RESEARCH ARTICLE

Development of a Novel Method for Quantitative Determination of Indomethacin

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ABSTRACT

A new green analytical chemistry method using a Ultraviolet–visible (UV-Vis) Spectroscopy is proposed to measure Indomethacin (as cuprous complex); Designate, sophisticated, and proved for determination pure mode of indomethacin, and at dosage forms respective.

The present study was prepared quantitatively of the Indomethacin complex, and the molar ratio between the cuprous and indomethacin (1:1) is measured by UV-Vis spectroscopy, the maximum absorbance peak of cuprous complex was measured at (319 nm) in wavelength range (190-900 nm), at range of the concentration (10–60µg.mL^{-1}) the linear calibration curve was painted and show regression equation (Y = 0.0036X – 0.0054) and correlation Coefficient (R^2 = 0.9999).

This study was validated and applied to the estimation of indomethacin in tablets from one company in Iraqi markets, when the wavelength and the analysis conditions were determined, no interference was found. It was concluded that the advanced method was accurate, sensitive, precise, and repeatable.

Keywords: Cuprous complex**,** Indomethacin, UV-Vis Spectroscopy.

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INTRODUCTION

Indomethacin is one of the Indole derivatives [1-(p-chloro-benzoyl)-5-methoxy-2-methyl-3-indolylacetic acid][1] as shown in Figure (1).

That belongs to the non- steroidal anti-inflammatory drugs (NSAD); It acts as an analgesic, antipyretic and anti-inflammatory, this activity is due to the non-selective inhibition of cox-cyclooxygenase enzyme which effects on prostaglandin synthesis;[2,3] This drug is mainly used for the treatment of rheumatoid arthritis, and peptic ulcer is considered one of its main side effects.[3,4]

Indomethacin undergoes degradation by decomposition into two products: p-Chlorobenzoic acid (as impurity A), and 5-Methoxy-2-Methyl-3-indole acetic acid as shown in Figure (2); This decomposition will effect on the activity of the drug itself in different dosage forms.[5,6]

Our aim is to determine the quality and quantity of the indomethacin in a specific sample. According to European pharmacopeia, which uses a titration method for the estimation of Indomethacin; absorption spectrophotometry is therefore used to determine the amount of the drug; also there are other different methods for the estimation including total leucocyte count (TLC), High-performance liquid chromatography (HPLC), Phosphomeric method, potentiometric, colorimetric

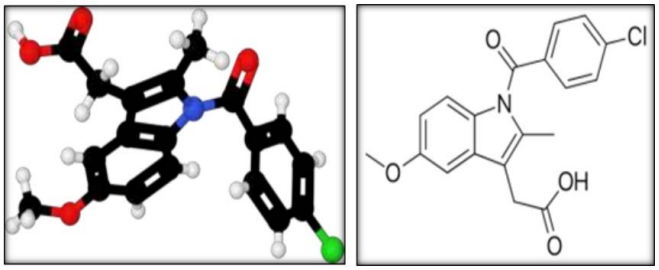

Figure 1: Chemical structure of indomethacin.

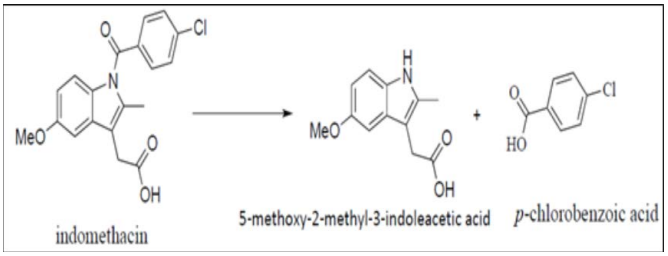

Figure 2: Decomposition of Indomethacin into 5 -methoxy- 2-methyl -3-indole acetic acid and *p*-Chloro-benzoic acid

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and phosphotungstic acid by continuing flow injection analysis via homemade ISNAG-fluorimeter.[7-15]

Since the discovery of bis-(phen) cuprous complex as the first cuprous based chemical nuclease, many attempts have been made to study cuprous complexes of ligands in combination with quinolone family drugs as antitumor, antibacterial, antimicrobial, DNA intercalating, artificial nucleases and superoxide dismutase (SOD) mimic agents; Mineral-based medicine is a research area of increasing importance for inorganic chemistry and medicinal chemistry and has focused much attention as an approach to an expansion of activating new drugs.[11] If major pharmaceutical companies do not do extensive work in the area of mineral complexes, many developments will be lost. There are three ways to develop new complexes that block the tumor[12]:

- Cisplatin derivatives (traditional and nontraditional).
- Tumor-inhibiting complexes (non-platinum).
- Platinum complexes, which are attached to transporter systems having the capability to collect the drug in tissues and organs.

