



## Original Research Article

# A Pioneered Homemade NAG-4SX3-3D Analyzer Coupled with Continuous Flow Injection Analysis New Approach for The On-Line Turbidimetric Measurements of Metronidazole in Pure and Pharmaceutical Formulations

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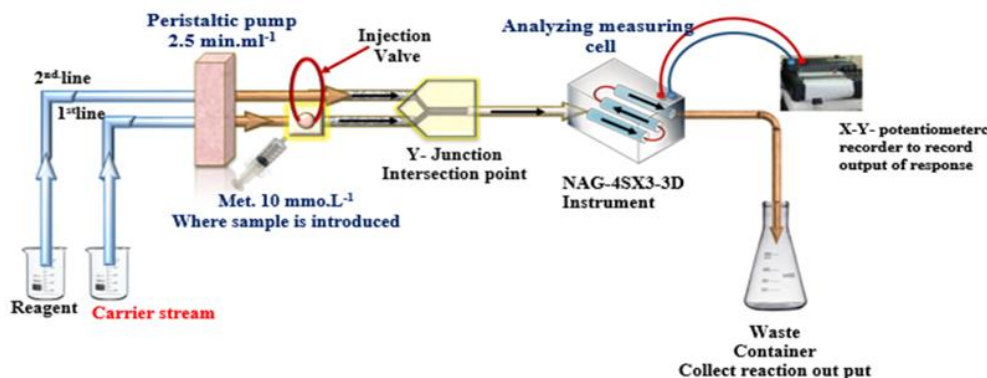
turbidity

NAG-4SX3-3D analyzer

## ABSTRACT

The detection of metronidazole (MTZ) in pure and pharmaceutical formulations is now possible with a newly developed analytical technique based on turbidimetric measurement (from 0 to 180 degrees) utilizing a NAG-4SX3-3D analyzer. The technique relied on the fact that phosphotungstic acid and metronidazole would precipitate white in an acidic medium, indicating the presence of an ion pair molecule. In order to measure turbidity, we used the attenuation of light after it collided with precipitated particles on surfaces with angles ranging from 0 to 180 degrees. We looked into both chemical and physical parameters. With a linear dynamic from 0.05 to 20 mmol.L<sup>-1</sup>, metronidazole has a correlation coefficient value @ of 0.9993. From the step-wise dilution for the least concentration in the linear dynamic range of the calibration graph with an RSD percent lower than 0.5 percent, we find that the detection limit for 3, 35 mmol.L<sup>-1</sup> (n = 8) metronidazole is 29.953 ng/sample. Four medicinal items have been successfully identified using this method. Standard addition and a t-test were used to compare the newly developed method of analysis to the tried-and-true methods (the HANNA equipment for turbidity measurement and the UV-spectrophotometric method). The researchers found no significant difference between the three methods at the 95% confidence level.

## GRAPHICAL ABSTRACT



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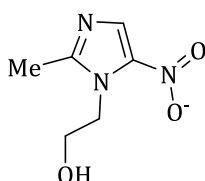
## Introduction

Metronidazole (MTZ), also known as 1-(hydroxyethyl)-2-methyl-5-nitroimidazole (Scheme 1), is a nitroimidazole derivative that was initially used as a systemic antitrichomonal drug in 1960 [1].

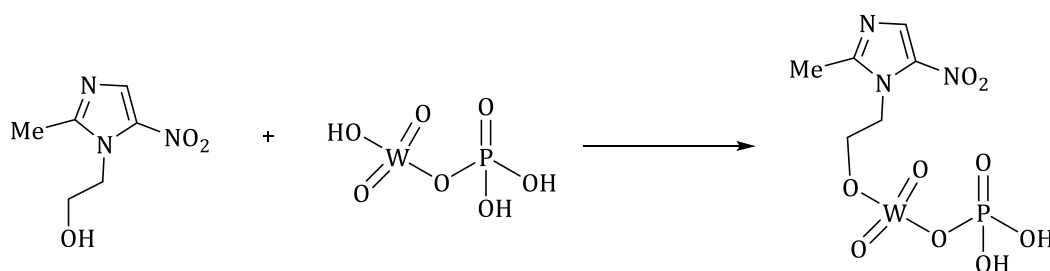
Metronidazole is one of the primary medications for treating infections [2] caused by anaerobic bacteria, protozoa, and microaerophilic bacteria. Microorganisms that can tolerate anaerobic conditions are cytotoxic. It treats various conditions, including gastrointestinal amebiasis, liver amebiasis [3, 4], bacterial septicemia; bone and joint infections; meningitis; brain abscess; endocarditis; endometritis; bacterial vaginitis; and surgical prophylaxis for colorectal procedures. The action mechanism is based on nitro group partial reduction. Some biological effects can be attributed to partially decreased metabolites due to their ability to attach to macromolecules in bacteria and cells. Bacterial DNA reacts with reactive metabolites to stifle

DNA and protein synthesis, eventually leading to cell death. DNA is known to interact with biological macromolecules in both humans and animals. Human DNA single-strand breaks were detected after a single therapeutic dose of metronidazole. In vitro, human lymphocyte cultures confirmed the same findings. High-performance liquid chromatography (HPLC)-spectrometry [5, 6], gas chromatography/mass spectrometry (GC/MS) [7, 8], capillary electrophoresis [9], and thin-layer chromatography (TLC) [10] are just some of the technologies that have been used to detect metronidazole.

The advantages of CFIA-Turbidity are its inexpensive price, fast response time, excellent sensitivity, and potential for downsizing. Scheme 2 shows the thought-out mechanism: metronidazole interacts with phosphotungstic acid as a precipitating agent in an acidic solution to form a white precipitate [11].



