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Research Article

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Molecular and atomic spectrophotometry and high performance liquid chromatographic determination of metronidazole in dosage forms via complex formation with Au(III) and Hg(II) ions in Solutions

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ABSTRACT

Uv-visible spectrophotometry, flame atomic absorption spectrophotometry and HPLC techniques have been developed for the assay of metronidazole in bulk drug and in dosage forms via complex formation with Au(III) and Hg(II) ions in solutions after fixing maximum reaction conditions . In the first two techniques, chloroform was used as a solvent for the two complexes with extraction percentages (96.652,93.975%) respectively. Stability constants for the two extracted complexes were ($1.697x10^4$, $5.118x10^5$)respectively. Beer's law was obeyed for (5-55), (2-22µg/ml) for uv-visible and atomic spectrophotometric methods respectively. The HPLC determination for metronidazole and its complexes was performed simultaneously without extraction or separation step with peak absorption at λ(272nm), (280nm) for Au and Hg complexes respectively. A rectilinear relationship between two mean peaks area for metronidazole and its complexes with concentrations was observed in the range (10-110 µg/ml). The correlation coefficients, regression equations, detection limits and recoveries for the two complexes in the three methods were calculated. These methods have been successfully applied to assay of metronidazole in preparations.

Keywords: metronidazole, spectrophotometric determination, optimum conditions HPLC, regression equations, direct methods

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INTRODUCTION

Metronidazole (MTZ) is chemically, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole. It is a well established antibacterial agent in the treatment of various bacterial infections and antiprotozoal agent. It is a therapeutic agent of choice for amoebiasis and is also used in combination with other antimicrobial drugs against yeast infections[1,2,3]. Therefore several methods have been reported for the determination of metronidazole in bulk and dosage forms or in biological fluids like voltammetry [4] , HPLC[1,5,6] , and liquid chromatography [7]. Assays of MTZ by spectrophotometric methods were also reported based on reduction of its nitro group [8, 2,3,9,10]. The studies on interaction of metronidazole with metal ions were based mainly on the synthesis and characterization of transition metal complexes of MTZ and the study of their biological activities as antitumor[11,12,13], antifungal [14] , antibacterial [11] genotoxic , mutagenic[13], and antiamoebic agents [15, 16] compared with the metal-free drug. However, research work on the assay of MTZ by complexation with metal ions in solution is very limited. This work describes fast methods for the determination and assay of MTZ in formulation and dosage forms by complexation with gold(III) and mercury(II) ions in solutions using molecular and atomic absorption spectrophotometry and HPLC techniques.

EXPERIMENTAL SECTION

Instruments:

UV-Visible spectra of solutions were recorded on Varian Gary 100 conc. UV-Visible spectrophotometer supplied with UV. Probe software., .FTIR spectra were recorded on Shimadzu FT-IR 8400S Fourier transforms spectrophotometer,. For Au(III) complexes Flame atomic absorption spectrophotometer (FAAS): GBC 933 plus was used, with hollow cathode lamp H.C.L., D_2 Lamp (GBC), flame auto-sampler, and air-acetylene flame. High Performance Liquid Chromatography was performed on HPLC, Shimadzu with uv-visible detector Shimadzu LC 2010A and column 150 X 4.65 mm supelcosil LC-18, mobile phase methanol: H_2O (25:75), flow rate (2 ml/min), injection volume 200 µl, and detection wavelength 272nm. pH of solutions was measured by using HANA, HI 98150 GLP PH/ORP- pH meter .

Materials and reagents:

All chemicals used were of analytical reagent grade and metronidazole standard material was provided from state company for drug industries and medical appliance(SDI) Samarra Iraq.

Preparation of antibiotic standard solutions (100 µg.ml^{-1}):-

A stock drug aqueous solution (1000 µg.ml^{-1}) was prepared by dissolving 0.1000 gm antibiotic standard powders in 100 ml distilled water. working standard drug solutions were prepared by diluting 10 ml of stock solution to 100 ml with doubly distilled deionized distilled water (DDW) in a 100 ml volumetric flask.

Metal ions standard solutions (100 µg.m^{-1}):-

Standard 100 µg.ml^{-1} Au(III) solutions were prepared by diluting 10 ml of , 1000 µg.ml^{-1} Au(III) stock solution provided for atomic absorption spectrometric analysis to 100ml with DDW. A stock solution (1000 µg.ml^{-1}) which was prepared by dissolving 0.1350 g of $HgCl_2$ in 100 ml (DDW) in 100 ml volumetric flask. Working solutions for Hg(II) ion were prepared by diluting 10 ml of stock solution to 100 ml.

a-Complexometric Determination by Molecular Absorption(UV-Vis) Spectrophotometry

A series of standard solutions of MTZ (5-55 µg.ml^{-1}) and 10 µg.ml^{-1} of metal ions were prepared by pipetting 0.5-5.5ml of (100 µg.ml^{-1}) standard solution in (10 ml) volumetric flasks containing (1 ml) of metal ion standard solutions (100 µg.ml^{-1}) and the volume was completed to 10ml with DDW . After experimental conditions have been adjusted to the respective optimum values of concentration, pH and temperature. Complexes in aqueous solutions were extracted with chloroform with a volume ratio of 1:5 chl:aq. The absorbance of complexes in each case was recorded at the recommended λ_{max} and plotted against the concentration of the antibiotic.

