International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Development Method for Determination of Metronidazole in Pharmaceutical Preparation via Flow Injection Technique Using Phosphotungstic Acid as a Precipitant Agent

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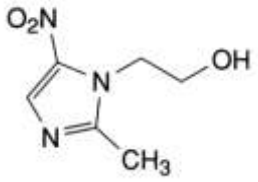
Abstract: ***The applications of flow injection-turbidimetric technique for the determination of Metronidazole (MTZ) are described.The method is based on the using the phosphotungstic acid as a precipitating agent for MTZ to form a turbid solution of ion pair MTZ-phosphotungstic acid (PTA).Turbidity values were measured via locally made turbidimeter(6SX1-T-2D Solar Cell) that fabricated with flow injectionsystem. Lots ofchemical and physical parameters such as precipitant concentration, salts effect, flow rate and sample volume have been investigated.Theresults of proposed methodshowa relatively wide range of MTZ concentration (0.1-5.0) mmol.L^{-1}and correlation coefficient (r) =0.9982. The detection limit thatdependson the dilution of minimum concentration in drug variation curve is 0.946 µg/sample while thelimit of quantitation (L.O.Q) was 12.05µg/sample. The proposed method has good repeatability of3.0and 3.5 mmol.L^{-1}of MTZsolution and was found less than 1% for n=6.The paired t-test value at 95% confidence interval was conducted and the results indicate that there is no significant difference between the proposed and classical methods.The developmentmethod can be used for theroutine and quantitativeassay of MTZ in pharmaceuticaldos ages.***

Keywords: Turbidimetric method, Metronidazole, phosphotungstic acid, flow injection analysis

1. Introduction

Metronidazole (MTZ) is one of thenitroimidazole derivatives and chemically knowns as 1-(hydroxyethyl)-2-methyl-5-nitroimidazole (Scheme (1)), which being largely used as asystemic antihelminthicdrugagainst anaerobic microorganisms[(1)].



Scheme (1): Chemical structure of Chloramphenicol

Metronidazole (MTZ) has 171.15 g.mol^{-1}molecular weight and the molecular formula$C_6H_9N_3O_3$. It's awhite or yellowish crystalline powder, slightly soluble in water, acetone, alcohol and in methylene chloride[(2)].In the literature, several of quantitationmethods for determination of metronidazolehave been reported such asUV-Visible Spectrophotometric[(3-6)], High-PerformanceLiquid Chromatography[(7,8)],Flow injection analysis-Spectrophotometric[(9)], turbidimetric method[(10)],Liquid chromatography-mass spectrometry[(11)], and Electrochemical[(12,13)].

The purpose of this work is to describe a fast, simple, sensitive and reliable approach for determination of metronidazole in pharmaceutical formulations. Flow injection analysis (FIA) technique based on the turbidimetric measurements was used for monitoring theprecipitation reaction with the use of Ayah 6SX1-T-2D solar cell. The method depended on the formation of an ion-pair complex with white precipitate by phosphotungstic acid with MTZ in anaqueous medium.In the literature, Flow injection analysis that combines with turbidimetric method was used for analysis several drugs[(14-17)].The proposed method was developed and applied to the analysis of the drug in tablets.

2. Materials and Methods

2.1 Chemicals

All of chemicals were of analytical grade and double distilled water was used inall the dilution processesin this procedure and the solutions were alwaysfreshly prepared.

Phosphotungstic acid (PTA) stock solution ($H_3PW_{12}O_{40}$, 2880.2 g.mol^{-1}, Hoplin & Willias) (0.005 mol.L^{-1})was prepared by dissolving 7.2005 g/500 mL distilled water.A stock solution of Metronidazole (MTZ) ($C_6H_9N_3O_3$, 171.15g.mol^{-1}, SDI) (0.05 mol.L^{-1}) was prepared by dissolving 0.8557 g in 10 mL of 0.01 M Hydrochloric acid then completed to 100 mL with distilled water, the solution was kept in the refrigerator.