**Scheme 1:** Chemical structure of metronidazole



**Scheme 2:** The proposed mechanism of reaction metronidazole with phosphotungstic acid

## Material and Methods

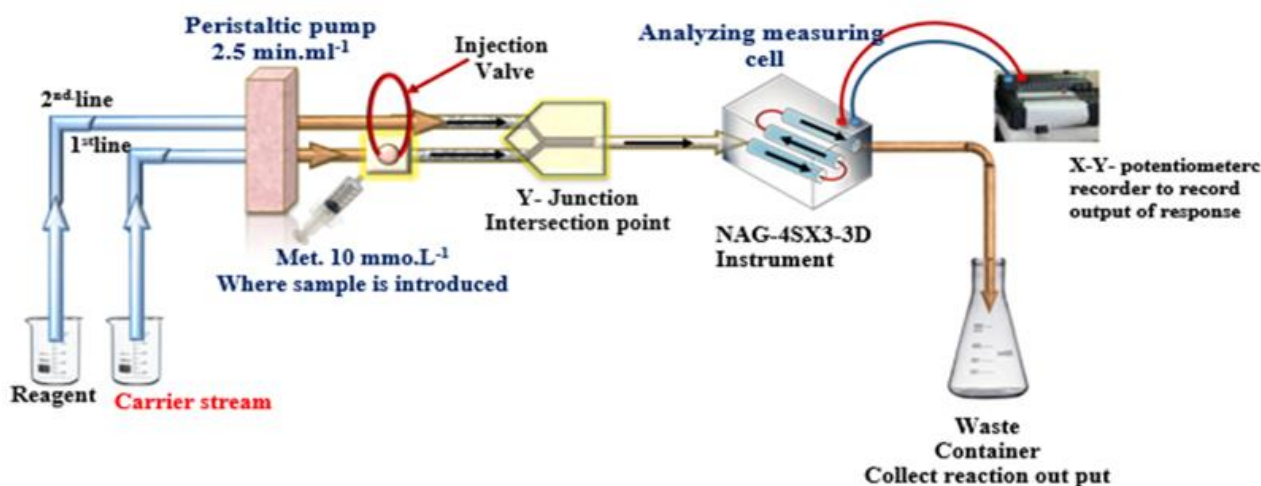
The chemicals used to make the solutions were of analytical reagent quality, and all of the water used was distilled. Dissolving 0.58875 g of metronidazole (molecular weight 171.15 g.mol<sup>-1</sup>, BDH) in 100 mL of distilled water yielded a 0.05 M metronidazole standard solution (C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>).

To create a 0.012 M standard solution, 250 mL of distilled water was combined with 8.6406 grams of phosphotungstic acid (H<sub>3</sub>PW<sub>12</sub>O<sub>40</sub>) with a molecular weight of 2880.2 g.mol<sup>-1</sup> (Hopkin and Williams LTD).

### Instruments

A flow cell derived from a home-built NAG-4SX3-3D analyzer was utilized [12-14]. The output of incoming light's 0-180 °attenuation was recorded (Figure 1). A potentiometric recorder (Siemens, Germany) captured the signal outputs. With a

sample loop and a six-port injection valve, an Ismatic peristaltic pump (Teflon, variable length). For the conventional procedures, turbidimetry and UV spectrophotometric (Shimadzu, Japan) equipment were utilized.



**Figure 1:** Diagram of the manifold used to measure metronidazole, which is determined by the white precipitate that is produced when metronidazole reacts with phosphotungstic acid

### Optimization of reaction parameters

#### Chemical parameters

Chemical parameters such as medium type, medium acid concentration, and phosphotungstic acid concentration as precipitating agents were investigated to optimize optimal settings for maximum response and sensitivity. It was discovered that 7 mmol/L of phosphotungstic acid was the best concentration, as shown in Figure 2. A, sulfuric acid was selected as the reaction medium, as shown in figure 4. B, and the concentration of sulfuric acid was 70 mmol.L<sup>-1</sup>. This concentration of sulfuric acid was found to be the best concentration of sulfuric acid as a carrier stream for the (metronidazole-phosphotungstic acid) system because it gave the highest response and will be used in subsequent studies, as shown in Figure 2c.