Mole ratio method for complexes formation

To determine the ratio of metal to ligand in the formed complexes, 5.08 X 10^{-3} M solutions of both ligand and metal ions were used. 1ml aliquots of metal ion solutions were transferred to a series of 10 ml volumetric flasks containing (0.25, 0.5, 0.75, 1.0, 2.0, 3.0, and 4.0 ml) of ligand solution, and all other experimental conditions were set to their optimum values before measuring the absorbance
of the extracted complex.

Determination of antibiotic- Au(III) complexes in dosage form by direct method

0.1gm of powder obtained from 20 tablets of (medazole 500 mg) was weighted and dissolved in 100 ml distilled water in volumetric flask. 10 ml of the resulted solution was diluted to 100 ml with DDW in volumetric flasks. (1, 2, and 3 ml) of this solution were transferred to (10 ml) volumetric flasks and (1 ml) of (100 µg.ml^{-1}) Au^{3+} solution were added . The volume was completed to the mark with distilled water .After adjusting to the optimum conditions. The absorbance of these solutions were measured against blank solution. The concentration of the studied analyst was calculated depending upon the respective standard direct calibration curve,

Determination of dosage MTZ-Au(III) complexes by standard addition method

This method depended on the addition of fixed volume (1 ml) of (100 µg.ml^{-1}) of the dosage antibiotic solution to a series of solutions containing mixtures of a fixed volume of (1 ml) of (100 µg.ml^{-1}) Au(III) ion solutions and various amounts of standard antibiotic solution(100 µg.ml^{-1}) (0.5- 5.0 ml) in 10 ml volumetric flask .The volume was completed to the mark . After adjusting the optimum conditions in each case, the absorbance were measured and the relationship between absorbance and concentration were plotted to construct standard addition curves.

Direct method for determination of MTZ-Hg (II) complexes

Solution mixtures of MTZ standard solutions (5-50 µg.ml^{-1}) containing fixed volume of mercury ion solutions (1.5 ml) (100 µg.ml^{-1}) standard solution of mercury ion were prepared in (10 ml) volumetric flasks , after

adjustment to all optimum conditions , the absorbance values were plotted against concentration to produce standard calibration curve.

Determination of dosage MTZ-Hg complexes by direct calibration method

0.1gm of powder from 20 medazole tablets (500mg) was dissolved in 100 ml distilled water in volumetric flask. Then (10ml) of this solution was diluted to (100 ml) with DDW in volumetric flasks. (1 , 2 , and 3 ml) of this solution were transferred to (10 ml)volumetric flasks , and 1.5 ml of (100 $\mu g.ml^{-1}$) mercury ion solutions were added. The volume was then completed to the mark .All optimum conditions were adjusted and the absorbance values of these solutions against blank solution were measured. The concentration of solutions were calculated depending on direct standard calibration curve.

Standard addition method for determination dosage MTZ-Hg(II) complexes

This method depended on addition of constant volume (1 ml) of (100$\mu g.ml^{-1}$) drug solutions prepared from dosage form (500 mg medazole tablets) to a mixture of 1.5 ml of 100 $\mu g.ml^{-1}$ mercury ion solutions and 0.5-4.5 ml MTZ standard solutions (100$\mu g.ml^{-1}$) in 10ml volumetric flasks .The optimum conditions were adjusted, and the absorbance values were measured. The relationship between absorbance of complexes and concentration of drug samples were calculated plotted to construct standard addition curves.

b-Flame Atomic Absorption Spectrometry FAAS of MTZ-Au(III) complexes:

This method was based on measuring the absorbance of standard solutions containing (2- 22 $\mu g.ml^{-1}$) for MTZ prepared from (100 $\mu g.ml^{-1}$) standard solution of ligand (antibiotic) , mixed with fixed volume (0.6 ml) of gold ion standard solutions (100 $\mu g.ml^{-1}$) in (10 ml) volumetric flasks. After adjusting all optimum conditions , the absorbance were measured and the relationship between absorbance and concentration were plotted to produce standard calibration curve**.**

Determination of antibiotic Au-complexes in dosage form by direct FAAS method:-

A powder of 20 tablet of MTZ (0.1gm) were weighted and dissolved in (100 ml) DDW in volumetric flask. Then (10 ml) of this solution(1000 $\mu g.ml^{-1}$) was diluted of to (100 ml) in a volumetric flasks by DDW. Then 0.6 , 0.8 , and 1.0 ml) of end solution were transferred to (10 ml) volumetric flasks containing 0.6 ml of 100 $\mu g.ml^{-1}$ gold solutions , the volumes were completed to the mark, and all optimum conditions were adjusted .The absorbance of these solutions against blank solution were measured , the concentration of solutions were calculated depending on standard direct calibration curve .

Determination of antibiotic Au-complexes in dosage form by standard addition method:-

In this method constant volume (0.6 ml) of (100 $\mu g.ml^{-1}$) of dosage drug solution prepared from dosage MTZ was added to a mixture of (0.6 ml) Au(III) standard solution (100 $\mu g.ml^{-1}$) and (1-22 $\mu g.ml^{-1}$) of standard drug solution. After fixing all optimum conditions the absorbance was measured and relationship between absorbance and concentration were plotted to produce standard addition curves.

c-High Performance Liquid Chromatography (HPLC)

Chromatographic optimum conditions for determination of antibiotics and their metal complexes were studied simultaneously in aqueous solution mixture without extraction with chloroform**.** Standard calibration curves were obtained using solution mixtures of 10-110 and 10-100 $\mu g.ml^{-1}$ of MTZ with 10 and 15 $\mu g.ml^{-1}$ of Au(III) and Hg(II) ions ions .