In this work, we are mainly focused on the UV-Vis spectroscopy method which is done by the addition of cuprous solution to an indomethacin solution. Subsequently, the absorbance of the solution will be changed due to the formation of Cuprous-Indomethacin complex and the intensity of the absorbance will depend on the complex formation.

The reason behind using Cuprous solution in our procedure is that it's a trace metal that exhibits the most important role in the formation of drug complexes, and these complexes are easy to be recognized in vitro-vivo study.[16,17]

MATERIALS AND METHODS

Apparatus

UV-VIS Spectroscopy (Shimadzu 1650 PC), recording spectrometer 160 digital double-beam, and quartz cells (1cm); the properties of measurement, range of wavelength (190–900 nm), speed of scan: medium, sampling interval: 0.5, auto sampling interval: enabled, scan mode: single, measuring mode: absorbance, slit width: 2.0 nm, light exporter alteration wavelength 340.8 nm, S/R interchange: normal, and software name: UV Probe, electric balance (four digital) and micropipette.

Chemical Reagents

Indomethacin RS was used as standards (assigned purity 99.8%), was supplied by the S.D.I. (Iraq), Copper (I) Chloride (Sigma-Aldrich) 99.99%, other reagents of chemical were analyR, and the tablets containing (25 mg) of Indomethacin were obtained commercially in the Iraqi market (Table 1).

Standard stock and standard solution preparation

To prepare stock solution (1000 µg. mL-1) of Indomethacin, dissolving (0.1002 gm) of pure Indomethacin (99.8%) by (5 mL) of NaOH (0.5N) in beaker, then transfer to volumetric flask (100 mL), adding (15 mL) of HCl (0.1N), and completing by distilled water.

To prepare nine standard solutions (10, 14, 16, 18, 20 ,30, 40, 50 and 60 $\mu g.mL^{-1}$) of indomethacin, in nine volumetric flasks (20 mL), by micropipette transferring (0.2, 0.28, 0.32, 0.36, 0.4, 0.6, 0.8, 1.0 and 1.2 mL) of the indomethacin stock solution, for each one added (1 mL) of CuCl (100 $\mu g. mL^{-1}$), with distilled water completed (Table 2).

Sample solution preparation

For indomethacin tablets were used by one company in the Iraqi market shown in (Table 1), an average amount (0.22 g) of the tablets equivalent to (25 mg) of indomethacin, dissolving with (1.5mL) of NaOH (0.5N), then transfer into a volumetric flask (25 mL), added and (4 mL) of HCL (0.1N), then diluting with distilled water to the mark (sample stock solution).

For estimate prepared five sample solutions in the volumetric flask (20 mL) through transferring (0.6 mL) of sample stock solution by micropipette, and add (1mL) of CuCl (100 $\mu g. mL^{-1}$) then diluted with distilled water to the mark ($\approx 30 \mu g.mL^{-1}$).

Table 1: Indomethacin Tablet (25mg) in the Iraqi markets.

Commercial name	*Manufacture company*	*Mad D.*	*Expiration date*	*Batch no.*	**Average mass (gm)*
Indomethacin Tablet	INDYLON 25 INDOMETHACIN BP MEDOCHEMIE LTD-CYPRUS (EUROPE)	12/2014	12/2019	A8M114	0.22 gm

* Average ten tablets of indomethacin

Table 2: Data of standard solutions (concentration and absorbance)

Concentration ($\mu g.mL^{-1}$)	*Absorbance*	*Notes*
0	0	Y = 0.0036X - 0.0054
10	0.0309	Correlation Coefficient R^2 = 0.9999
14	0.0454	Chi Square =0.99999995
16	0.0526	Standard Error of Estimate(SEE) = 0.000128
18	0.0597	Residual Standard Deviation = 0.035914
20	0.0671	Multiple Correlation Coefficient R^2 = 0.999998
30	0.1032	Slope = 0.0036
40	0.1388	Intercept = - 0.0054
50	0.1749	
60	0.2110	

Absorbance Spectrum

For Indomethacin

To prepare (10 µg.mL^{-1}) indomethacin solution from stock solution (1000 µg.mL^{-1}), transfer (0.2 mL) by micropipette to volumetric flask (20 mL), then volume completed to the mark with distilled water, the absorbance spectrum measured at range (190-900 nm), the absorbance of the solution shows λ_{max} at (280.5nm) as in Figure 3.