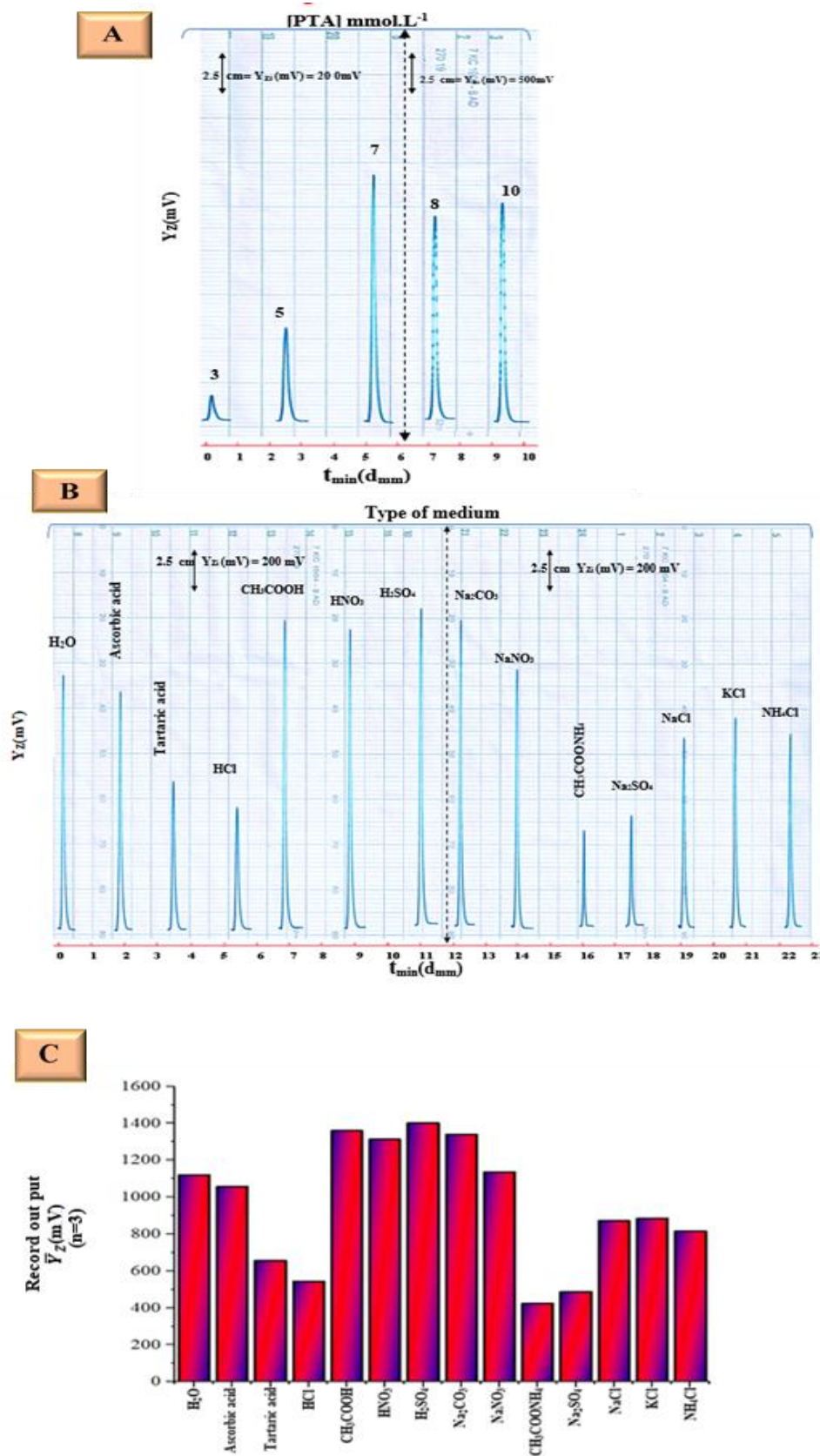
#### Physical parameters

Physical parameters such as flow rate, length of reaction coil, injected volume, and Y-junction

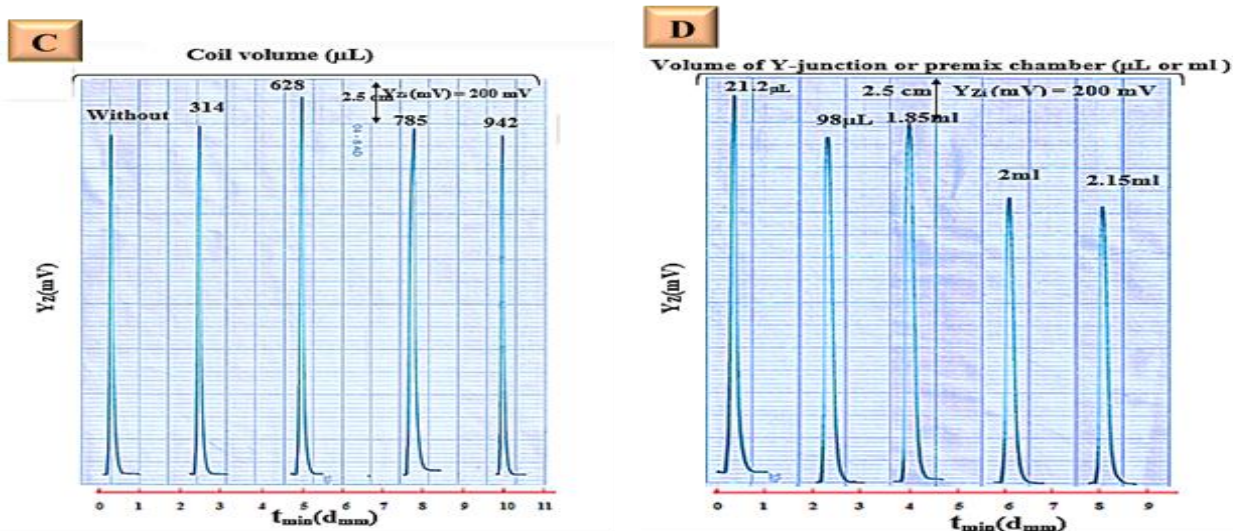
were studied for their impacts on the response of metronidazole to phosphotungstic acid. It was discovered that the best conditions were: a flow rate of 2.5 ml.min<sup>-1</sup>, a sample volume of 175 µl, 314 µl of reaction coil, and a choice of 21.2 µl to obtain the best response with high sensitivity and selectivity with low material consumption. The results are represented in Figure 3a, b, c, and d.

#### Changes in light attenuation energy transducer output as a function of metronidazole concentration

The calibration graph also referred to as a standard curve in analytical chemistry, is a general procedure used to determine the chemical substance concentration in an unknown sample by creating sets of known concentrations and contrasting them with the unknown. The calibration curve was created for the proposed method by applying all of the best chemical and physical parameters that were selected. Following that, a series of metronidazole solutions (0.05–35 mmol. L<sup>-1</sup>) were made.



**Figure 2:** a) Effect of reagent concentrations on the response profile, b) Effect of medium type (acids and salts) on response profile " $Y_{zi} - t$  (min)" (dmm), c) Attenuation of energy transducer response for the detection of metronidazole by the precipitate formation



**Figure 3:** a) Effect of flow rate on the response profile, b) Effect of sample volume on response profile " $Y_{zi} - t_{min}$ " (dmm), c) Influence of reaction coil volume on the response profile, d) Light attenuation caused by Y-junction influence

