RESEARCH ARTICLE

# Simple and Rapid Method For Estimate of Propranolol With Bi (III) Via Long-Distance Chasing Photometer (NAG-ADF-300-2) Utilization Continuous Flow Injection Analysis

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## ABSTRACT

A simple, sensitive and rapid method was used for the estimate of: Propranolol with Bi (III) to prove the efficiency, reliability and repeatability of the long distance chasing photometer (NAG-ADF-300-2) using continuous flow injection analysis. The method is based on a reaction between propranolol and Bi (III) in an aqueous medium to obtain a yellow precipitate. Optimum parameters were studied to increase the sensitivity for the developed method. A linear range for calibration graph was 0.1-25 mmol/L for cell A and 1–40 mmol/L for cell B, and LOD 51.8698 ng/200 µL and 363.0886 ng /200 µL , respectively to cell A and cell B with correlation coefficient (r) 0.9975 for cell A, 0.9966 for cell B, RSD% was lower than 1%, (n = 8) for the determination of propranolol at concentration (0.5,10 and 25) mmol/L, respectively to cell A and cell B. Results were compared with classical methods UV-Spectrophotometric at $\lambda_{max}$ = 289 nm and turbidimetric method by using standard addition method via t-test at 95% level confidence. The comparison of data explains that long-distance chasing photometer (NAG-ADF-300-2) is the choice with extended stellar detection and broad application.

**Keywords:** Attenuation of light, Continuous flow injection analysis, Diverged light, Fluorescence, Propranolol.

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**Conflict of interest:** None

## INTRODUCTION

Propranolol [1-isopropylamino-3-(1-naphthyloxy)-2-propranolol] is a β-adrenergic blocking drug for type II of antiarrhythmic. Molecular Weight 259.349 g/mol, Molecular Formula $C_{16}H_{21}NO_2$, White, crystalline solid, which is ready soluble in water and ethanol.[1] Propranolol is commonly used in the management of hypertension, angina, pectoris, cardiac dysrhythmias, hypertrophic obstructive cardiomyopathy, myocardial infarction, anxiety, essential tremor and migraine. Structure of propranolol Figure 1.[2-4]

Several methods depend on continuous flow injection analysis[5-10] and Several methods have been reported for propranolol and include Spectrophotometric methods,[2,11] indirect Spectrophotometric,[12] Near Infrared Spectrometry,4 UV-VIS Spectra, 3 Titrimetric,[13] total leucocyte count (TLC),[14] high-performance liquid chromatography (HPLC),[15-17] oxidative cleavage,[18] Oxidation of propranolol,[19,20] Conductmetric Titration,[21] UV-Spectrophotometric,[22] Study of adsorption of drug on activated charcoal and coal of shell eggs.[23]

In this work, study and determination of propranolol with Bi (III), they obtained resultant signals which resulted from the attenuation of the incident light on particulate surfaces of yellow precipitate using a new long-distance chasing photometer for 300 mm length with 2 mm path length to chase and to accumulate output resulted from attenuated incident light 0-180° via two flow cells of 110 mm and 60 mm length (NAG-ADF-300-2).[24]

### Chemicals and Apparatus

*Reagents and chemicals*

The reagents of Each analytical chemical were used, and all the solutions dissolved by distilled water. A standard solution of 100 mmol/L of propranolol, molecular weight 259.349 g/mol,

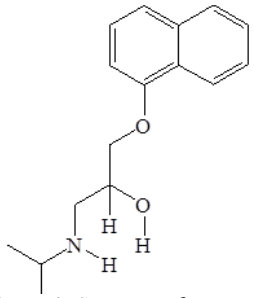


**Figure 1:** Structure of propranolol

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was prepared by Dissolving 2.5935 g in 100 mL. A series of Bi (III) solutions were prepared from the dilution of standard solution 50 mmol/L with distilled water.