Hydrochloric acid (HCl, 36.5 g.mol^{-1}, sp.gr. 1.18 g.mL^{-1}, BDH) (0.1 mol.L^{-1})was prepared by pipetting2.2 mL of concentrated HCl(35%)and completed the volume (250mL)with distilled water.A 0.5670 g of potassium chloride(KCl, 74.6 g.mol^{-1}, BDH) was dissolvedin 250 mLdistilled water to prepare 0.003 mol.L^{-1}.Sodium chloride standard solution (NaCl, 58.5 g.mol^{-1}, BDH) (0.03 mol.L^{-1}) was prepared by dissolving 0.4387g in 250 mLdistilled waterand dilute to the mark of volumetric flask.A 0.03 mol.L^{-1}of ammonium chloride (NH_4Cl, 40.5 g.mol^{-1}, Fluka) was prepared by dissolving 0.3037g in 250 mLdistilled water.A standard potassium nitrate solution (0.03mol.L^{-1})

(KNO_3, 101.1 g.mol^{-1}, BDH) was prepared by dissolving 0.7582in 250 mLdistilled water and dilute to flask mark.

2.2 Apparatus

Four channels peristaltic pump (Ismatec, ISM796, Switzerland) with variable speedwas used to fluid propulsion. Six-port with two-direction (medium pressure) injection valve (Rheodyne, USA) provided with 1 mm internal diameter with variable sample loop length.

Locally madeturbidimeter (Ayah 6SX1-T-2D Solar Cell) was used to turbidimetric measurements of precipitate particles at 0-180º. The local made Ayah 6SX1-ST-2D Solar cell supplied with awhite light emitting diode as a source with flow cell (glass tube) and two solar cells as a detector. The principle of turbidity measurements has measured the attenuation of incident light at 0-180º that product from the reflection and scatter light from surfaces of precipitate.

The locally made turbidimeterread out was composed of thex-t potentiometric recorder (KOMPENSO GRAPH C-1032) (Siemens, Germany) with measured range 1-500Vor 1-500 mV, and digital AVO-meter (auto range, China) with range 0.0 to 2.0V. Signalswere measured by peak height method. Shimadzuspectrophotometer model1800 (Japan)was used to measure UV-Vis spectra.

2.3 Methodology

The manifold system for determination of metronidazole in pharmaceutical formulations is composed of two lines as shown in Figure (1). The procedure includes the formation of a white precipitate from the reaction of MTZ with phosphotungstic acid in neutral medium.

The first line in manifold designis distilled water as a carrier stream with flow rate 1.3mL.min^{-1}, that lead to the injection valvefor carrying drugs samplesegment with sample loop79 µL.The second line provides the 0.32 mmol.L^{-1}phosphotungstic acid at flow rate 1.5 mL.min^{-1}. The two lines meet at a Y-junction point to form the white precipitate which passes through a locally made turbidimeter (Ayah 6SX1-T-2D Solar Cell) to record response signals in mV against time. Scheme (2) shows asuggested mechanism of ionpair for MTZ-PTA in anaqueous medium.

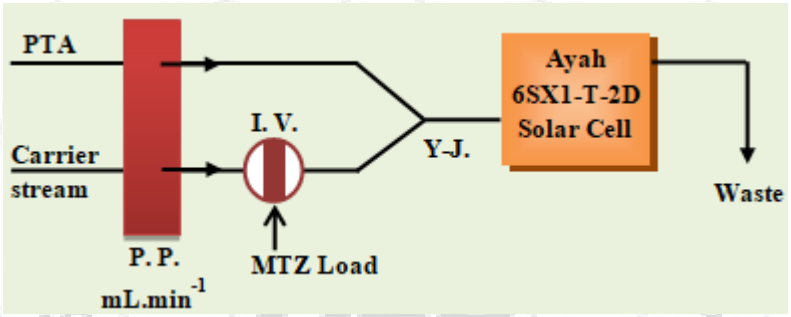
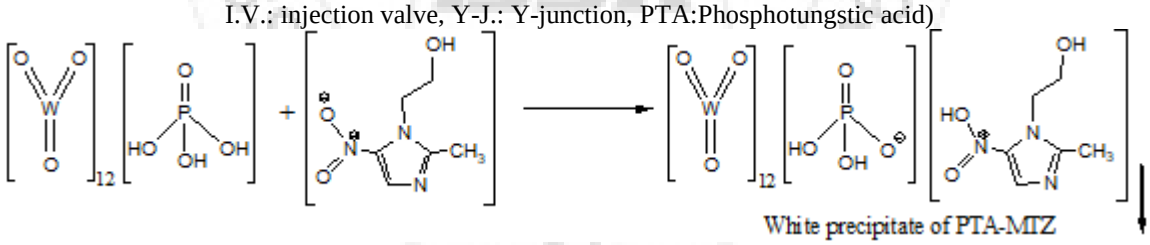