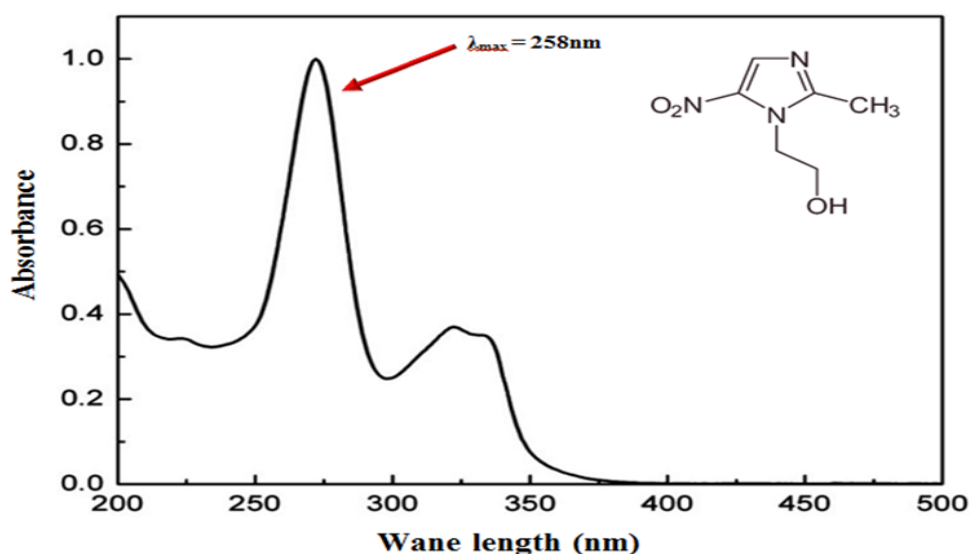
The NAG-4SX3-3D analyzer's energy transducer response changed linearly from 0.05 to 20 mmol.  $\text{L}^{-1}$  in response to metronidazole concentration, with a correlation of  $\bar{Y}_{zi}$  (Mv) versus  $t_{min}$  (dmm) of 0.9993 and a coefficient of determination of 0.9987. This linear response account for 99.87% of the results.

The evaluation of a newly created approach (i.e., the NAG-4SX3-3D analyzer) for the detection of metronidazole was compared with two methods described in the literature: the UV-spectrophotometric method at  $\lambda_{max} = 258 \text{ nm}$

(Figure 4) and the turbidimetric method as shown in Table 1.

#### Detection limit

Metronidazole's detection level was investigated from three aspects: practically, through increasing dilution; theoretically, through a slope value and experimentally, using a standard curve. The detection limit for the metronidazole-phosphotungstate-hydrogen sulfide (PTA- $\text{H}_2\text{SO}_4$ ) system is calculated in Table 2.



**Figure 4:** Absorbance of UV- Spectrum of metronidazole at a concentration of 0. 4mmol. $\text{L}^{-1}$  that shows  $\lambda_{max} = 258 \text{ nm}$

**Table 1:** Using a first-degree equation of the form  $\bar{Y}=a+ b x$  at optimal circumstances for developed technique and spectrophotometer and Turbidimetric method, this summary provides linear regression findings for the variability of the (S/N) energy transducer response as a function of metronidazole concentration (classical methods)

Type of mode	[Met] range mmol.L <sup>-1</sup> (n)	$\hat{Y}_{zi}=a \pm S_a t+b(\Delta y / \Delta x_{\text{mmol/L}}) \pm S_b t$ [Met] mmol.L <sup>-1</sup> at confidence level 95%, n-2	R r <sup>2</sup> R <sup>2</sup> %	t <sub>tab</sub> at 95%, n-2	Calculated t-value $t_{\text{cal}}=r/\sqrt{n-2} / \sqrt{1-r^2}$
Developed method (mV)					
UV-spectrophotometriy at $\lambda_{\text{max}}$ 258nm					
Turbidimetry (NTU)					
Linear range or linear dynamic range	0.05-20(13)	62.2965±48.0701+167.4564±4.0327	0.9993 0.9987 99.87	2.201 << 91.398	
	0.001-0.7 (19)	0.0225±0.0261+2.5218±0.0839	0.9979 0.9958 99.58	2.110<< 63.433	
	0.1-5(18)	-14.4774±31.1647+205.0840±11.2326	0.9947 0.9894 98.94	2.120 << 38.707	
Working range or calibration range	0.05-25 (14)	154.0493±206.5205+153.2867±15.5410	0.9873 0.9747 97.47	2.179 << 21.490	
	0.001-0.8 (20)	0.0375±0.0383+2.1498±0.1089	0.9959 0.9918 99.18	2.101 << 46.665	
	0.1-5.3 (19)	-2.3166±37.3362+197.8382±12.6068	0.9923 0.9847 98.47	2.110 << 33.109	
Analytical or Dynamic range	0.05-30(15)	262.3284±275.0384+139.0145±18.3433	0.9766 0.9537 95.37	2.160 << 16.372	
	0.001-0.85 (21)	0.0529±0.0502+2.3208±0.1286	0.9934 0.986 98.69	2.093 << 37.761	
	0.1-5.5 (20)	9.6142±42.9703+19.9648±13.6950	0.9897 0.9795 97.95	2.101 << 29.296	
Scatter plot	0.05-35(16)	403.4921± 353.5390+122.5150±20.	0.9586 0.9189 91.89	2.145 << 12.599	
	0.001-0.9 (22)	0.0708±0.0647+2.2136±0.1517	0.9894 0.9789 97.89	2.086 << 30.443	
	0.1-5.7(21)	25.3234±53.2387+182.2295±16.1083	0.9835 0.9672 96.72	2.93 3.678	