*Apparatus*

A homemade NAG-ADF-300-2 is a long-distance chasing photometer as a flow cell will have 300 mm as a distance with 2 mm as a path length to chaise and to accumulate the output resulted from attenuation of incident light 0-180° and diverged or fluorescence light at 0-90° via a flow cell. The first flow cell is of 110 mm length covered with 11 white snow LED (WSLED) followed by uncovered distance of 100 mm length then attached to another with 2 solar cell at each side of (0–180° and 0–90° ) cell (cell number 2), which is covered by six WSLED and a single photo cell (solar) of 55 mm length at each side was used with peristaltic pump (Ismatec, Switzerland) and six-port medium pressure injection valve (IDEX Corporation, USA) with sample loop (1 mm i.e., Teflon, variable length). Potentiometric recorder to estimate the output signals (Siemens, Germany). UV-Spectrophotometric (RF-1501, shimadzu, Japan) was used for classical methods. A turbidimetry instrument, HANNA Company (Hungary), which is used for the classical method measurement of turbidity at 0–180°.

## METHODOLOGY

Using a manifold of two lines connected with NAG-ADF-300-2 analyzer to an evaluation of propranolol via its reaction with Bi (III) as shown in Figure 2. The first line is as a carrier stream at 3.6,2 mL/min flow rate (distilled water), respectively to cell A and cell B passing through the injection valve to carry the segment of sample propranolol (10 mmol/L) to meet with Bi (III) (10,12 mmol/L) for cell A and cell B in the line of second at 5.8,3.1 mL/min flow rate, respectively to cell A and cell B at a Y-junction point before it is introduced to the NAG-ADF-300-2 analyzer.

The obtained resultant signals which resulted from the attenuation of the incident light on particulate surfaces (i.e.; the precipitated particulate is yellow).

It can be noticed that the result obtained from cell A are higher in sensitivity than the cell B output signals.

The higher no. of $WSLED_S$ and solar cell detection available for cell A gives most probably the reason of this higher sensitivity compared with reference to cell B.

Scheme 1. shows a proposed expected mechanism for the reaction of propranolol with Bi (III) in an aqueous medium.[19, 20]

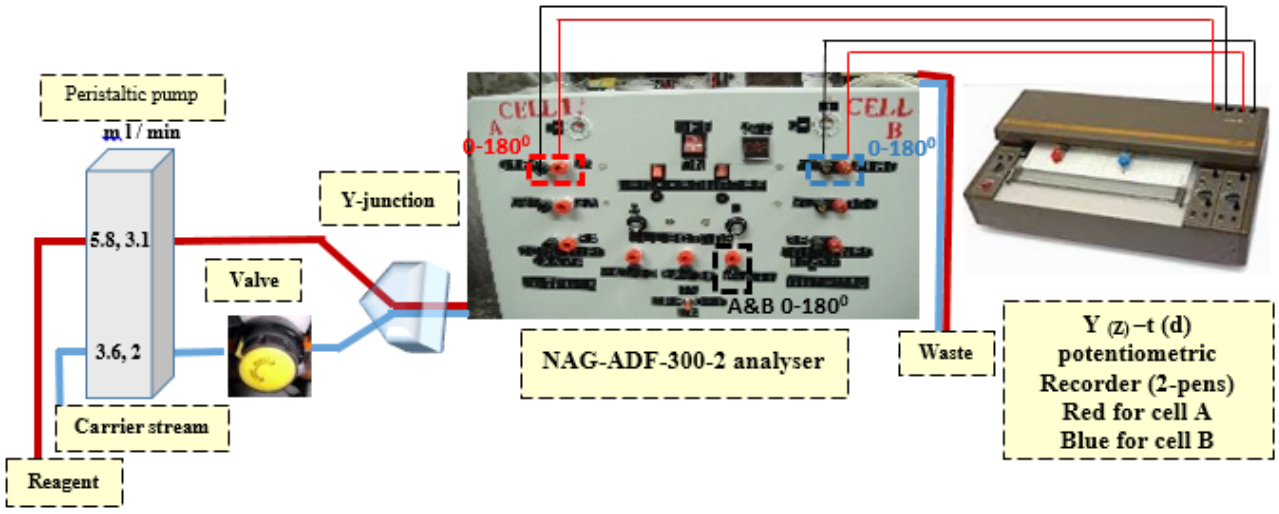
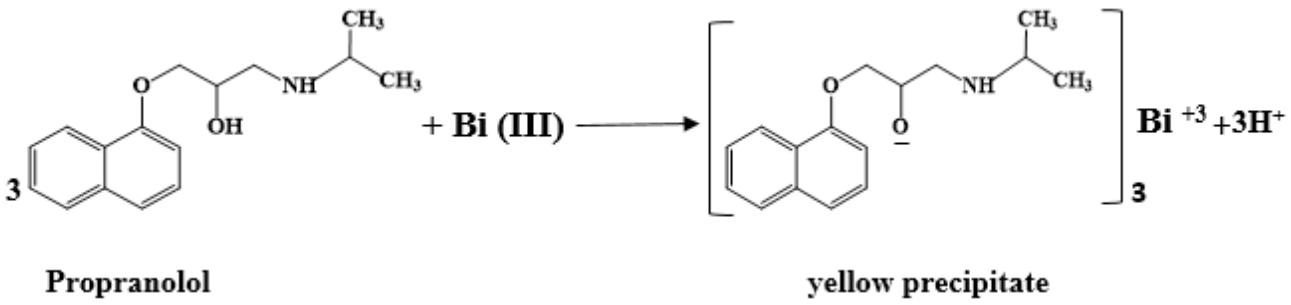