Direct method for determination of MTZ and complexes in dosage form by HPLC:-

Powder of 20 tablets was collected , 0.1gm of this powder was weighted and dissolved in (100 ml) distilled water in volumetric flask. Solution mixtures of (0.3 , 0.5 , and 1.0 ml) of this solution were transferred to (10 ml) volumetric flasks and 1 ml and 1.5ml of (100 $\mu g.ml^{-1}$) Au(III) and Hg(II) ion solution respectively were added. The volumes were completed , all optimum conditions were adjusted and peak area of these solutions were measured the concentration of solution was calculated depending direct calibration curve.

RESULTS AND DISCUSSION

Figure (1) shows the absorption spectra of 50 µg/ml standard aqueous solutions of MTZ and gold(III) and mercury (II) ion . The maximum absorption peaks of MTZ at λ (235,330) nm correspond mainly to $\pi \rightarrow \pi^*$ transfer transitions [7].The spectrum of the yellow gold(III) solution versus distilled water as a blank solution, exhibited two absorption bands a doublet appeared at λ 240,325 nm and a low intensity band at 385nm and were assigned to ligand to metal charge transfer and $^1A_1g \rightarrow {}^1Eg$ transitions of square planar tetrachloroaurate(III) anion $[AuCl_4]^-$ [18,19]. The spectrum of the Hg (II) ion exhibited a single high intensity band at λ 285nm and was

assigned to ligand→metal charge transfer for the d^{10} metal complexes which do not exhibit d-d transitions [18]. The spectrum of Metronidazole with the Au (III) ion exhibited hypsochromic shift of the high intensity π→ π * band and the appearance of two new bands , a low intensity band at λmax.350nm attributed to $^1A_1g \rightarrow {}^1Eg$ of square planar Au(III) complexes [20] and a higher intensity band at λmax 290 nm may be attributed to ligand to metal charge transfer transition [18,19] . The spectrum of the Hg (II) complex exhibited shifts of ligand bands to shorter wavelength and the appearance of additional band at λmax320 attributed to ligand to metal charge transfer transitions[18,19,20] .

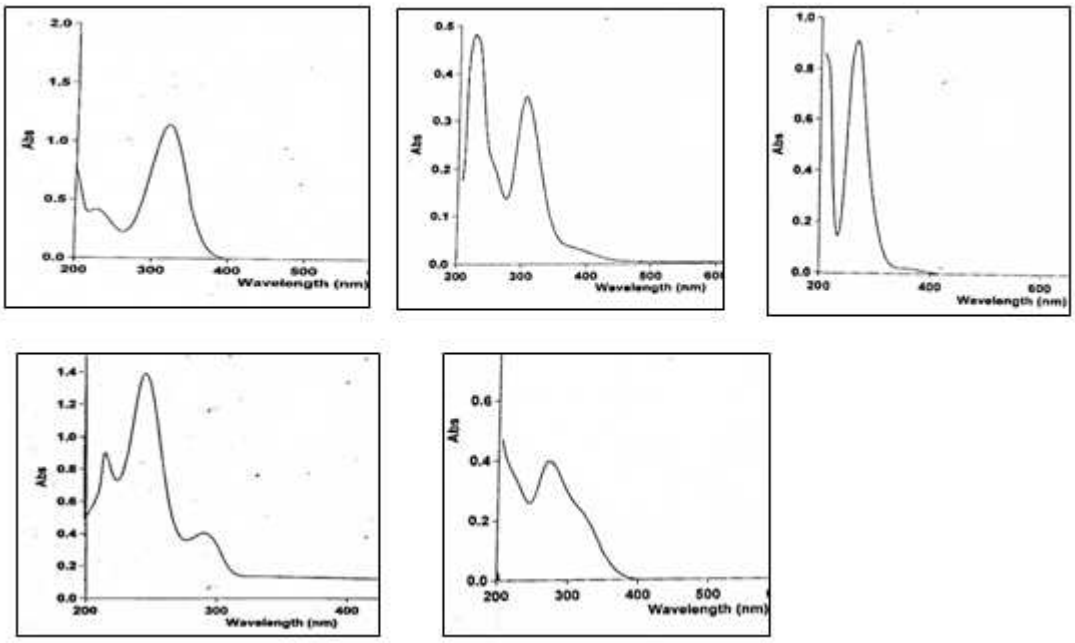
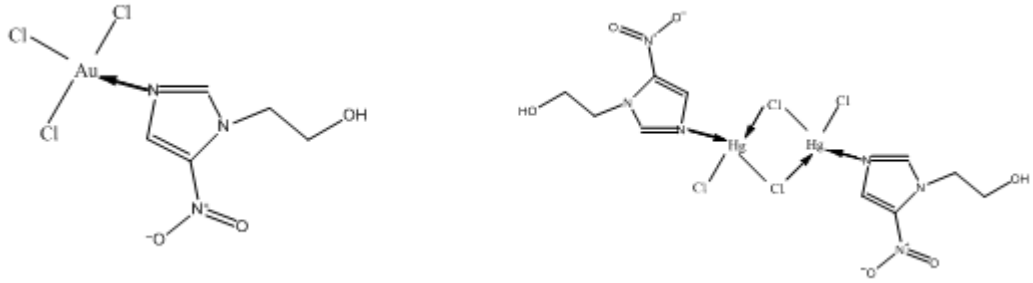


Fig 1: **The uv -visible spectrum of a- MTZ b- gold (III) ion and c- mercury(II) ion (50µg.ml^{-1} each) d- MTZ -Au(III) complex and e- MTZ -Hg(II) complexes (50µg.ml^{-1} each 1:1) in aqueous solutions.**

Results of molar conductivity and metal content obtained from the stoichiometric preparation of the two complexes in the solid state that will be published in details later[20] supported the mole ratio method in solution . The IR spectra showed that both metal ions are coordinated to the unsubstituted nitrogen of MTZ, leading to the suggested structures shown in scheme 1 [20].



