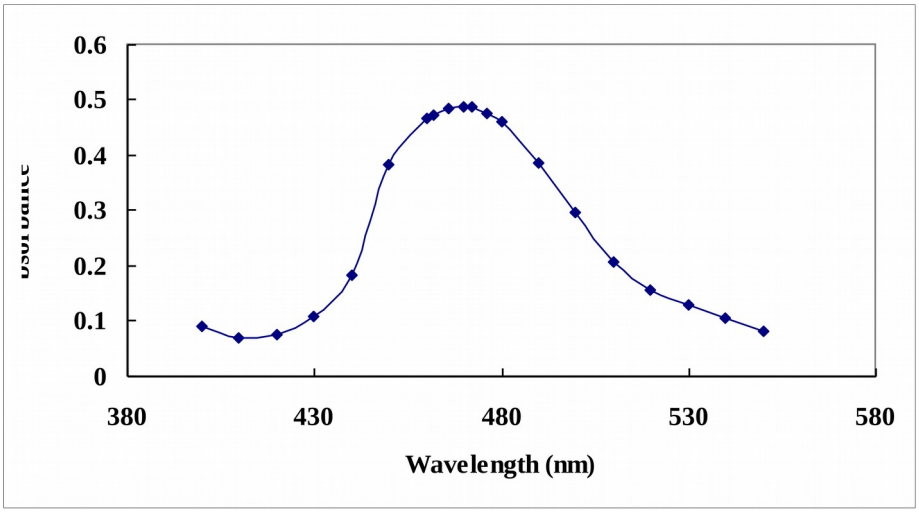


Figure (3). Absorption spectrum of Mo(V)-thiocyanate ion-pair with GRF

Optimization of reaction conditions

Optimum reaction conditions for quantitative determination complexes were established via various preliminary experiments

Effect of temperature and time

The effect of temperature on GRF determination using TCNQ and Mo(V)-thiocyanate reagents was studied at different temperatures. The results obtained show that, the absorbance is generally increased by temperature increase and reached a maximum value at 40 °C for using TCNQ and Mo(V)-thiocyanate reagents. Therefore, this temperature is chosen as the best temperature for determination of the drug under study.

The effect of time on the formation and stability of the complexes is studied carefully. The absorbance values remain almost unchanged with the increase of time. The optimum time for the completion of the reaction of GRF with TCNQ and Mo(V)-thiocyanate reagents is 40 and 10 minutes, respectively. The results indicate that complexes need the mentioned time for their complete formation.

Effect of reagent concentration

It is found that, when various concentrations of TCNQ solutions are added to a constant concentration of GRF, it is obvious that 300 µg mL^{-1} of TCNQ solution is found to be sufficient for quantitative determination of the drug under study.

Also, it is found that, the reduction probability of Mo(VI) to Mo(V) may occur by ascorbic acid or by SCN^- in acidic medium. The absorbance of the extracted ion-pair is increased by increasing the concentration of ascorbic acid till 150 µg mL^{-1}, and then remains unchanged with further increasing the concentration of ascorbic acids. Therefore, 150 µg mL^{-1} of ascorbic acid is sufficient for complete conversion of Mo(VI) to Mo(V) and hence suitable for determination of the drug under investigation. The absorbance of the extracted ion-pair is increased by increasing the molybdate concentrations till 200 ﻩg mL^{-1}, and then remains constant by increasing the volume of molybdate reagent. 200 ﻩg mL^{-1} of ammonium thiocyanate gave the maximum pronounced effect on the absorbance of the ion-pair used in the determination of GRF drug.

Effect of solvent:

In order to select the suitable solvent for CT complex formation, the reaction of TCNQ with drug is made in different solvents. These solvents include acetonitrile, acetone, tetrahydrofuran, 1,4-dioxane, ethyl alcohol, and diethyl formamide. The results obtained are shown in Table (1). From these results, it is found that acetonitrile is considered to be an ideal solvent for the colour reaction as it offers solvent capacity for TCNQ and gives the highest yield of the radical anion as indicated by high (ε) value.

Table (1). The absorbance and molar absoptivity (ε) values for the determination of GRF drug using TCNQ reagent in different solvents at λ = 842 nm.