**Figure 2:** Flowgram manifold flow injection analysis system used for the determination of propranolol. Using volume of sample 200 µL, [propranolol] = 10 mmol/L, [Bi (III)] = 10, 12 mmol/L for cell A and cell B respectively, flow rate of carrier stream=3.6, 2 ml/min respectively for cell A and cell B, speed of recorder of recorder=60 cm/hr., Intensity I=3 for cell A, I=2 for cell B with enlarged NAG-ADF-300 -2 unit (working keys).




**Scheme 1:** Probable mechanism pathway for the reaction of propranolol with Bi (III).

## RESULTS AND DISCUSSION

### Study of the Optimum Intensity Used for Cell A and Cell B

A study was conducted for the effect of intensity of incident light for the irradiation sources on the S/N – response of the energy transducer via the selector switch (C.F diagram of NAG-ADF-300-2 analyzer Figure 2). The selector switch gives 0-1-2-3-4, i.e., four choices plus the off position for both cells individually controlled.

It was noticed from Table 1 that a selection of three positions (i.e., I = 3) was a very convenient intensity for cell no.1 (cell A) (larger number of the selector switch means more light intensity), while position 2 (I = 2) of the selector switch was convenient intensity. The higher intensity (I = 3) for cell A refers to that precipitated particulate that is formed are line particles and dense; therefore, the high light intensity is required. While it was not necessary to use high light intensity for cell B due to the conglomeration of precipitated particulates and interspaces are formed. Therefore, low intensity of height is required for cell B.

### Study of the Optimum Parameters

The flow injection manifold system, as shown in Figure 2. Was investigated in the relation of chemical and physical variables, to obtain optimum conditions for the system. They were optimized by making all variables constant and varying one at a time, i.e., fixed, variable optimization.

### Chemical Variables

*Bi (III) concentration*

Different concentrations of precipitating reagent 1–12 mmol/L were prepared of 100 µl volume of sample was injected through the carrier stream (distilled water). 10 mmol/L concentration of propranolol was injected with 3.2, 4.6 mL/min flow rate for carrier stream and reagent, respectively. In addition to I = 3 for cell A, I = 2 for cell B. From Figure 3. A and B, it was found that an increase in peak height expressed as attenuation of incident light with an increase of Bi (III) concentration.

It is possible that might be attributed to the increase of colored precipitate particulate, which in turn work on the attenuation of part of the incident irradiation light plus it's

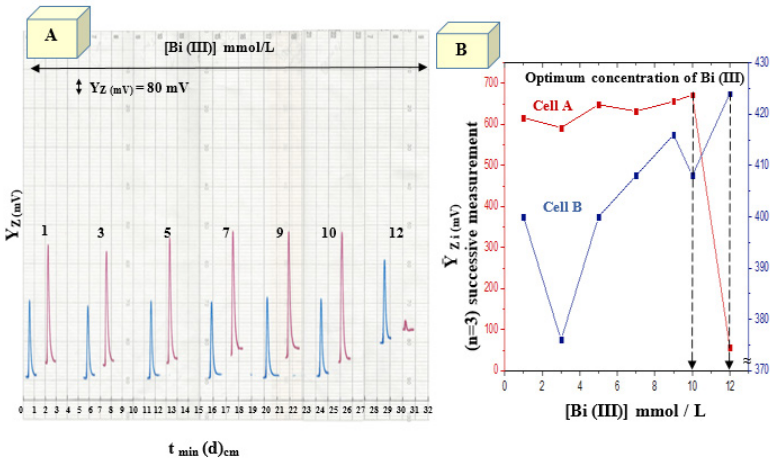



**Figure 3. A:** Effect of variable concentration of Bi (III) on S/N energy transducer response.
**B:** Attenuation of incident light expressed as an average peak height versus Bi (III) Concentration.