Optimization of spectrophotometric determination

Figures 2 ,3 and 4 show the variation of absorbance of MTZ -Au(III) and Hg(II) complexes at λ 290 and 320nm respectively with variation of metal ion concentration pH, temperature solvent extraction percentage [21] and extraction time . Many solvents have been tested for extraction of complexes and best results were obtained on using chloroform

Table1: Optimum experimental conditions for MTZ complexes with Au (III) and Hg (II) ions.

complex	Ion conc. (µg/ml)	pH	Heating temperature (ºC)	Heating Time (min.)	Phase ratio (org:aq)	Extraction time (min.)	Extraction% (E%)
MTZ -Au	10	4	90	15	1:5	1	96.247
MTZ-Hg	15	2-4	90	15	1:5	1	93.975

Calculation of formation constant (*k*) of complexes :-The calculated formation constant values for complexes of the drugs with Au(III) and Hg (II) ions are described in table (2), depending on mole ratio curves shown in figure 5 and according to the following equation:

$$k = \frac{(A_1 - A_3)(A_2 - A_3)}{(A_2 - A_1)^2 C}$$ where k =formation constant, C= Molar concentration

A_1= Absorbance which represents two tangents intercept, A_2= Absorbance which represents the highest point of fixing absorbance, A_3= Absorbance which represents first point.

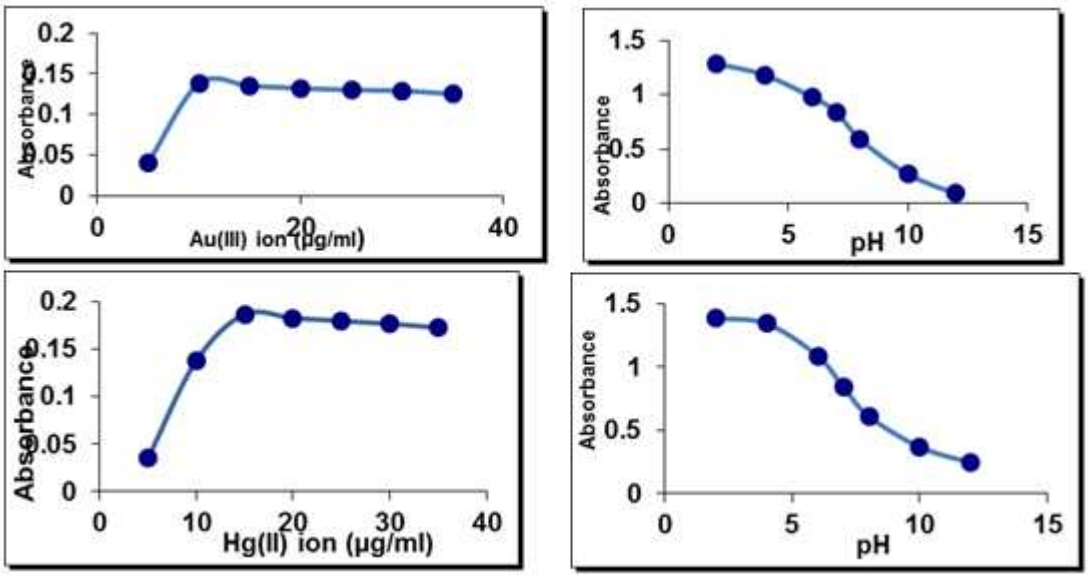


Fig 2: Effect of metal ion concentration and pH on the absorbance of Au(III) (a and b) and Hg(II) (c and d) complexes of MTZ (50µg/ml) at λmax. 290 and 320 nm respectively