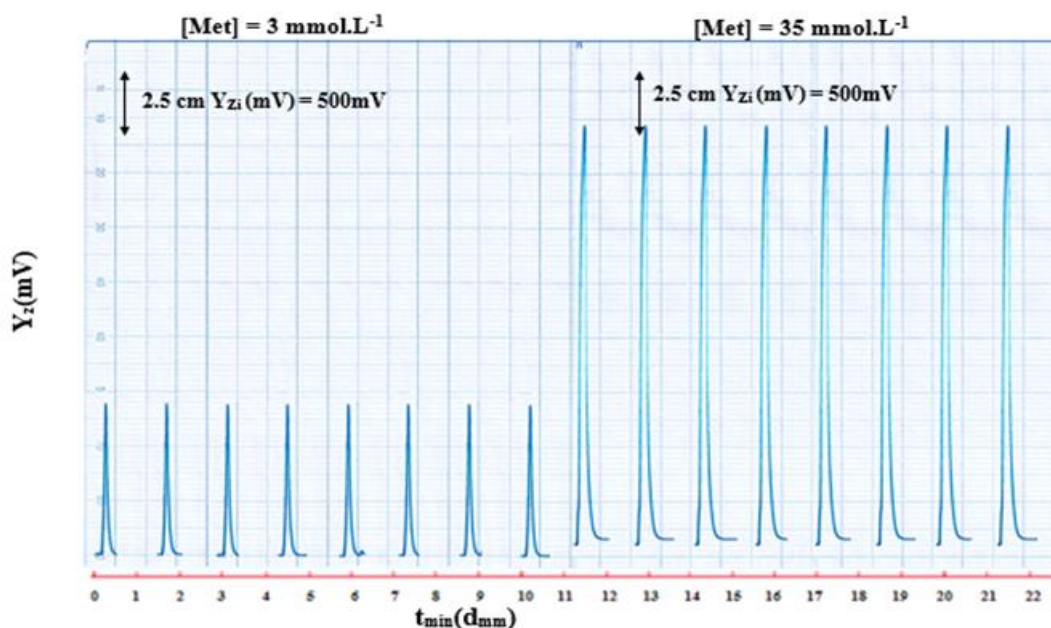
### Repeatability

The repeatability of a proposed or new method is measured by comparing the results from different preparations of the same analyte. The same operator, instrument, laboratory, and materials are used for this measurement; the only

variable is the number of measurements for the same analyte concentration. Therefore, eight injections were evaluated at a constant metronidazole concentration. Two distinct intensities were present (3 and 35 mmol.L<sup>-1</sup>). The relative standard deviation was less than 5%, as shown in Figure 5 and Table 3.

**Table 2:** Detection Limit of Metronidazole -PTA - H<sub>2</sub>SO<sub>4</sub> system Using 175 µL injection Sample and Optimal parameters

Scatter plots practically show the lowest concentration decreasing over time.		From a theoretical standpoint (and taking into account the slope's value), $x = 3S_b / \text{slope}$	Linear-equation-based theory $\hat{Y} = Y_b + 3S_b$	Limit of quantitative
Newly developed method (0.001)mmo.L <sup>-1</sup>	Spectrophotometric analysis, a classic technique (0.0008) mmol.L <sup>-1</sup> for Met.-PTA system ----- Turbidity approach (0.05) mmol.L <sup>-1</sup> Met.-PTA system			
29.953 ng/sample	0.1369 µg/ sample ----- 85.5800 µg/ sample	0.5806 µg/sample	24.7702 µg/sample	82.5655 µg/sample



**Figure 5:** A profile of eight successive measurements with repeatability of profile for 3 mmol

**Table 3:** Repeatability of Met-PTA-H<sub>2</sub>SO<sub>4</sub> system

[Met] mmol.L <sup>-1</sup>	$\bar{Y}_{zi}$ (mV) (n=8)	RSD %	The Interval of Confidence at 95%
3	560	0.34	560±1.6135
35	3830	0.07	3830±2.3826

L<sup>-1</sup> and 35 mmol. L<sup>-1</sup> concentration of [Metronidazole]  
 $t_{0.05/2,8}=2.3646$ , n= number of injection

### Determination of metronidazole in drugs using NAG-4SX3-SD analyzer

The devised approach was applied to four samples of medication from four different manufacturers (Flazol, Tabuk, Saudi Arabia, 500 mg); (Metrosule, Ajanta, India, 500 mg); (Negazole, Julphar, U.A.E., 500 mg); and (Metronidazole, Ajanta, India, 500 mg) (Flagyl, Sanofi, Spain, 500 mg). Using UV-spectrophotometric by measuring absorbance at  $\lambda_{\text{max}} = 258\text{nm}$  [15] and the turbidimetric method, the attenuation of incident light at  $0-180^\circ$  for a white precipitate of metronidazole that reacted with phosphotungstic acid in an acidic medium was studied.

Using a newly developed technology (the NAG-4SX3-3D analyzer) and two other techniques (UV-spectrophotometric absorbance measurement at  $\lambda_{\text{max}} = 258\text{ nm}$  and turbidimetric measurement), the data was mathematically processed and displayed in Table 4. (a). See Table 4 instead of (t-test) (Figure 6) [16], which compares two possible outcomes. At a 95% level of confidence and with high efficiency, B supplies the active component's usable content. Table 4. B shows the results of comparing the NAG-4SX3-3D analyzer's calculated t-values with the official stated value (500 mg) for each company.

One way to estimate a hypothesis is to start with the null hypothesis, which states that there is no statistically significant difference between the sample averages.

( $\bar{W}_i$  and quoted value  $\mu$  i.e.,  $H_0: \bar{W}_i = (500\text{ mg})$  for (Flazol, Tabuk, Saudi Arabia, 500 mg)), (Metrosule, Ajanta, India, 500 mg), (Negazole, Julphar, U.A.E., 500 mg), and (Flagyl, Sanofi, Spain, 500 mg).

A large discrepancy exists between the means and the stated number in favor of the null hypothesis (500 mg).

There is a statistically significant discrepancy between the stated and measured amounts of the active ingredient since  $t_{\text{cal}} > t_{\text{tab}} (4.303)$  at a

confidence level of 95% and a degree of freedom of  $= 2$ . This means the null hypothesis was rejected, and the alternative hypothesis was accepted.

The new method performs conventional addition techniques when applied to this foundation.

Since the computed t-value is smaller than the tabulated t-value, this further indicates no statistically significant discrepancies between the established method and the method claimed by the corporation.

As a result (Figure 7), the newly developed method can be utilized as a replacement method for detecting metronidazole in pharmaceuticals.