Figure 1: Schematic flowgram for flow injection manifold used for determination of Metronidazole. (P.P.:peristaltic pump, I.V.: injection valve, Y-J.: Y-junction, PTA:Phosphotungstic acid)



Scheme 2: Suggested mechanism for the reaction of Metronidazole with Phosphotungstic aid

3. Results and Discussions

Variable Optimization

All chemicals and physicals parameters such as the concentrationof Phosphotungstic acid pH effect, sample volume, flow rate have been investigatedto set the optimum parameters.

4. Chemical Parameters Optimization

1) Effect of Phosphotungstic Acid Concentration

A series of solutions of (0.1-1 mmol.L^{-1}) of phosphotungstic acid were prepared and using the preliminary conditions of5mmol.L^{-1} of MTZ with79µL sample volume on the carrier stream (distilled water).The flow rate of 1.3, 1.5 mL.min^{-1}for both carrier stream and PTA line respectively.Each measurement was repeated for three successive times. Table (1) tabulates all the obtained results while Figure (2) shows the response profile of variation of Phosphotungstic acid. From Figure (2), it can be noticedthat an increase in PTAconcentrations causes an increase in the reflection of the incident light on thesurfaceof particles[18]up to 0.32mmol.L^{-1}. Therefore, the optimum concentrationof PTA was0.32 mmol.L^{-1}.

Table 1: Variation of phosphotungstic acid on attenuation of incident light for determination of metronidazole

Concentration of PTA mmol.L^{-1}	Attenuation of incident light $\bar{y}_i$ (n=3) (mV)	Standard Deviation σ_{n-1}	Repeatability %RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
0.08	106.67	2.31	2.17	106.67±5.74
0.1	114.33	2.08	1.82	114.33±5.17
0.13	442.00	2.00	0.45	442.00±4.97
0.15	480.33	1.53	0.32	480.33±3.79
0.2	704.17	1.76	0.25	704.17±4.36
0.25	1033.53	1.50	0.15	1033.53±3.73
0.3	1247.33	1.15	0.09	1247.33±2.87
0.32	1282.00	1.73	0.14	1282.00±4.30
0.35	1122.33	2.52	0.22	1122.33±6.25

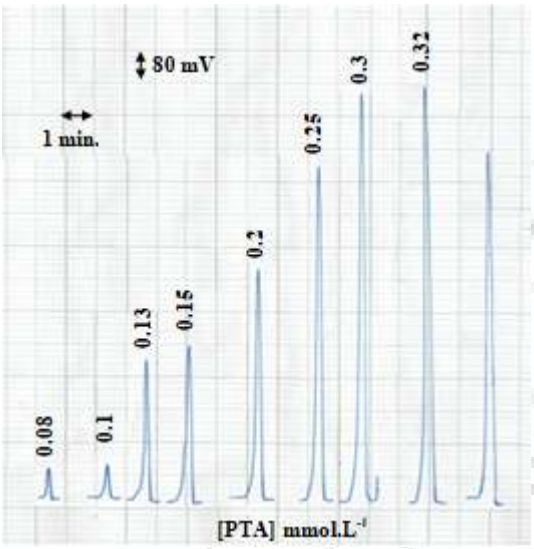


Figure 2: Response profile of variation of Phosphotungstic acid on precipitation of metronidazole

2) Variation of acidityon MTZ-PTA Precipitation Reaction

The effect of acidity medium was examined using optimum concentration of PTA solution (0.32 mmol.L^{-1}).Series of diluted solutions of HCl (1-20) mmol.L^{-1} were prepared which was used as a carrier stream.The experiment was carried out using the preliminary concentration 5 mmol.L^{-1} of MTZ with sample volume79µL. The obtained results are summarized in Table (2), which briefs the expected behaviour that the solubility for formed precipitate is increased in acid medium thus might cause passage more light to detector and decrease in peak height.Therefore,it was concluded that the distilled water is the most suitable medium and give high response peak.