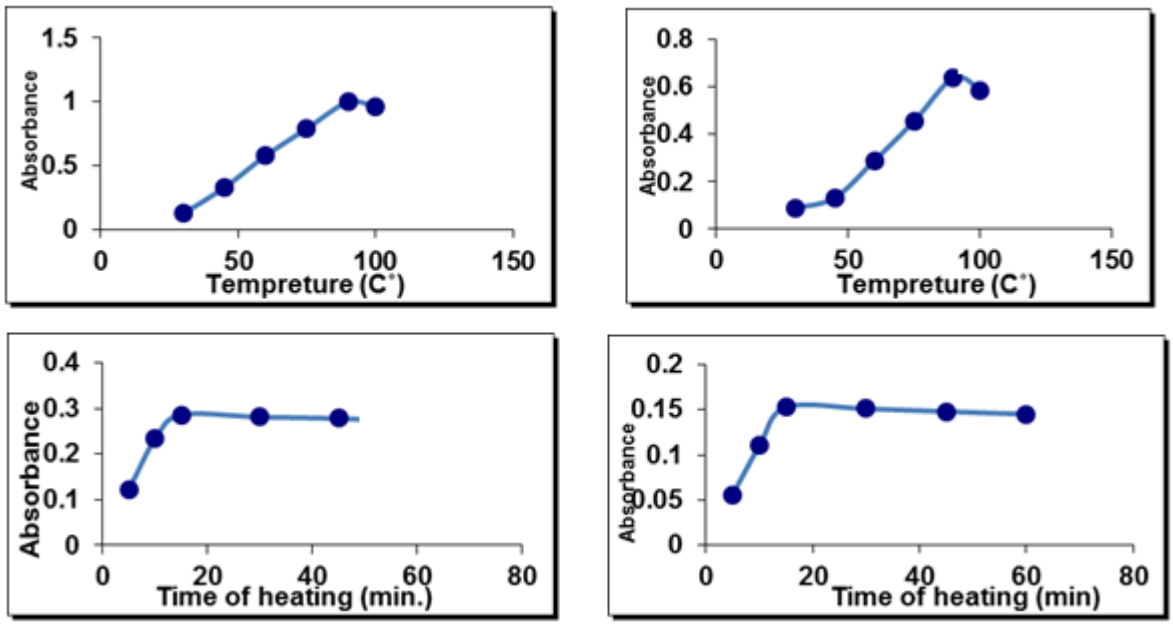


Figure 3: Effect of temperature, and heating time on the absorbance of MTZ-Au(III) and MTZ- Hg(II) complexes at λmax. 290 and 320 nm for the two complexes respectively.

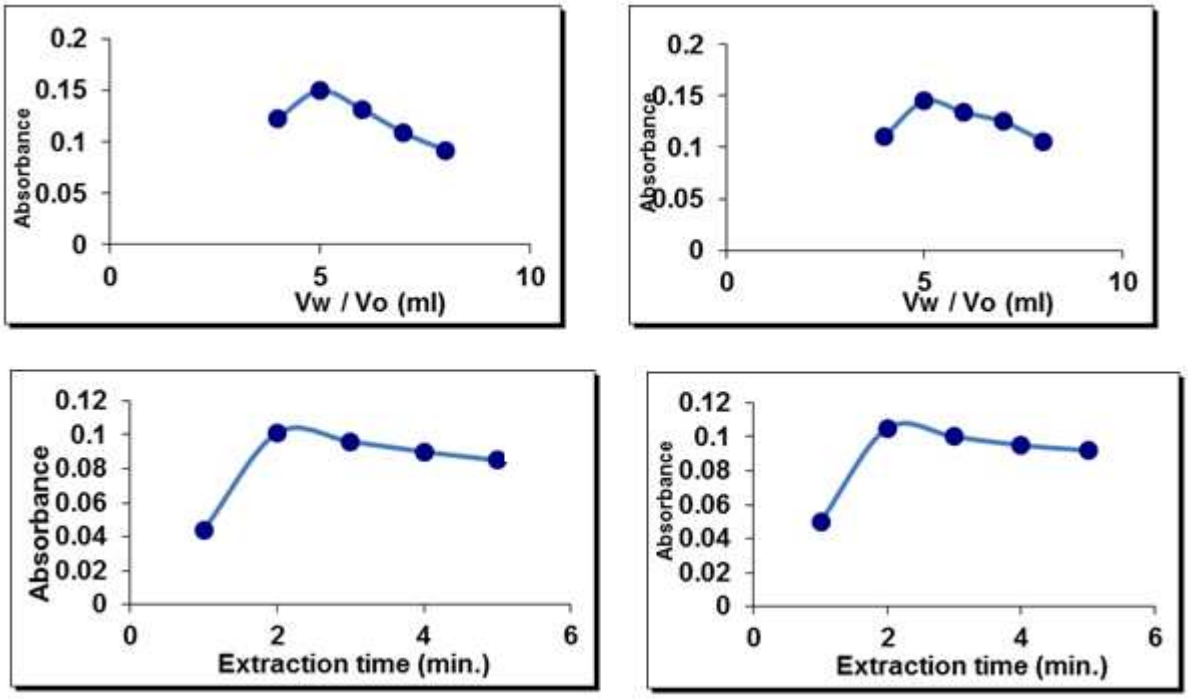


Fig 4: **Effect of aqueous solution: chloroform ratio (V/V) and extraction time on absorbance of MTZ -Au(III) (a and b respectively) and MTZ -Hg complexes(c and d respectively)**

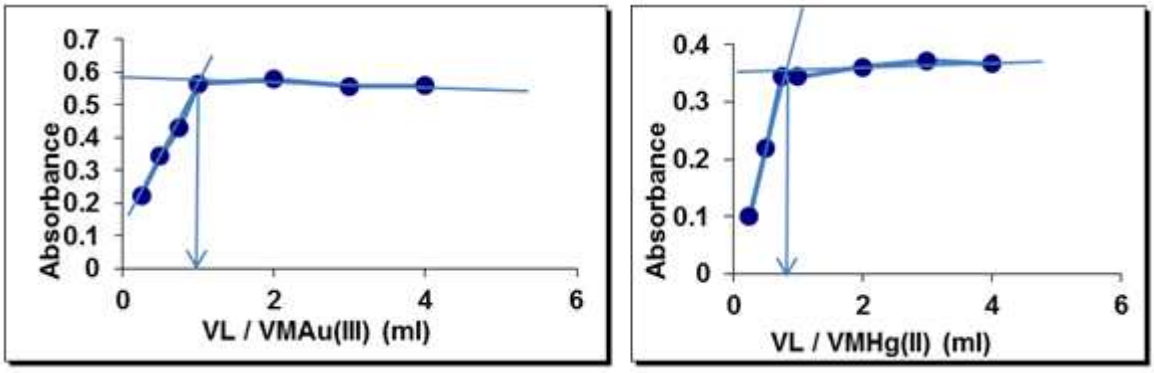


Fig 5 : Mole ratio plots for a-MTZ.-Au and b-MTZ.-Hg complexes

Table 2: Formation constants (*k*) and molar absorptivity of MTZ-Au and MTZ-Hg complexes