Second, a paired t-test with a significance level of  $= 0.05$  (2-tailed) was used to compare the newly developed methodology with the NAG-4SX3-3D analyzer to two traditional methods: UV-spectrophotometry with a Shimadzu (double beam UV-1800) spectrophotometer and turbidity with a turbidity meter (HANNA, Hungry). Table 4 displays the results of a measurement of the attenuation of incident light from 0 to 180 degrees. Assumption B: All medications from all manufacturers are for the same population, i.e., no individual differences exist between the manufacturers.

The average of the two approaches shows no discernible difference.

Another possibility is that the mean results from the conventional approach and the NAG-4SX3-3D analyzer differ significantly.

An Optional Hypothesis H1: The NAG-4SX3-3D Analyzer as the Standard Reference Method for Measuring Turbidity.

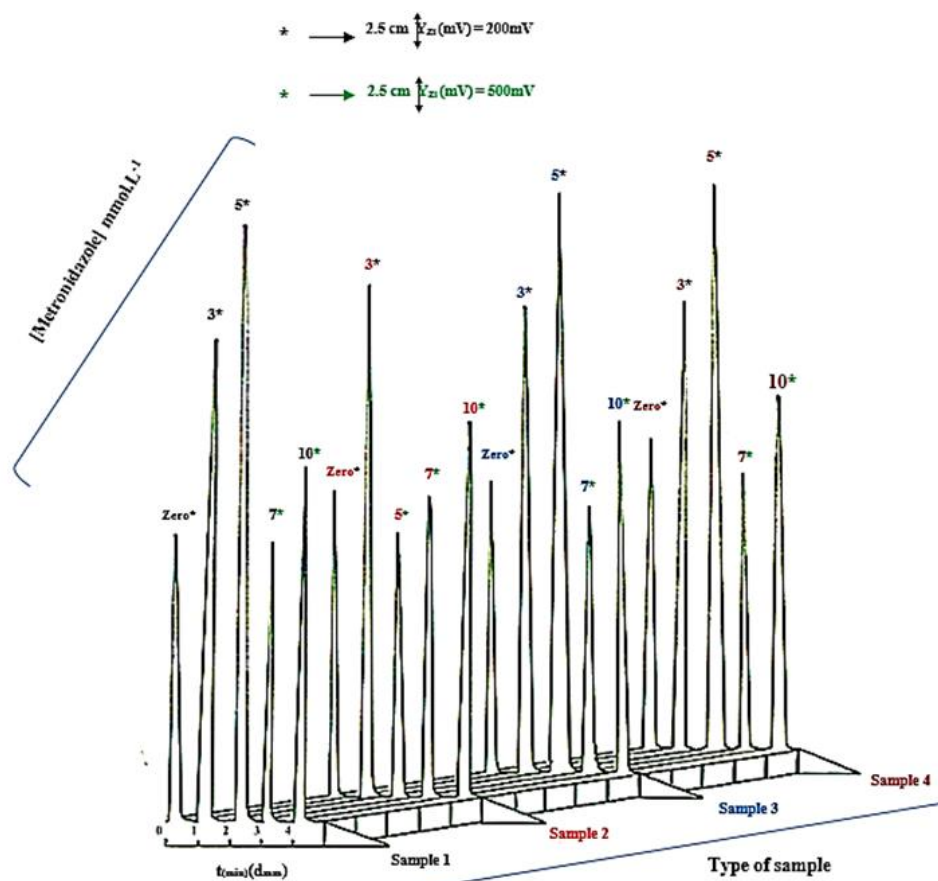
Results obtained reveal no significant difference (at the  $95\% = 0.05$  level of confidence) between the devised approach, the UV-spectrophotometric method, and the turbidity method (traditional methods) (Scheme 2 and Table 4 (b)).

**Table 4 (a):** The NAG-4SX3-3D metronidazole-phosphotungstic acid system analyzer was used to determine the concentration of metronidazole in four medication samples. The results were compared to those obtained using two traditional methods