**Table 1:** Effect of variable intensity on the attenuation of incident light for the reaction between [Propranolol] 10 mmol/L with [Bi (III)] 7 mmol/L, sample volume 100 µL, flow rate of Carrier stream = 3.2 mL/min and speed of recorder =60 cm/hr.

| *Intensity(I)* | *Attenuation of incident light expressed as an average peak heights (n = 3)* $\bar{Y}_{Z\,i\,(mV)}$ | *RSD%* | *Confidence interval at (95%)* $\bar{Y}_{Z\,i\,(mV)} \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| Cell A | | | |
| 1 | 264 | 0.3485 | 264 ± 2.2856 |
| 2 | 376 | 0.1277 | 376 ± 1.1925 |
| 3 | 632 | 0.1915 | 632 ± 3.0060 |
| 4 | 128 | 0.9531 | 128 ± 3.0309 |
| Cell B | | | |
| 1 | 372 | 0.2715 | 372±2.5092 |
| 2 | 380 | 0.2974 | 380 ± 2.8073 |
| 3 | 104 | 0.9808 | 104 ± 2.5340 |
| 4 | 64 | 0.9063 | 64 ± 1.4409 |
| | Cell A & Cell B | Cell A & Cell B | Cell A & Cell B |
| Cell A(I = 2) and Cell B (I = 3) | 408 and104 | 0.4461 and 0.9712 | 408±4.5215 and 104±2.5092 |
| Cell A(I = 3) and Cell B (I = 4) | 624 and 64 | 0.2452 and 0.8125 | 624±3.8010 and 64±1.2919 |
| Cell A(I = 3) and Cell B (I = 2) | 632 and 408 | 0.3022 and 0.3750 | 632 ± 4.7451 and 408±3.8010 |

$t_{0.05/2,2} = 4.303$, $\bar{Y}_{Z\,i\,(mV)}$: (S/N) energy transducer response of cell A and cell B in mV for n = 3

absorption due to its coloration. Also, a decrease in the resultant intensity due to its penetration to precipitate more particulate suffering from many internal reflections and refraction, which in turn to attenuate the incoming penetrating light toward the detection area. While at higher concentrations (i.e.,> 10, 12 mmol/L for cell A and cell B respectively) might lead to increase agglomeration of precipitated particulate and an increase of inter-spatial distances, which help to increase penetration light toward the detector and a decrease in the response height. Therefore 10, 12 mmol/L respectively to for cell A and cell B was selected as optimum concentration for either cells.

*Effect of different medium*

Propranolol (10 mmol/L) reaction with Bi (III) (10,12 mmol/L) respectively to for cell A and cell B was studied in different medium (ammonium chloride, ammonium acetate, sodium chloride, potassium bromide, sodium sulfate, sodium chloride, sodium hydroxide) at 50 mmol/L concentration in addition to aqueous medium as a carrier stream. It was noticed the salt causes to a decrease of S/N-response; this might be attributed leads to its effect in an increasing the agglomeration i.e.; increase the density of aggregates and compactness with each other than increase the intensity of transmitted light as there will be more vacant spaces in between agglomerates of particulate. On this basis, it was (the salts) canceled throughout this work /and distilled water as a carrier stream in the next studied. The set of data were summarized in Table 2.

**Physical variables**

*Flow rate*

Variable of flow rate study was carried out utilization manifold of two lines, as shown in Figure 2, to assess the $Y_{Z\,(mV)}$-$t_{min}$ $(d)_{cm}$ response profile. It was noticed that an increase in S/N-response profile from cell A & cell B up to 3.6, 2 mL/min for carrier stream for cell A and cell B, respectively. This may be attributed to an increased opportunity for the crystal that are formed to grow up relatively, while there is a time lag difference between cells used.