Formation constant (*k*) (M^{-1})		ε_{max} ($l.mol^{-1}.cm^{-1}$)	
MTZ-Au (III)	MTZ-Hg(II)	MTZ-Au(III)	MTZ-Hg(II)
$1.6976x10^{4}$	$5.1181x10^{5}$	$3.258x10^{3}$	$3.2648x10^{3}$

Spectrophotometric determination of MTZ-Au(III) and Mert.-Hg(II) complexes from direct and standard addition calibration curves

Direct and standard addition curves for the spectrophotometric determination of MTZ-Au(III) and MTZ-Hg(II) complexes are shown in figures 6 and 7. Regression equation, correlation coefficient(r), t-test, detection limits, recovery%, and error% were calculated and described in tables 3and 4.

From the comparison between t-calculated and t-tabulated in tables (3,4) that t-tabulated is more than t-calculated which indicates that the results of applied method were accepted. The slope of standard addition calibration curve was parallel to slope of direct method which means that no matrix interference exists in these methods [21,22]. The values of correlation coefficients are within the accepted limits. The detection limit for the Au(III) complex in this method is higher than the Hg(II) complex. The percentage errors obtained from direct method for both complexes were less than those of standard addition method . The accuracy may be affected by extraction process and choice of solvent[22]

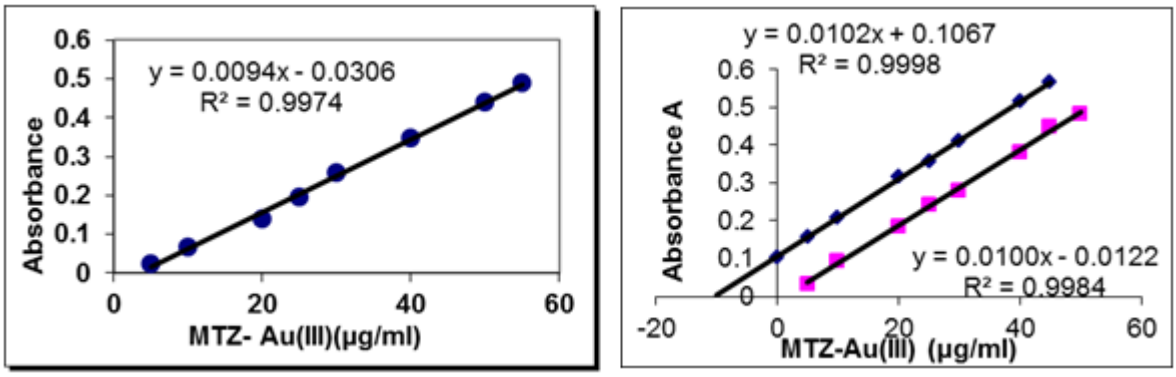


Fig 6: Direct and standard addition calibration curve for uv-visible spectrophotometric determination of MTZ.-Au(III) complex .

Table 3: Regression Equation, Correlation coefficient, t-test concentration ranges, detection limits and RSD% using direct calibration curve for MTZ-Au complex.

Assay method	Regr.eq. Y=Bx+A	Corr. coef. (r)	Linear range (µg/ml)	D.L. (µg/ml)	t-test statistic (calculated)	Tabu-lated t-test two tailed %95 C.I.	RSD (n=4)	Rec. %
Direct method.	Y=0.0094x-0.0306	0.9987	5-55	0.5076	0.6013	2.365	1.023	101.070
Standard addition method	Y=0.0102x+ 0.1067	0.9999	5-50		1.172	2.365	0.8859	106.500

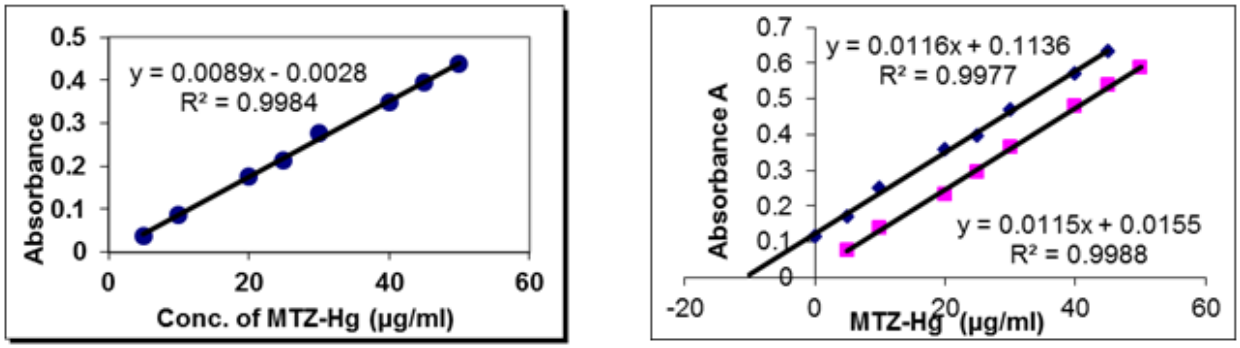


Fig 7 :Direct and standard addition calibration curve for determination of MTZ.-Hg complex by UV-Visible spectrophotometry

Table 4: Regression Equation, Correlation coefficient, caulated and tabulated t-test linear ranges, detection limits, and RSD% obtained from direct and standard calibration curves for MTZ-Hg complexes by uv-visible spectrophotometry .