Solvent	Absorbance(A)	ε (L mol^{-1} cm^{-1})
Acetonitrile	1.13	4×10^3
Ethylalcohol	0.266	9×10^2
Acetone	0.765	3×10^3
Dimethyleformamide	0.590	2×10^3
1,4-Dioxane	0.211	7×10^2
Tetrahydrofurane	0.030	1×10^2

The solvents like acetonitrile, 1,4-dioxane, methanol, acetone, absolute ethyl alcohol and DMF cannot be used for the extraction of the ion-pair formed, while chloroform, methylene chloride and dichloroethane extract that ion-pair quantitatively. On comparing the molar absorptivity value of the different solvents, it is found that, the molar absorptivity values (ε) for the ion-pair in dichloroethane are higher than that in methylene chloride at = 470 nm for the investigated drug Table (2).

Table (2). The absorbance and molar absorptivity (ε) values for the determination of .GRF drug using Mo(V)-thiocyanat in different solvents

Solvent	Absorbance	ε (L mol^{-1} cm^{-1})
Chloroform	0.416	$10^3\times3$
Methylene chloride	0.588	$10^3\times4$
Ethylene chloride	0.728	$10^3\times5$

Stoichiometry of the reaction of GRF with TCNQ and Mo(V)-thiocyanate reagents

Molar ratio and Job's continuous variation methods [18, 19] are applied in order to determine the suitable ratio between GRF drug and TCNQ reagent. Figures (4, 5) show that the interaction between this drug and reagent occurs in equimolar basis, i.e. the straight line is intersected at 1:1 [Drug]: [Reagent]. This means that 1:1 complex is formed between the drug and TCNQ reagent.

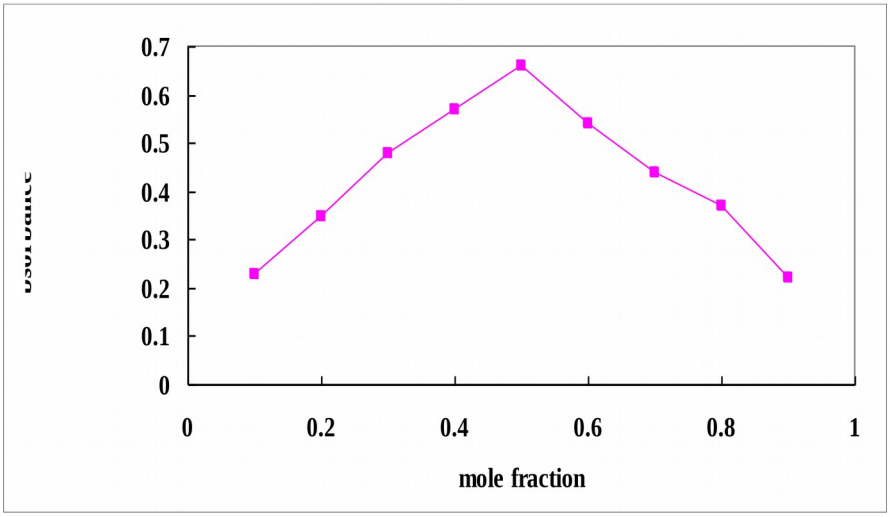


Figure (4). Job's method for GRF-CT complex with TCNQ in acetonitrile.

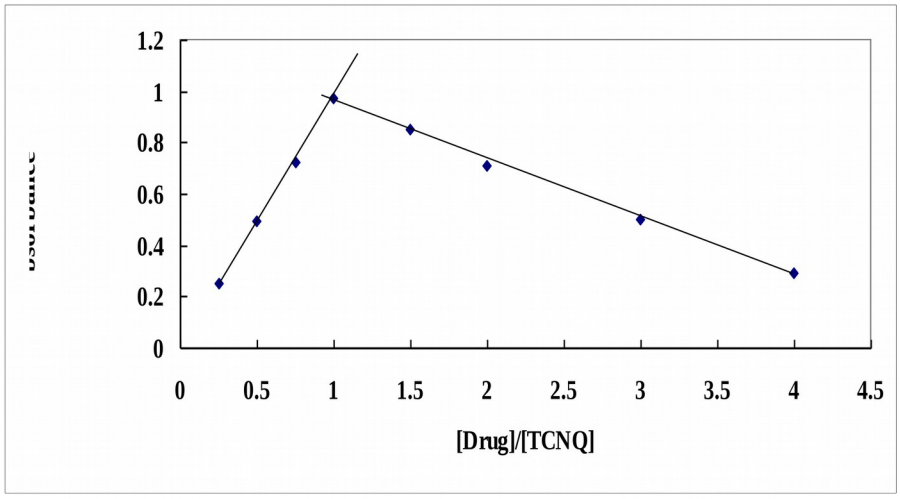
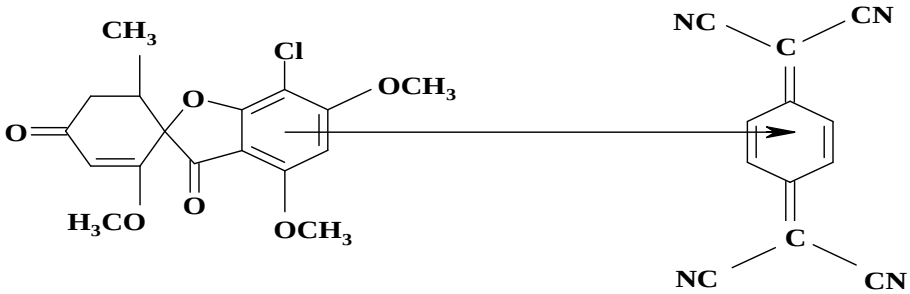