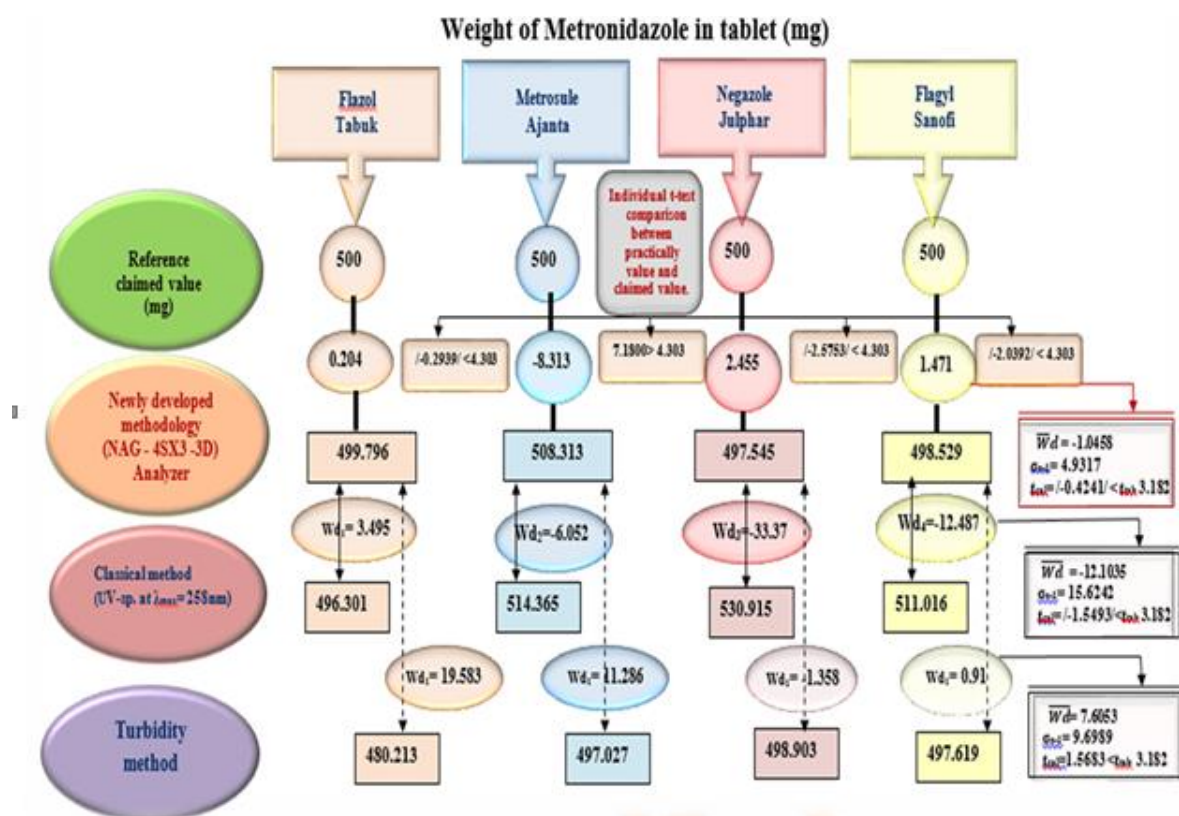
No. of sample	Commercial Name, Company Content Country	Type of method									
		Developed approach									
		UV Spectrometer with a Maximum Wavelength of 258 nm.									
		Classical turbidity (NTU)									
		Confidence interval for the average weight of Tablet $\bar{w} \pm 1.96 \sigma_{\bar{w}} / \sqrt{n}$ at 95% (g)	Weight of Sample equivalent to 0.3423 gm (20 mmol.L <sup>-1</sup> ) of the active ingredient	Theoretical content for the active ingredient at 95% (mg) $W \pm 1.96 \sigma_W / \sqrt{n}$	[Met] mmol.L <sup>-1</sup>					Equation of standard addition at 95% for n-2	
					0 ml	0.6 ml	1 ml	1.4 ml	2 ml	$\hat{Y}_i = a \pm s_{at} + b \pm s_{bt} [\text{Met}] \text{mmol.L}^{-1}$	$r$ $r^2$ $R^{2\%}$
					0	3	5	7	10		
					0 ml	0.01 ml	0.03 ml	0.05 ml	0.07 ml		
					0	0.05	0.15	0.25	0.35		
					0 ml	0.1 ml	0.2 ml	0.3 ml	0.4 ml		
					0 mm	0.5 mm	1 mm	1.5 mm	2 mm		
1	Flazol Tabuk 500mg Saudi Arabia	0.6576±0.0028	0.4502	500±1.9615	920	1540	1900	2230	2830	941.7586±76.9512+188.4483±12.7196	0.9993 0.9987 99.87
					0.111	0.262	0.559	0.848	1.131	0.1158±0.0129+2.9151±0.0629	0.9999 0.9999 99.99
					200	287	350	510	580	188.8000±64.8113+196.6000±52.9182	0.9894 0.9790 97.90
2	Metrosule Ajanta 500mg India	0.6403±0.0031	0.4383	500±2.4193	980	1640	2080	2400	3000	1018.2759±116.6869+200.3448±19.2877	0.9986 0.9973 99.73
					0.112	0.235	0.493	0.761	1.021	0.1073±0.1044+2.6071±0.0510	0.9994 0.9998 99.98
					190	213	326	420	500	164.4000±62.2225+165.4000±50.8045	0.9863 0.9728 97.28
3	Negazol Julphar 500mg U.A.E	0.9614±0.0041	0.6582	500±2.1478	930	1480	1840	2160	2800	918.7241±92.5650+184.6552±15.3005	0.9990 0.9980 99.80
					0.098	0.232	0.421	0.662	0.920	0.0979±0.04651+2.3045±0.2270	0.9986 0.9971 99.71
					193	260	358	440	555	180.4000±34.5419+180.8000±28.2034	0.9964 0.9928 99.28
4	Flagyl Sanofi 500mg Spain	0.6795±0.0128	0.4652	500±9.4314	988	1420	1800	2200	2820	921.4621±162.4792+184.8276±26.8570	0.9969 0.9938 99.38
					0.121	0.262	0.611	0.893	1.181	0.1249±0.0461+3.0545±0.2248	0.9920 0.9984 99.84
					225	300	420	530	640	211.0000±32.4502+212.0000±26.4954	0.9977 0.9954 99.54

**Table 4 (b):** An overview of the metronidazole-phosphotungstic acid system's practical application, efficiency (Rec%) in determining Metronidazole in four medication samples, and individual t-test in comparing mean weight to quoted value

No. of sample	Developed method using NAG - 4SX3 - 3D Analyzer			Individual t-test between claimed value & practical value ( $\bar{W}_{i(mg)}-\mu$ ) $\sqrt{n}/\sigma_{n-1}$	Paired t –test Compared between three methods			
	UV- Spectrophotometer at $\lambda_{max}= 258nm$ .							
	Classical turbidity							
	Practical concentration (mmol.L <sup>-1</sup> ) in 10 ml ----- Practical concentration (mmol.L <sup>-1</sup> ) ----- In100 ml ----- Practical weight of Met. in (g)	Weight of Met in each sample (g) $\bar{W}_{i(g)} \pm 4.303 \sigma_{n-1}/\sqrt{n}$	Efficiency of determination Rec%					
		Weight of Met in tablet $W_{i(g)} \pm 4.303\sigma_{n-1}/\sqrt{n}$			$t_{cal}= \bar{W}_d \sqrt{n} / \sigma_{n-1}^*$	$t_{tab}$ at 95%confidence level (n-1)		
1	4.9974	0.4998±0.003	99.96	/ -0.2939/ < 4.303	Newly developed methodology + quoted value ( reference method )  ----- $\bar{W}_d = - 1.0458$ $\sigma_{n-1}^* = 4.9317$ / -0.4241/ << 3.182			
	19.9890	499.796±2.987						
	0.3421							
	0.0397	0.4963±0.004	99.26					
	19.8500	496.301±4.32						
	0.3398							
2	0.9603	0.4802±0.009	96.04	7.1800 > 4.303	Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182			
	19.2070	480.213±8.97						
	0.3287							
	5.0826	0.5083±0.005	101.66				Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182	
	20.3340	508.313±4.982						
	0.3479							
0.0411	0.5144±0.007	102.87	Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182					
20.5726	514.365±6.83							
0.3521								
0.9939	0.4970±0.003	99.41			Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182			
19.8790	497.027±3.121							
0.3403								
3	4.9750	0.4975±0.004	99.51	/-2.5753 / < 4.303			Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182	
	19.9010	497.545±4.102						
	0.3406							
	0.0425	0.5309±0.007	106.18		Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182			
	21.2360	530.915±7.423						
	0.3635							
0.9978	0.4989±0.003	99.78	Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182					
19.9558	498.903±2.53							
0.3416								
4	4.9860	0.4985±0.003			99.71	/-2.0392 / < 4.303	Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182	
	19.9420	498.529±3.104						
	0.3413							
	0.0409	0.5110±0.008	102.20	Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182				
	20.4415	511.016±7.98						
	0.3499							
0.9953	0.4976±0.003	99.52	Newly developed methodology and UV- spectrophotometric ( classical method )  ----- $\bar{W}_d = -12.1035$ $\sigma_{n-1}^* = 15.6242$ /-1.5493/ < 3.182					
19.9057	497.619±3.298							
0.3407								