Assay method	Regr.eq. Y=Bx+A	Corr. coef. (r)	Linear range (µg/ml)	D.L. (µg/ml)	t-test statistic (calcd)	t-test tabulated two tailed %95 C.I.	RSD% (n=4)	Rec. %
Direct	Y=0.0089x-0.0088	0.9992	5-55	0.3854	0.4078	2.365	1.3340	101.166
Standard addition	Y=0.0118x+ 0.1136	0.9984	5-50		2.1256	2.365	1.2669	102.720

Table 5: Relative error percentage for determination of MTZ-Au(III) and MTZ.-Hg(II) complexes by direct and standard addition uv-visible method

Complex	State of Drug	Stated concentration (mg per unit)	Found direct calb (mg per unit)	%Erel.	Found st.add. calb. (mg per unit)	%Erel
MTZ-Au(III).	tablet	500	505.350	+1.070	475.215	-4.957
MTZ-Hg(II)	tablet	500	505.830	+1.166	475.680	-4.864

Indirect determination of MTZ-Au(III) complex by flame atomic absorption spectrophotometry FAAS:-

The flame atomic absorption spectrophotometry was applied to determine metronidazole indirectly by complexation with Au(III) ion . Maximum absorbance was achieved when the ion concentration was 6 µg/ml At higher concentrations the value of absorbance was almost unchanged as is shown in figure 8

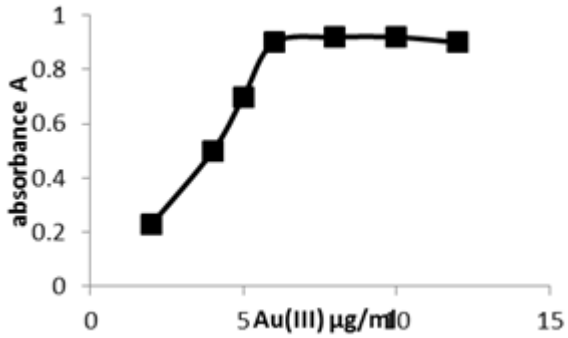


Fig 8: Effect of gold(III) ion concentration on the absorbance of MTZ-Au(III) complex by flame atomic absorption spectrophotometry.

The data described in table 6 show that t-tabulated is more than t-calculated which indicates that the results of applied method were accepted. The slope of standard addition method was parallel to slope of direct method which means that no matrix interference exists in these methods [22]. The detection limits in this method is much lower than those obtained by uv-visible spectrometry and the correlation coefficients are within the accepted limits which refers to higher sensitivity.

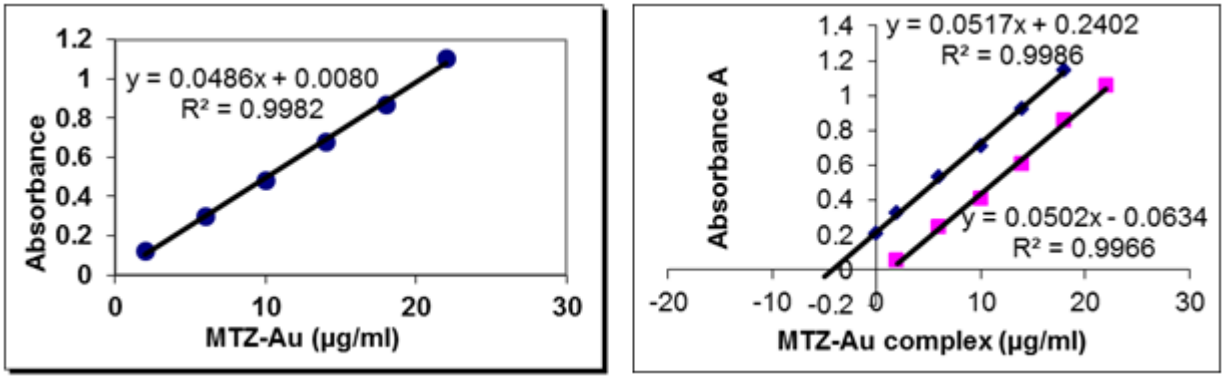


Fig 9: Direct and standard addition FAAS calibration curves of MTZ.-Au complex

Table 6: Regression equation, correlation coefficient, t-test, linearity ranges, detection limits, and RSD% data obtained from FAAS direct and standard addition calibration curves of MTZ- Au complexes

Assay method	Regr.eq. Y=Bx+A	Corr. coef. (r)	Linear range (µg/ml)	D.L. (µg/ml)	t-test statistic (calcd)	t-test tabulated two tailed %95 C.I.	RSD % (n=4)	Rec. %
direct	Y=0.0486x+ 0.0080	0.9991	2-22	0.0245	2.1741	2.571	1.1318	104.909
Stand. addition	Y=0.0517x+ 0.2402	0.9993	2-20		0.5190	2.571	3.4593	96.553

However the relative assay errors by FAAS (table 7) are higher than the previous technique which may be attributed to technical errors such as the extraction process and the choice of extracting solvent

Table7: Relative percentage error for determination MTZ-Au complex by direct and standard addition methods by FAAS.