Figure (5). Molar ratio of GRF-CT complex with TCNQ in acetonitrile.

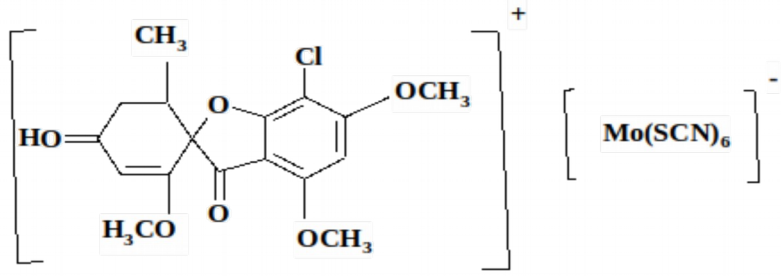
The CT complex formed between TCNQ and GRF drug take place through the π-π* CT complex is formed through the benzene ring (electron rich group) of the GRF as electron donor and the electron-acceptor reagent (TCNQ) [20]. The structure of the CT complex formed between the drug under study and reagent is shown in scheme (1).



GRF - TCNQ

Scheme (1). The structure of the complex formed between the drug and TCNQ

The nature of the binding of Mo(V) to drug in the presence of excess amount of ammonium thiocyanate is determined by the continuous variation [18] and the molar ratio [19] methods to check the ratio between Mo(V) and GRF drug to select the optimum conditions for its determination. The data obtained are given in Figures (6, 7). The result indicate that a 1:1 Mo(V):GRF ion-pair is formed through the electrostatic attraction between positive protonated drug GRF^{+} and thiocyanate negative complex $[Mo(SCN)_6]^{-}$ [21] as shown by the proposed structure (scheme 2).



Scheme (2). The proposed structure of the GRF-Mo(V) thiocyanate ion - pair.

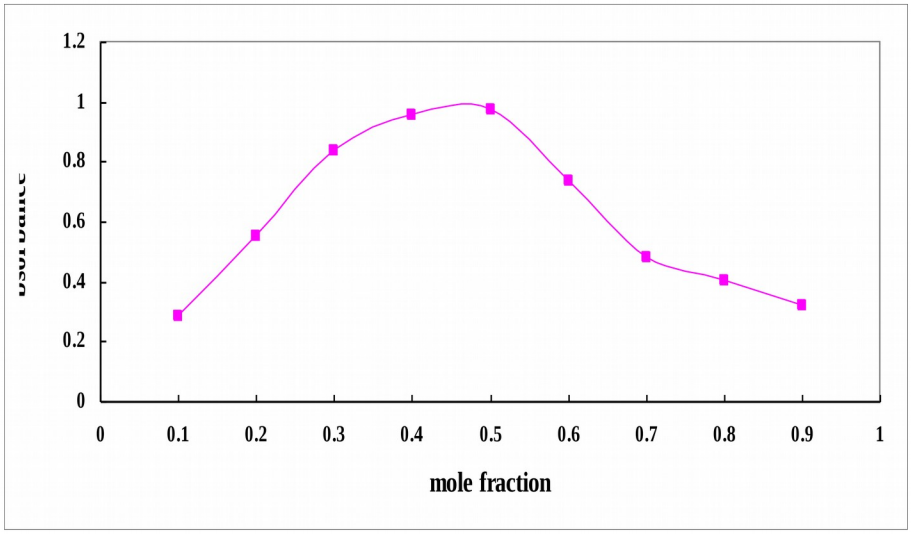


Figure (6). Stiochiomertric ratio of the reaction of Mo(V)-thiocyanate with GRF drug using mole fraction method at λ_{max} = 470 nm.