Table 2: Effect of hydrochloride acid on the measurements of turbidity response

[HCl] mmol.L^{-1}	Attenuation of incident light $\bar{y}_i$ (n=3) (mV)	Standard Deviation σ_{n-1}	Repeatability %RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
D.W	1282.67	2.31	0.18	1282.67±5.74
1	1250.17	2.75	0.22	1250.17±6.84
5	1112.33	2.08	0.19	1112.33±5.17
10	988.00	2.00	0.20	988.00±4.97
15	852.67	3.06	0.36	852.67±7.59
20	724.67	1.53	0.21	724.67±3.79

3) Influence of Different Media on Precipitation System

The precipitation of MTZ (5 mmol.L^{-1}) by PTA (0.32 mmol.L^{-1}) was investigated in differenttypes of salts medium like sodium chloride, potassium chloride,ammonium acetate, potassium bromide, potassium nitrate and ammonium chloride at 0.03 mol.L^{-1}that addition to aqueous medium as a carrier stream. The reason for using different salts is because ofexpectedincrease the formation of a dense precipitate. Figure (3) shows the obtained results. It can observe that the effect of using this salt as a carrier stream did not indeed improve the obtained signal.Therefore, the aqueous medium was used for further experiments.

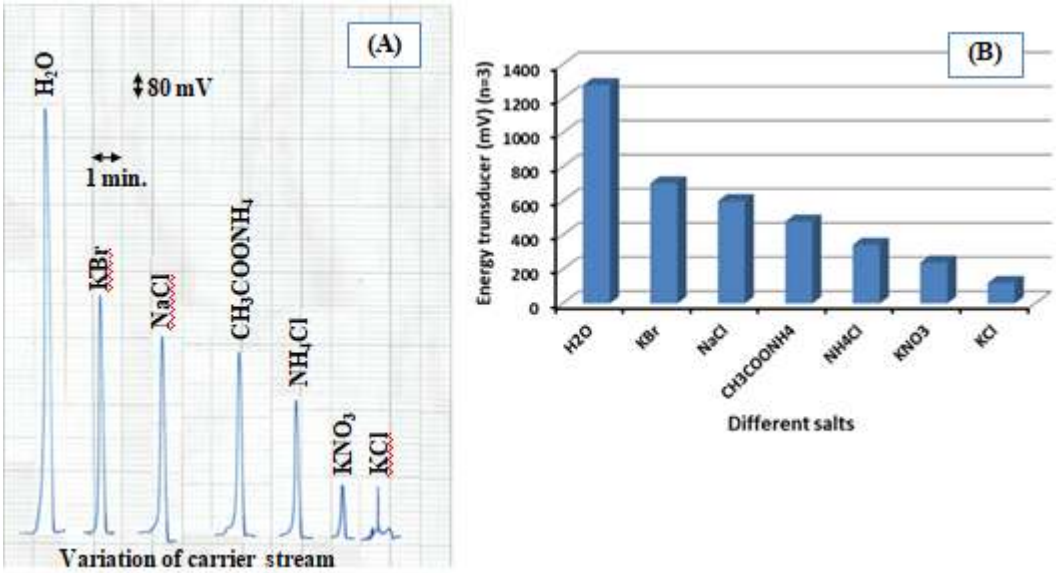


Figure 3: Effect of the different media on (A): Response profile of precipitation reaction for the determination metronidazole, (B) variation of different salts on response peak height

5. Physical Variables Optimization

1) Flow rate

The flow rate was achieved under the optimum chemical parametersin previous sections. The experiment was carried out using (5 mmol.L^{-1}) MTZ and (0.32 mmol.L^{-1}) PTA with avariation of flow rates by control the peristaltic pump speed (5-40 rpm) as shown in Table (3) and Figure (4).The obtained result shows at low flow rates, an increase inwidth of peak base (Δt_B) which can be due to the dilution and dispersion.While the high flow rate > 20 pm leads to decrease in (Δt_B) and peak height due to the precipitate of MTZ-PTA remained for a very short time in the measuring cell. Therefore, theflow rate at 20 rpm indication approximatethatequivalent to (1.3and 1.5) mL.$min^{-1}$for distilled water as carrier stream and phosphotungstic acid was given arelatively suitable responsewith minimal dilutionand short analysis time.