For Cuprous Chloride

To prepare (100 µg. mL^{-1}) CuCl solution from stock solution (1000 µg. mL^{-1}), transfer (2 mL) by micropipette to volumetric flask (20mL), then volume completed to the mark with distilled water, the absorbance spectrum measured at range (190–900 nm), the absorbance of the solution shows λ_{max} at (814nm) as in Figure 4.

For Complex

Preparation of cuprous complex by transfer 0.4 mL of indomethacin (1000 µg. mL^{-1}) by micropipette up to (20mL) volumetric flask and add 1mL from CuCl (100 µg. mL^{-1}) then diluted with distilled water to the mark the absorbance spectrum measured at range (190–900 nm), the absorbance of the solution shows λ_{max} at (319 nm) as in Figure 5.

Calibration Curve

The method was estimate at nine levels concentration (10, 14,16, 18, 20,30, 40, 50 and 60µg.mL^{-1}) for indomethacin, by drawing a calibration curve between the concentration of indomethacin (µg. mL^{-1}) and absorbance, and calculated the line equation, the average of six determinations of absorbance for each concentrate solution, linear absorbance data for calibration curve was shown in Table 2, and Figure 6.

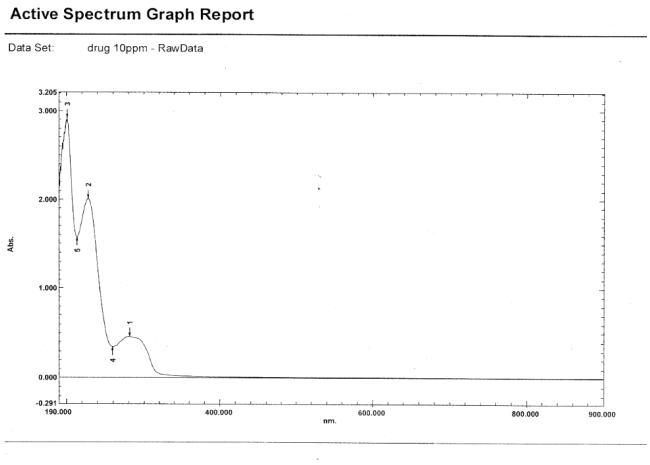

Figure 3: Spectrum of Indomethacin Absorbance (λ_{max} 280.5 nm)

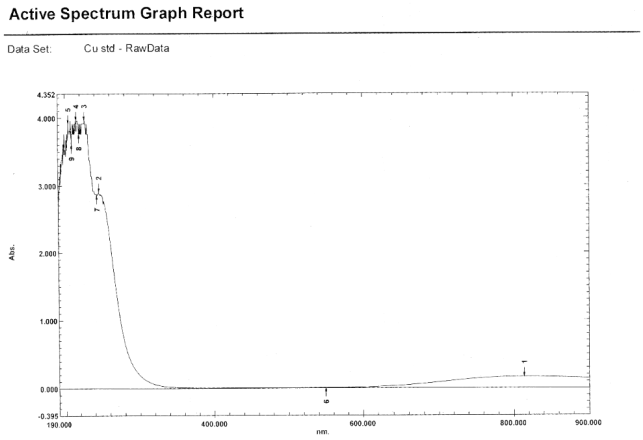

Figure 4: Absorbance spectrum of CuCl (λ_{max} 814 nm)

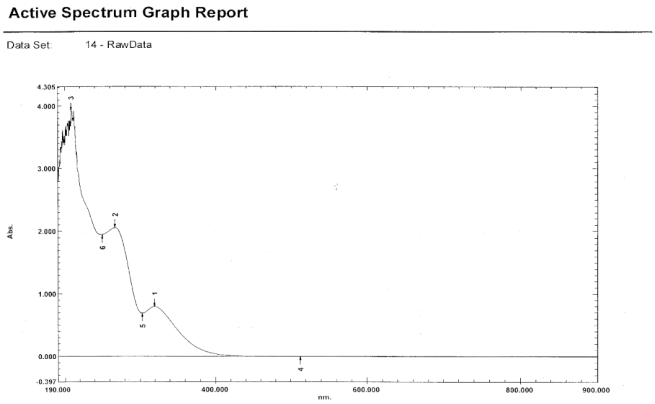

Figure 5: Absorbance spectrum of complex (λ_{max} 319 nm)