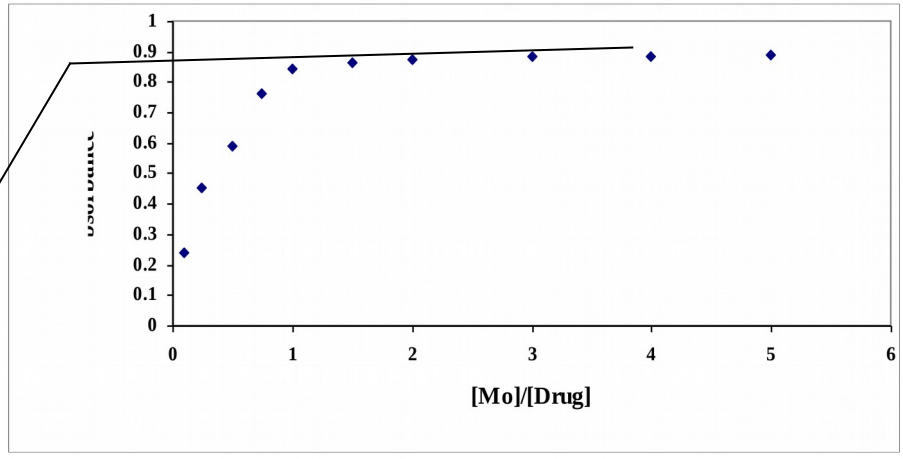


Figure (7). Stiochiomertric ratio of the reaction of Mo(V)-thiocyanate with GRF drug using molar ratio method at λ_{max} = 470 nm.

Validity of Beer's law:

Spectrophotometric determination of GRF drug is carried out under the favourable conditions of acidity, reagent concentration, time, temperature, ratios, wavelength and extracting solvent. The results of determination of the drug under investigation are shown in Table (3). It is found that, Beer's law is valid over the concentration ranges from 5-200 and 2-150 µg mL^{-1} of GRF drug using TCNQ and Mo(V) thiocyanate reagents, respectively (Figures 8 and 9).

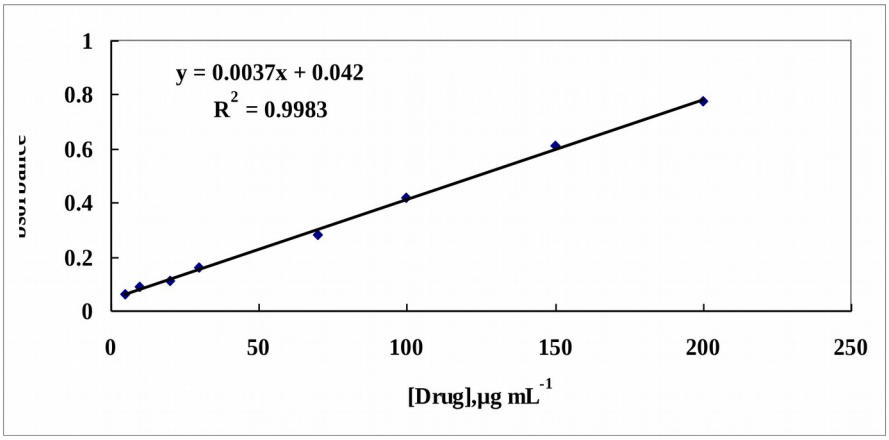


Figure (8). Validity of Beer's law of the CT reaction between TCNQ and GRF drug at selected optimum conditions.