At high speed (i.e., more than 3.6, 2 mL/min (carrier stream) for cell A and cell B respectively) does not offer a time lag period causing increase attenuation of incident light that is measured by the detector. So at high speed, it is noticed that a decrease in response height might be attributed to incomplete or immature precipitation. Therefore, 3.6,2 & 5.8, 3.1 mL/min flow rate for carrier stream and reagent respectively for either cell.

*Volume of sample*

Variable length of Teflon tube 2.6-25.47 cm of diameter 1 mm was used in the decision of optimum volume of sample that will be used throughout the procedure. Table 3 shows various aspects of obtained results, including arrival time, and average peak heights in mV for each various aspects of used volume of sample. All these obtained data were tabulated in Table 3, we notice the increase of the off base width by increasing the sample volume Δ t, probably due to a long duration of carrier stream to passes through injection valve causing a restriction of the flow, which lead to increase of dispersion of the precipitate particles segment and increase of base width (Δ t). A compromise was made in this study to choose 200 µL as a suitable most convenient of sample size level.

The departure time of the sample segment from the injection valve

A study was carried out to measure and determine the time required for the sample segment to departure totally from the injection valve. 2–14 sec were tried, also open valve (18 sec) through the whole measurement was studied. It was noticed

**Table 2:** Effect of different medium on the measurement of energy transducer response for Determination of propranolol.

| *Type of medium [50] mmol/L* | *Attenuation of incident light expressed as an average peak height (n = 3)* $\bar{Y}_{Z\,i\,(mV)}$ | *RSD%* | *Confidence interval at (95%)* $\bar{Y}_{Z\,i\,(mV)} \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| Cell A | | | |
| $H_2O$ | 648 | 0.1867 | 648 ± 3.0060 |
| $NH_4Cl$ | 560 | 0.2589 | 560 ± 3.6023 |
| $CH_3COONH_4$ | 544 | 0.2463 | 544 ± 3.3290 |
| NaCl | 536 | 0.2910 | 536±3.8756 |
| KBr | 584 | 0.3048 | 584 ± 4.4221 |
| $Na_2SO_4$ | 600 | 0.3200 | 600 ± 4.7699 |
| $Na_2CO_3$ | 576 | 0.3490 | 576 ± 4.9935 |
| NaOH | 560 | 0.4000 | 560 ± 5.5649 |
| Cell B | | | |
| $H_2O$ | 440 | 0.2750 | 440±3.0060 |
| $NH_4Cl$ | 432 | 0.4282 | 432 ± 4.5960 |
| $CH_3COONH_4$ | 424 | 0.3844 | 424 ± 4.0495 |
| NaCl | 432 | 0.3565 | 432 ± 3.8259 |
| KBr | 376 | 0.5106 | 376 ± 4.7699 |
| $Na_2SO_4$ | 344 | 0.4157 | 344 ± 3.5526 |
| $Na_2CO_3$ | 320 | 0.6000 | 320±4.7699 |
| NaOH | 232 | 0.8534 | 232±4.9190 |

$t_{0.05/2,2}$=4.303, $\bar{Y}_{Z\,i\,(mV)}$: (S/N) energy transducer response of cell A and cell B in mV for n=3

**Table 3:** Effect of the variation of sample volume on the Attenuation of incident light by 10 mmol/L concentration of propranolol and 10, 12 mmol/L concentration of Bi (III), Speed of recorder 60 cm/hr. and flow rate of the carrier stream 3.6, 2 ml/min for cell A and cell B.