Table 3: Flow rate variation of MTZ-PTA precipitation system

Pump speed (indication approximate) (rpm)	Flow rate (mL.min^{-1})		Attenuation of incident light $\bar{y}_i$ n=3 (mV)	σn-1	%RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$	Sample segment arrival time (sec.)	Width of base ΔtB (min.)
	Carrier stream (H_2O)	Precipitation reagent (PTA)						
5	0.5	0.5	1017.90	1.85	0.18	1017.90±4.60	78	3.5
10	0.8	0.9	801.33	2.31	0.29	801.33±5.74	42	1.7
15	1.1	1.1	1305.17	2.02	0.15	1305.17±5.02	30	1.2
20	1.3	1.5	1504.83	2.36	0.16	1504.83±5.87	24	0.9
25	1.8	1.9	1457.13	1.96	0.13	1457.13±4.88	18	0.85
30	2.2	2.4	1401.67	2.08	0.15	1401.67±5.17	15	0.75
35	2.8	2.9	1377.44	2.50	0.18	1377.44±6.21	12	0.65
40	3.5	3.6	1344.96	1.66	0.12	1344.96±4.13	8	0.55

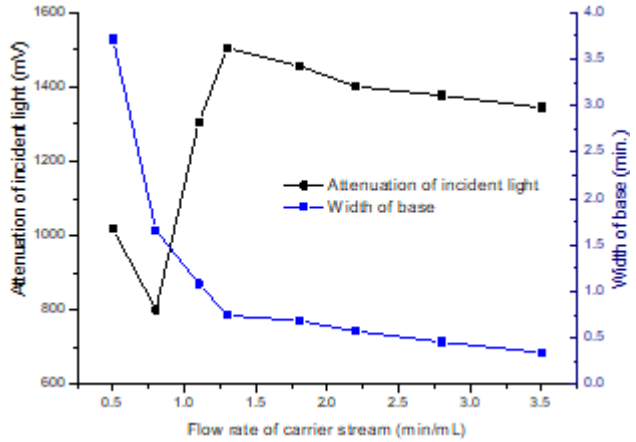


Figure (4): Variation of flow rates versus output responseof Ayah 6SX1-T-2D Solar Cell in mV unit

2) Sample Volume

Using the manifold system is shown in Figure (1) with previously optimum parameters for the development methodology adopted in the determination metronidazole. The study was carried out by a series of variable length of sample loopwith 1 mm internal diameter that used todetect theoptimum volume. The obtained results show an increase in peak height with increase sample volume but the response broadening at lager sample loop as apparent in Figure (5).Therefore, a small size of sample volume (79 µL) was adopted throughout this work.