State of Drug	Stated concentration (mg per unit)	Found direct calb (mg per unit)	%Erel.	Found st.add. calb. (mg per unit)	%Erel
tablet	500	524.5345	+4.909	470.588	-6.000

HPLC determination of MTZ –Au (III) and Hg(II) complexes by direct method:-

In this method, the determination of the drug and its complexes were studied directly and simultaneously without extraction with chloroform . The peaks corresponding to the complexes appeared at higher retention time than the original drug. The plot of direct calibration curves of the peak area against concentration for the two complexes are shown in figure 10,11 respectively. The calculated data described in tables 8 and 9 showed higher linear range compared with the two previous techniques. The t-calculated were lower than tabulated, the correlation

coefficients and percentage recovery and percentage error values are within the accepted limits of determination which indicates that the results of the applied method are acceptable [22].

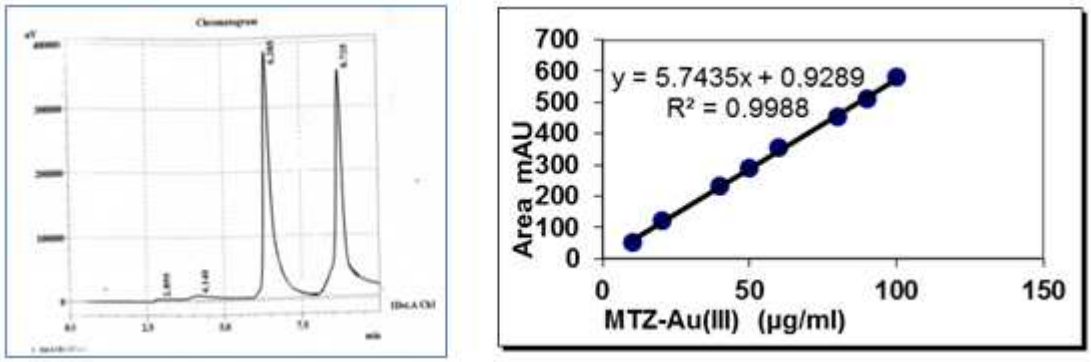


Fig 10: a-Chromatogram of MTZ and MTZ-Au complex at retention times (6.385, 8.735min) respectively at λmax.270nm. b- Direct calibration curve of MTZ.-Au complex by HPLC

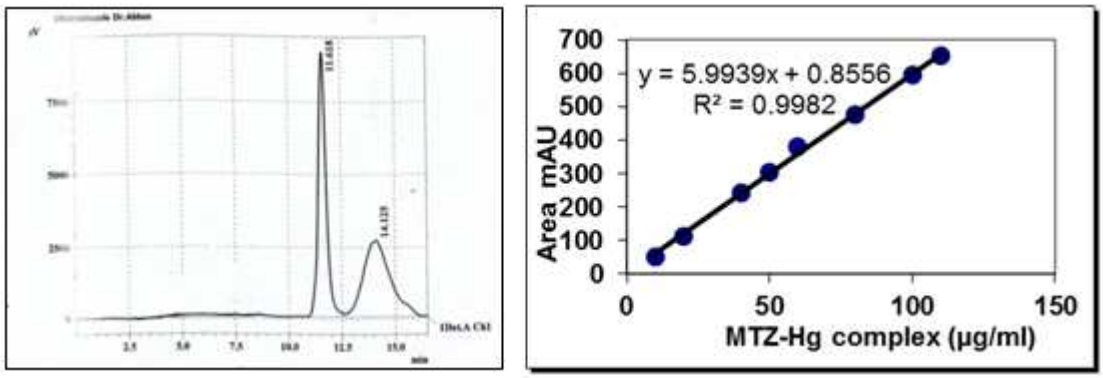


Fig 10: a-chromatogram of MTZ and MTZ-Hg complex with retention times (11.618, 14.125min) respectively and λmax.280 nm.b-HPLC calibration curve of MTZ-Hg complex

Table 8 :Regression Equation, correlation coefficient, t-test , ranges, detection limits, and RSD% obtained from HPLC direct calibration curves for MTZ-Au(III) and MTZ-Hg(II) complexes

complex	Regr.eq. Y=Bx+A	Corr. coef. (r)	Linear range (µg/ml)	D.L. (µg/ml)	t-test statistic (calc.)	t-test tabulated two tailed %95 C.I.	RSD% (n=4)	Recovery %
MTZ-Au(III)	Y=5.7435x+ 0.9289	0.9994	10-100	3.484	1.4836	2.365	1.0394	101.544
MTZ-Hg(II)	Y=5.9939x+ 0.8556	0.9991	10-100	2.850	1.2390	2.365	1.1636	101.500

Table 9: Relative percentage error for determination MTZ- Au and MTZ-Hg complexes by HPLC

complex	State of Drug	Stated concentration (mg per unit)	Found (mg per unit)	%Erel.	Retention time (min)		Detection Wavelength nm
					MTZ	complex	
MTZ-Au(III)	tablet	500	508.444	+1.544	6.385	8.735	270
MTZ-Hg(II)	tablet	500	507.944	+1.5444	11.618	14.125	280

CONCLUSION

This study presents fast methods for the determination of dosage MTZ by uv-visible and atomic absorption spectrophotometries and by HPLC via complexation of the drug with Au(III) and Hg(II) ions in solutions . The Percentage errors obtained from uv-visible direct method was lower than standard addition method . Lowest detection limits and linearity were recorded by FAAS but the relative percentage error was highest compared with the other two applications. The choice of solvent and extraction process may have affected these results .The The HPLC method was applied successfully in a shorter period of time without solvent extraction process and showed best results of percentage recovery, linearity, and percentage error but the detection limit was highest More work is required to optimize conditions for better evaluation.

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