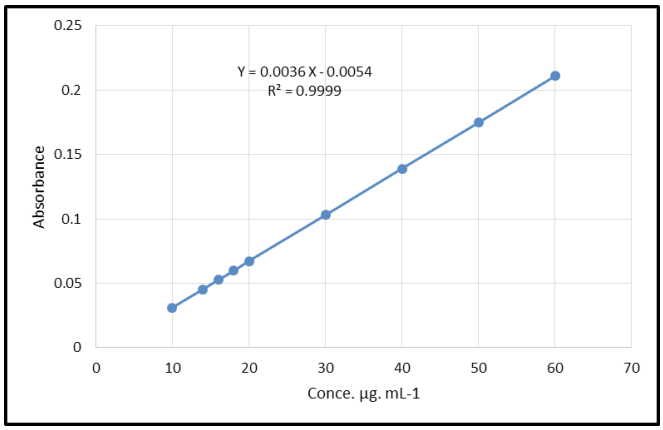

Figure 6: Calibration curve of the complex

Table 3: Volumetric flask of Mole Ratio

No. Vol. Flask	*1*	*2*	*3*	*4*	*5*	*6*
CuCl (100 µg /mL) 0.001M	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Indomethacin (100 µg /ml) `0.00028M	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL
Absorbance	0.306	0.465	0.665	0.825	0.826	0.827
Mole Ratio (Cu : IND)	1: 0.28	1: 0.56	1: 0.84	1: 1.12	1: 1.4	1: 1.68

Mole Ratio

The method was determined at six volumetric flask content each one as shown in Table 3, the best mole ratio at (1:1) for this complex shown in Figure 7, and the best condition measured, the best pH at (6.2), and the beast temperature at (15.7^0C).

RESULT AND DISCUSSION

The UV-Vis spectrum shows us the absorbance values over varying wavelengths 2 nm apart, ranging from (900–190 nm). Brand peak (Maximum absorbance) of indomethacin at (λ_{max} = 280.5 nm), maximum absorbance of CuCl at (λ_{max} = 814 nm), and maximum absorbance of the complex at (λ_{max} = 319 nm). For the characterization of metal complex, UV–Vis was analyzed. The electrons donation to the atom of metal transition (cupreous) will reduce the energy state, and thus a longer wavelength change occurs, a bathochromic and hypochromic transformation occurred in the case of cupreous complex, when; However, absorbance values did change at λ_{max} which is of concern for us because it represents the peak absorptive value of indomethacin according to complex or cupreous chloride; The brands with the highest to lowest peaks are listed respectively as shown in figure 3, 4, and 5. And a bathochromic and hypochromic transformation occurred in the case of cupreous complex.

To determine the reaction ratio between cupreous and indomethacin, a series of solutions was preparing in which the concentration of cupreous chloride was constant while the concentration of indomethacin changes, as in Table 3. The absorbance was measured for each solution prepared from the series of solutions (of the reactants), and the relationship between absorbance and molar ratio was plotted, as shown in Figure 7, through the Figure, we observe an increase in absorbance with an increase in the mole ratio of reactivity between the reactors until it reaches 1:1, after which the absorbance is increased slightly, the reaction equation is:

$$Cu^{+} + IND \rightarrow [Cu—IND]^{+}$$

Linearity

The linear relationship between absorbance and concentration range of indomethacin (10-60 µg.mL^{-1}) (as cupreous complex), the regression analysis of the linear equation was performed in the above concentration it was found that linear calibration curve, linear equation (Y = 0.0036 X - 0.0054), correlation coefficient (R^2 = 0.9999), slope (m = 0.0036), intercept =(-0.0054), chi square (0.99999995), standard error of estimate (SEE = 0.49843143), relative standard deviation (RSD = 0.896799), and multiple correlation coefficient (R^2 = 0.9988524), as in (Table 2).

Precision and Accuracy

The precision of analytical method was exact by carry out six consecutive propagate solution of the same standard solution, the low values of standard deviation (SD) and the low values of RSD obtained are listed in Table 4, indicated that the method is precise.