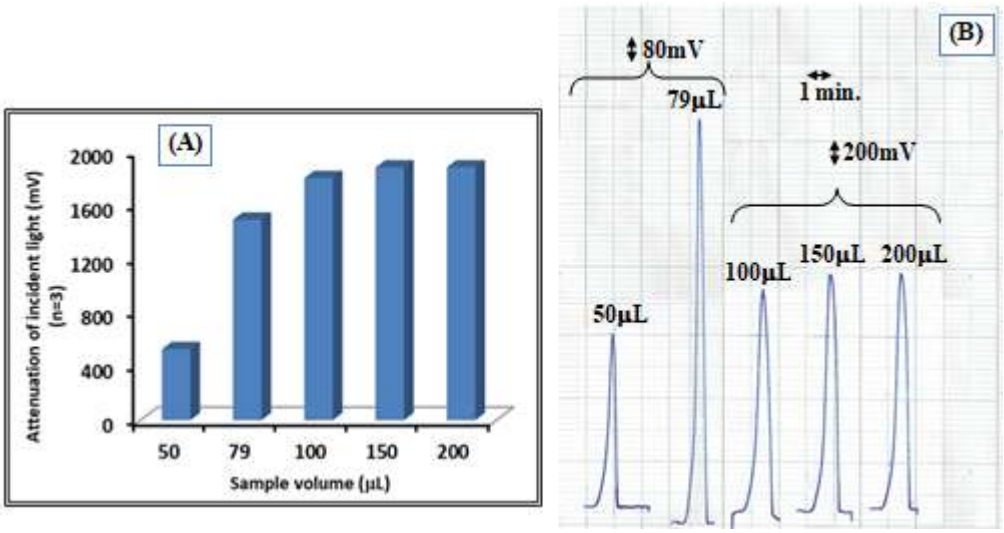


Figure 5: Effect of variation of injection sample volume versus (A) energy transducer output response, (B) response profile variation

Variation of MetronidazoleConcentration

The study carried out under all optimum conditions. The calibration graph of turbidimetric-flow injection analysis method wasconstructed using the local made turbidimeterAyah 6SX1-T-2D Solar Cell. The variations of Metronidazole solutions (0.1-6.0) mmol.L^{-1} were prepared. Each measurement was repeated for three successive measurements.Calibration curve of various concentrations w shown in Figure(6). A straight-linear response ranging from (0.1-5.0) mmol.L^{-1} with correlation coefficient of 0.9982 with percentage of linearity (%r^2)= 99.65%, and coefficient of determination was 0.99.65. Table (3)illustrates the brief resultsofanalysis of metronidazole using Ayah 6SX1-T-2D-Solar cell with flow injectionshows the values

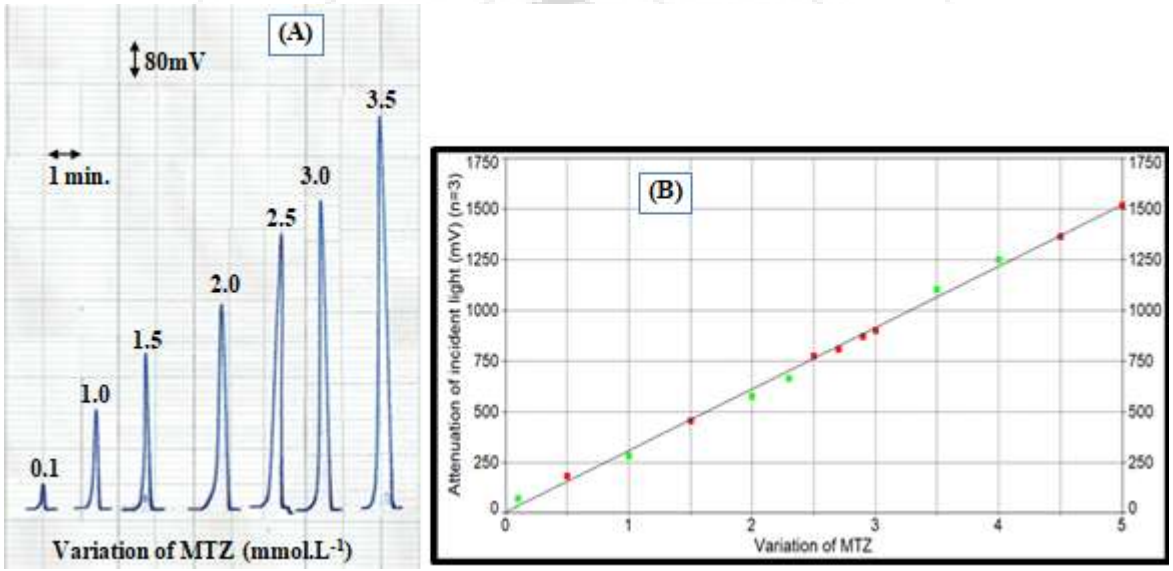


Figure 5: (A) Response profile for variation of metronidazole concentration against time. (B) Calibration graph for metronidazole using developed precipitation method.