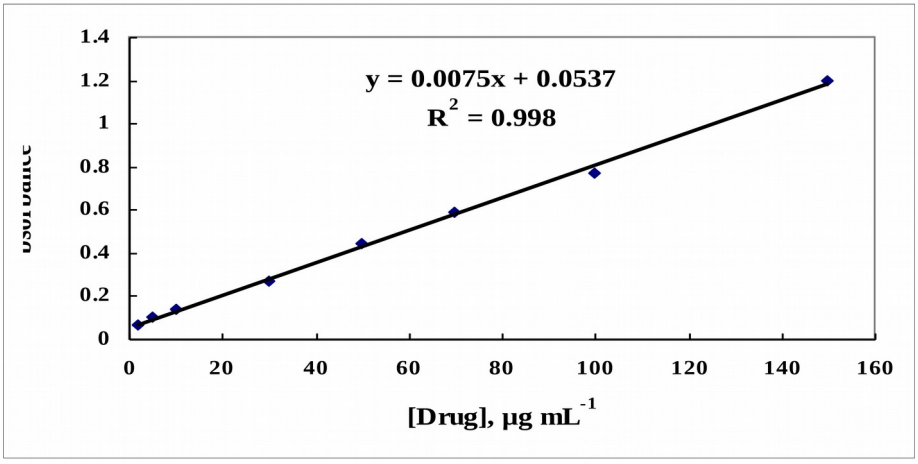


Figure (9). The validity of Beer's law for the determination of GRF with Mo(V)-thiocyanate at λ_{max} = 470 nm.

Table (3). Analytical parameters for the determination of GRF drug using TCNQ, and Mo-thiocyanat reagents.

		Method A	Method B
Parameters		**TCNQ**	**Mo(VI)**
λ_{max} (nm)		842	470
time (min)		40	10
T (^{0}C)		40	40
[GRF] (µg mL^{-1})		5-200	2-150
ε (L mol^{-1}cm^{-1})		4× 10^3	5×10^3
Sandell Senstivity (µg cm^{-2})		0.71	0.57
A=mC+Z	**m**	0.0037	0.0075
	Z	0.042	0.0537
Correlation coefficient (r)		0.998	0.999
SD		0.03-0.06	0.04-0.09
RSD (%)		0.16-0.22	0.14-0.31
LOD (µg mL^{-1})		9.73	7.6
LOQ (µg mL^{-1})		32	25
Percentage recovery (%)		100-100.7	100-100.5

Between-day measurements:

In order to prove the validity and applicability of the proposed method and the reproducibility of the results mentioned, four replicate experiments at three concentrations of GRF drug are carried out. Table (4) shows the values of between-day relative standard deviations for different concentrations of the drug obtained from experiments carried out over a period of four days.

Table (4). Between-day precision for the determination of GRF drug using TCNQ and Mo(V)-thiocyanate reagents.

[GRF] Taken µg mL^{-1}	[GRF] Found µg mL^{-1}	% Recovery	SD*	RSD* (%)
TCNQ:				
10.00	10.07	100.7	0. 043	0.427
30.00	29.82	99.40	0. 062	0.210
70.00	70.00	100.0	0. 086	0.120
Mo(V)-thiocyanate:				
10.00	10.05	100.5	0.053	0.530
30.00	30.00	100.0	0.025	0.083
70.00	70.24	100.3	0.034	0.134

Spectrophotometric determination of GRF drug in pharmaceutical preparation using Mo(V)-thiocyanate, and TCNQ reagent.

The validity of the proposed method was tested for the determination of GRF drug in dosage form manufactured in the local companies. The concentration of the drug in the dosage forms was calculated from the appropriate calibration graphs. Table (5) shows the results obtained from the determination of drug in the dosage form. These results compared with those obtained by applying the official method [22].

Conclusion

Two simple, rapid and accurate spectrophotometer methods were suggested for the determination of GRF drug in raw material and pharmaceutical preparation. These two methods based on charge transfer formation using π-acceptors such as TCNQ reagent or using Mo(V)-thiocyanate in acid medium.

Different experimental factors extensively studied from which the optimum conditions stabilized. The concentration limits of each drug using both methods are also studied. It is obvious from the results that GRF drug can be determined in a wide concentration range using ion-pair and charge transfer methods. The smaller values of SD and RSD indicate the reliability, accuracy and precision of the suggested procedures.

Table (5) Spectrophotometric etermination of GRF drug in pharmaceutical preparation by proposed and official methods.

Sample	Reagent	Proposed		Official		% Recovery		SD*	SD**
		[Drug] μgmL^{-1}		[Drug] $mgmL^{-1}$					
		Taken	Found	Taken	Found	Proposed	Official		
Ultra GRF	TCNQ	110.00 70.00	9.89 69.57	10.00	9.95	98.90 99.93	9.50	0.07 0.08	0.04
	Mo(V))-thiocyanate.	30.00 100.0	29.83 100.0	10.00	9.95	99.40 100.0	9.50	0.05 0.09	0.04

*Proposed method.
** Official method.

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