**Figure 6:** Profile - time for standardized Met-PTA system addition procedure involving four companies  
standardized Met-PTA system addition procedure involving four companies



**Figure 7:** Set of results for comparing actual content to claimed content by (Individual t-test) and Paired t-test

Figure 8, four-by-four ANOVA [17, 18], describes the treatment of the data obtained for analyzing four drugs with the same active ingredient content manufactured by different supplier companies. The columns represent the drug sample supplied by different suppliers, while the rows represent methods. While table 5 tests the effect between subjects (i.e., Supplier Company) and shows the interaction of methods and different samples by ANOVA table that shows the sum of squares, degree of freedoms, means squares, and F- value. The assumption is made to accept  $H_0$  (Null hypothesis).

( $H_0$ :  $\mu_{\text{Flazol}} - \text{Saudia Arabia} = \mu_{\text{metrosul}} - \text{India} = \mu_{\text{Negazole}} - \text{UAE} = \mu_{\text{Flagyl}} - \text{Spain}$ ). If the significant level is greater than 0.05 at a 95% confidence level (i.e.,  $F_{\text{cal}} < F_{\text{tab}}$ ) there is no difference between all used mean

of each sample from different companies concerning the output of results. The alternative proposed is that there is a significant difference between all means of values (i.e.,  $H_1$  (alternative hypothesis))

$H_1$ :  $\mu_{\text{Flazol}} - \text{Saudia Arabia} \neq \mu_{\text{metrosul}} - \text{India} \neq \mu_{\text{Negazole}} - \text{UAE} \neq \mu_{\text{Flagyl}} - \text{Spain}$

The obtained results that were summarized in Table 5 show that there is no significant difference between the means of samples, the value of  $F_{\text{cal}}$  (1.127981)  $<<$   $F_{\text{tab}}$  (3.490295).

Therefore Null hypothesis will be accepted and will reject the alternative hypothesis. These mean this means there are no significant differences between the four companies for used samples (four samples).

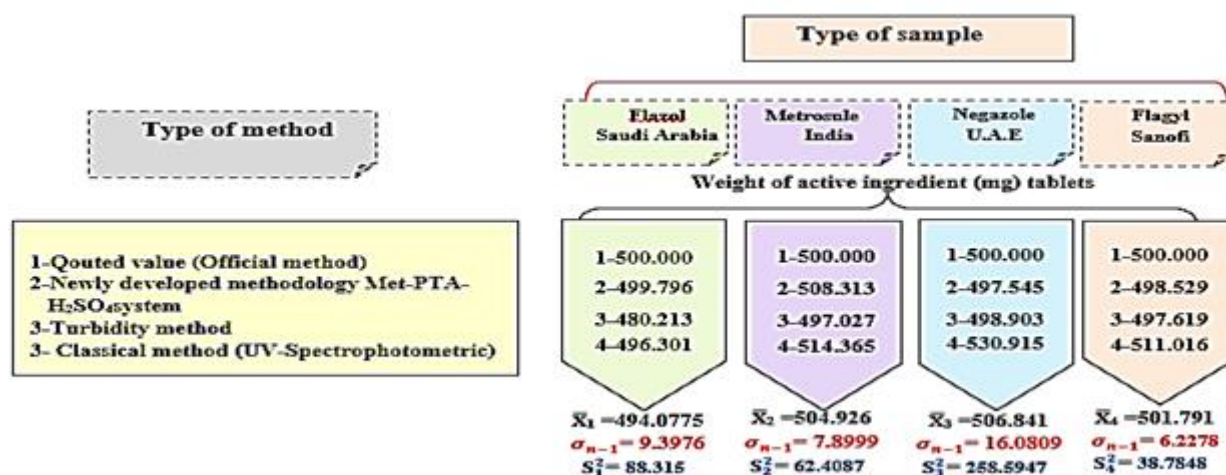


Figure 8: Results obtained via utilizing ANOVA

Table 5: Comparison of four samples from two companies using analysis of variance

Source	Sum of squares (SSq)	Df	Mean square (MSq)	$F_{\text{cal}}$	$F_{\text{critical}}$
Between group	$SS_B = 379.0891$	3	$MS_B = 126.3630$	1.127981 < 3.490295	
Within groups	$SS_W = 1344.31$	12	$MS_W = 112.02587$		
Total		15			

## Conclusion

The conclusion indicates that the four used data of different drugs using continuous flow injection analysis method with NAG-4SX3-3D Analyzer can be used as the reference method that gave a satisfactory result when it was compared with the standard reference method.

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### Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

### Conflict of Interest

We have no conflicts of interest to disclose.

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