Table 3: The results of calibration graph for the estimation of MTZ using precipitation method

Measured [MTZ] mmol.L^{-1}	Straight Line range mmol.L^{-1}	$\hat{Y}_{(mV)}=(a\pm S_a t)+(b\pm S_b t)$ [Metronidazole] mmol.L^{-1} at confidence level 95%, n-2	r r^2 %r^2	t_{tab} at 95% confidence level, n-2	Calculated t-value $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
0.1-6.0	0.1-5.0 n=14	$\hat{Y}$=5.34±32.63+302.91±11.28 [X] mmol.L^{-1}	0.9982 0.9965 %99.65	2.179 << 58.53	

r :correlation coefficient, r^2: coefficient of determination, r^2%: linearity percentage

Table (4) tabulated the calculations of Analysis of varianceand the calculations were indicated that

$F_{tab.} = F^{v_1}_{v_2} = F^{1}_{11} = 4.84 << F_{Stat.} = 3140.37$, therefore, a significant relationship between the concentrations of Metronidazolewith response obtained.

Table 4: Analysis of Variance (ANOVA) for linear regression equation results[18-19]

Source	Sum of squares	D_f	Mean square	$F_{stat}=S_1^2/S_2^2$
Regr. ($\hat{y}_i$-$\bar{y}$)	2498669.18	V_1=1	2498669.18	3140.37
Error (y_i-$\hat{y}_i$)	8752.266	V_2=11	795.66	
Total (y_i - $\bar{y}$)	2507421.42	12		

Limit of Detection (L.O.D)

Limit of detection for metronidazole was accomplishedby three different methods.The first one is depended on the dilution of the minimum concentration in avariation of MTZgraph while the second method based on the value of slope and the third onefrom the linear regression plot as tabulated in Table (5)with asample volume of 79µL.

Table 5: Calculations summary for limit of detection and limit of quantity to determination of MTZat optimum parameter

Minimum concentration (mmol.L^{-1})	Practically based on minimum concentration in calibration graph	Theoretically based on the slope value L.O.D=$3S_B$/slope	Based on the linear equation $\hat{Y}=Y_B+3S_B$	Limit of Quantity L.O.Q $\hat{Y} = Y_B+10S_B$
0.07 mmol.L^{-1}	0.946 µg/Sample	4.017 µg/Sample	3.616 µg/Sample	12.054 µg/Sample

Repeatability

Repeatability of the measurements performance was studied under optimum chemical and physical conditions. Six successive measurements were carried out for 3.0and 3.5mmol.L^{-1} of metronidazole.Table (6) shows the percentage relative standard deviation (%RSD) was less than 1% whichindicatesthe methodologyis reliable trusted method. Response profile of 3.0 and 3.5 mmol.L^{-1} was shown in Figure (6).

Table (6): Repeatability of the determination of MTZ using homemade turbidimeter

[MTZ] mmol.L^{-1}	Number of injection	Attenuation of incident light $\bar{y}i$ (mV)	σ_{n-1}	Repeatability RSD%	Confidence interval of the average at (95%) $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
3.0	6	890.66	7.06	0.786	890.66±7.35
3.5	6	1107.6	3.14	0.284	1107.6±3.30

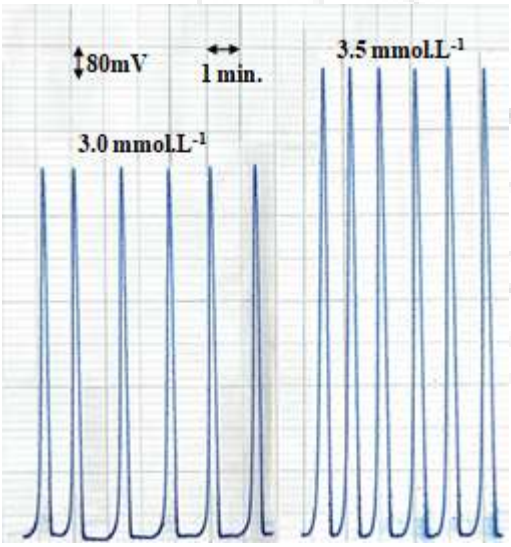


Figure 6: Six successive response profiles versus time for measurements of 3.0 and 3.5 mmol.L^{-1} of metronidazole

TheAnalysis of Pharmaceutical Preparations

The developed method for the determination of metronidazole was used to analysisof drugsin apharmaceutical formula that available in the local market. The solutions of pharmaceutical preparationswere preparedby weighted, crashed, and grindedthirteen tabletsfor each company. Then an amount of powder was weighted as 0.2222 g, 0.2198 g and 0.3244 g for Medazole-SDI, Metrosul-Ajanta, Negazole-Julphar. The weighing powder was dissolvedin distilled water to prepare 0.02 mol.L^{-1}.