By estimating indomethacin, the accuracy (**recovery**) of the method was investigated, a concentration of indomethacin

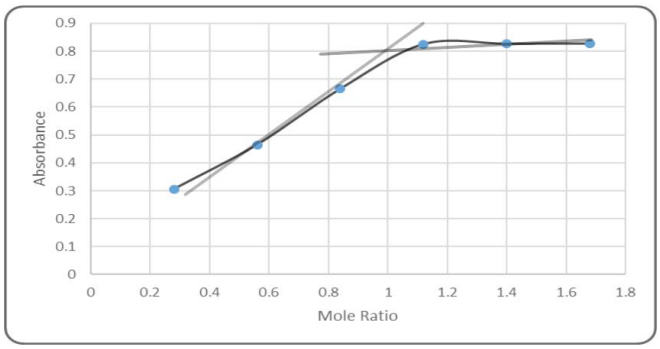

Figure 7: Mole Ratio

Table 4: Precision and accuracy

Concentration of indomethacin (µg.mL^{-1})							
Taken	*Found**	*Recovery* %*	*SD*	*R.S.D* %*	*Error* %*	*LOQ*	*LOD*
10	9.9676	99.6749	0.0296	0.2974	0.3251	90.20921	29.76904
14	14.0046	100.033	0.0373	0.2665	-0.033	113.5836	37.48259
16	16.0046	100.0289	0.0296	0.1852	- 0.0289	90.20921	29.76904
18	17.963	99.7938	0.0524	0.2916	0.2062	159.3911	52.59905
20	20.037	100.1848	0.0262	0.1307	- 0.1848	79.69553	26.29952
30	30.0463	100.1541	0.1909	0.6353	-0.1541	580.876	191.6891
40	39.9537	99.8841	0.1908	0.4778	0.1159	580.876	191.6891
50	49.9722	99.9444	0.0578	0.1157	0.0556	175.963	58.06778
60	60.0115	100.0193	0.0644	0.1073	- 0.0193	195.8478	64.62978

*Six consecutive replicate solutions of standard solution.

Table 5: Parameters and Optical characteristics of the method

No.	*Characteristics and parameters*	*Result*
1	Absorbance maxima (λmax)	319 nm
2	Linear equation	Y = 0.0036 X - 0.0054
3	Regression coefficient	0.9999
4	Linearity	10 - 60 µg.mL^{-1}
5	LOD (µg.mL^{-1})	191.6891 – 26.29952
6	LOQ (µg.mL^{-1})	580.876 - 79.69553

Table 6: Estimation of indomethacin tablets company

Trade name	*Company*	*Found* ($\mu g.mL^{-1}$)*	*SD*	*Found* In one tablet (mg)*	*Wt.%*
indomethacin Tablets	Copruse	18.444	1.224916	25.617275	102.4691%

***** Average of six consecutive replicate sample solution.

solutions containing (C = 10–60 $\mu g.mL^{-1}$) and (1 mL) of CuCl, with no noticeable impurities was barbed with the indomethacin substances at appropriate concentrations, the recovery (Wt.%) and RSD obtained are registered in the method confirmed satisfactory accuracy (Table 4).

Limit of Quantification (LoQ), and Limit of Detection (LoD)

$$LoQ = 10 (SD/m)$$
$$LoD = 3.3 (SD/m)$$

The LOQ and LOD were calculated by the above equations from the standard deviation (SD) of responses and slope (m= 0.0036), as shown in Table 4, the LOQ for Indomethacin was up to (580.876 - 79.69553 $\mu g. mL^{-1}$), while LOD were (191.6891 – 26.29952 $\mu g. mL^{-1}$).

Validation of Method

In Table 5 as shown, this method was validated out of precision, accuracy, sensitivity and linearity.

Estimation of Active Indomethacin Tablet in the Iraqi Market

This supported method was used to estimate commercially available brands of indomethacin tablets make, one company only in Iraqi markets. The results revealed were acquiescence with the amount requirement (90–110%) with regard to the classified claim in (Table 6), in this method, each concentration, and absorbance was given for six sample solutions, and each samples compared to the standard calibration curve, then calculated SD and recovery percentage, the method originality could be show by estimate authentic samples of the drug.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The successful method developed UV-Vis spectrometer was fast, easy, simple, efficient, inexpensive and perfect, with high values of precision and accuracy, the parameters of validation were rated as per Iraqi Ministry of Health instructions, the acceptable detecting of this work mention that this technique of analysis might be applied for quantitative determination of Indomethacin from medicinal drugs; also this method may be employed in routine quality control aspects, The later studies expected the structure of the complex.

The experimental practical above-mentioned characterize a new synthesize pathway for metal complexes of cuprous indomethacin.

Recommendations

Recommendations for further research are:

- Investigation of other drugs in Iraqi markets using UV-VIS spectroscopy method.
- Investigation of other drugs in Iraqi markets using the FTIR method.
- A comparison between above methods can be made.
- The metal complexes characterization was analyzed by melting point, FT-IR, UV-Vis, AAS, and DSC.

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