Comparison of development method with classical method was carried out via the measurementsof turbidity by HANNA instrument.All obtained results wheresummariesin Table (7-A)and standard additions method was adopted in theanalysis of the three pharmaceutical samples. While Table (7-B) shows the paired t-testcalculations for adeveloped and classical method which appears the calculated t-test is less than tabulated t-testthatexhibits no significant difference between the newlyprecipitation method and the classical method.

Table (7-A): Summary for determination of metronidazole in pharmaceutical samples using standard addition method

Sample No.	Commercial name, Company, Country, Content,	Confidence the interval for the average tablet weight $\bar{W} \pm 1.96\frac{\sigma_{n-1}}{\sqrt{n}}$ (g)	Weight of Sample equivalent to 0.1712 g of active ingredient to obtain 0.02 mol.L^{-1} (g)	Theoretical content of active ingredient at 95% (mg)	Development method using Ayah 6SX1-ST-2D solar cell turbidimeter combine with CFIA (mV) / Classical method using HANNA turbidimeter (NTU)		
					Equation of standard addition curve at 95% for n-2 $\hat{Y}_{(mV)}$=a + bX [MTZ]mmol.L^{-1}	r r^2	found content of active ingredient at 95% (mg)
1	Medazole, SDI, Iraq, 500 mg	0.6491 ±0.0137	0.2222	500 ± 0.034	$\hat{Y}_{(mV)}$= 89.73 + 224.53 [X] mmol.L^{-1}	0.9905 0.9239	499.51 ±1.16
					$\hat{Y}$=111.89+77.65[X] mmol.L^{-1}	0.9978 0.9957	496.78 ± 1.53
2	Metrosul, Ajanta, India, 500 mg	0.6422 ±0.0061	0.2198	500 ± 0.052	$\hat{Y}$ = 168.16 + 403.78[X] mmol.L^{-1}	0.9932 0.9865	521.15 ± 2.44
					$\hat{Y}$ =125.66+82.27[X] mmol.L^{-1}	0.9965 0.9913	512.29 ± 1.68
3	Negazole, Julphar, UAE, 500 mg	0.9475 ±0.0040	0.3244	500 ± 0.041	$\hat{Y}$ = 110.76 + 269.32[X] mmol.L^{-1}	0.9941 0.9883	511.04 ± 1.27
					$\hat{Y}$ =95.42+97.41[X] mmol.L^{-1}	0.9970 0.9942	509.87 ± 1.21

Table (8-B): Summary of the efficiency and comparison of the development method with classical method using paired t-test for determination the determination of MTZin pharmaceutical formulations

Sample No.	Practical content (mg)		Paired t-test $t = \frac{\bar{x}_d\sqrt{n}}{\sigma_{n-1}}$	t_{tab} at 95% confidence interval n-1	Efficiency of determination (%Rec)
	Development method	Classical method			
1	499.51	496.78	2.46< 4.303		99.902
2	521.15	512.29	2.73< 4.303		104.23
3	511.04	509.87	2.24< 4.303		102.208

6. Conclusion

The proposed method demonstrated the ability for the determination of MTZ in real samples by precipitation reaction of the MTZ with phosphotungstic acid using a fabricationsimple flow injection with alocally made turbidimeter (Ayah 6SX1-ST-2D solar cell). The adopted method is characterized by thehigh precision with %RSD less than 1%, sensitive, and speed.

The developed turbidimetric-FIA procedure which is used for analysis of MTZ in pharmaceutical preparationshas shownnointerferencewith inactive ingredients commonly accompanying the drug in those formulations. Therefore, the proposed method can use as an alternative method for analysis of metronidazole in apharmaceutical preparation.

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