| *Length of Sample segment Cm r=0.5m* | *Sample volume µL $V=\pi r^2 h$* | *Attenuation of incident light expressed as an average peak heights (n=3) $\bar{Y}_{Z\ i\ (mV)}$* | *RSD%* | *Confidence interval at (95%) $\bar{Y}_{Z\ i\ (mV)} \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$* | *t (sec)* | *Base width Δ t (sec)* | *$V_{add}$ (ml) at flow cell* | *Concentration mmol/L at flow cell* | *D f at flow cell* |
|---|---|---|---|---|---|---|---|---|---|
| Cell A | | | | | | | | | |
| 2.60 | 20 | 344 | 0.3256 | 344 ± 2.7825 | 3.0 | 18.0 | 2.840 | 0.0704 | 142.05 |
| 3.10 | 24 | 520 | 0.3500 | 520 ± 4.5215 | 4.5 | 24.0 | 3.784 | 0.0634 | 157.73 |
| 4.10 | 32 | 480 | 0.4000 | 480 ± 4.7699 | 6.0 | 27.0 | 4.262 | 0.0751 | 133.16 |
| 7.00 | 55 | 536 | 0.3713 | 536 ± 4.9438 | 7.5 | 30.0 | 4.755 | 0.1157 | 86.43 |
| 9.00 | 71 | 592 | 0.2568 | 592 ± 3.7762 | 9.0 | 31.5 | 5.006 | 0.1418 | 70.52 |
| 12.74 | 100 | 656 | 0.2774 | 656 ± 4.5215 | 10.5 | 33.0 | 5.270 | 0.1898 | 52.69 |
| 20.40 | 160 | 760 | 0.2605 | 760 ± 4.9190 | 16.5 | 36.0 | 5.800 | 0.2759 | 36.25 |
| 25.50 | 200 | 840 | 0.2369 | 840 ± 4.9438 | 18.0 | 48.0 | 7.720 | 0.2591 | 38.60 |
| Cell B | | | | | | | | | |
| 2.60 | 20 | 216 | 0.5185 | 216 ± 2.7825 | 9.0 | 36.0 | 3.080 | 0.0649 | 154.08 |
| 3.10 | 24 | 304 | 0.4046 | 304 ± 3.0557 | 12.0 | 42.0 | 3.594 | 0.0668 | 149.70 |
| 4.10 | 32 | 336 | 0.4226 | 336 ± 3.5278 | 15.0 | 48.0 | 4.112 | 0.0778 | 128.54 |
| 7.00 | 55 | 376 | 0.4096 | 376 ± 3.8259 | 18.0 | 54.0 | 4.645 | 0.1184 | 84.46 |
| 9.00 | 71 | 432 | 0.3750 | 432 ± 4.0246 | 21.0 | 57.0 | 4.916 | 0.1444 | 69.25 |
| 12.74 | 100 | 456 | 0.3114 | 456 ± 3.5278 | 24.0 | 60.0 | 5.200 | 0.1923 | 52.00 |
| 20.40 | 160 | 552 | 0.3315 | 552 ± 4.5463 | 27.0 | 72.0 | 6.280 | 0.2548 | 39.25 |
| 25.50 | 200 | 640 | 0.3109 | 640 ± 4.9438 | 30.0 | 78.0 | 6.830 | 0.2928 | 34.15 |

t: Arrival time from injection valve reaching to measuring cell (sec), Δ t: Base width of peak(sec), $t_{0.05/2,\ 2}$ =4.303, Df: Dilution factor at the flow cell

that 18 sec is the most suitable time for loading the manifold with sample segment from sample loop in the injection valve. The above text is represented in Figure 4.A, B and C. The optimum purge time (open valve at 18 sec) of propranolol (10 mmol/L)-Bi (III) (10,12mmol/L for cell A and cell B) system for obtaining clear undisturbed in case of converting injection valve from injecting the sample segment to reach in the manifold to loading position.

### Effect of reaction loop length

The reaction coil of any type whether it is made of glass or teflon as turns (depending on length, loop diameter, and tube diameter used). Glass is preferred material due to its easily cleaneing specilization via pumping a certain cleaning solvent to remove precipitated particulate (usually at high concentration of reactants or even sedimentation of precipitate particulate due to high molecular weight of precipitated reaction product or precipitated reagent that is used (e.g., change of valence). Usually any addition of extra length to the manifold will increase dispersion via the different process (e.g., convection at high speed of flow rate or diffusion at low speed of flow rate).

On this basis, reaction coils are avoided unless it serves the purpose of using or verifying its use. The volume of the reaction coil can be found as it is a cylinder of cross-section diameter of ϕ =2r (where r = radius; i.e., tube radius). A volume of a cylinder =$\pi r^2 L$ (where L= length of the used tube for e.g., is ϕ=1, the r=0.5 mm for 100 mm length. The volume will be equal to 3.14 (0.05 cm) $^2$ × 20 cm=0.157 $cm^3$=157 µL.

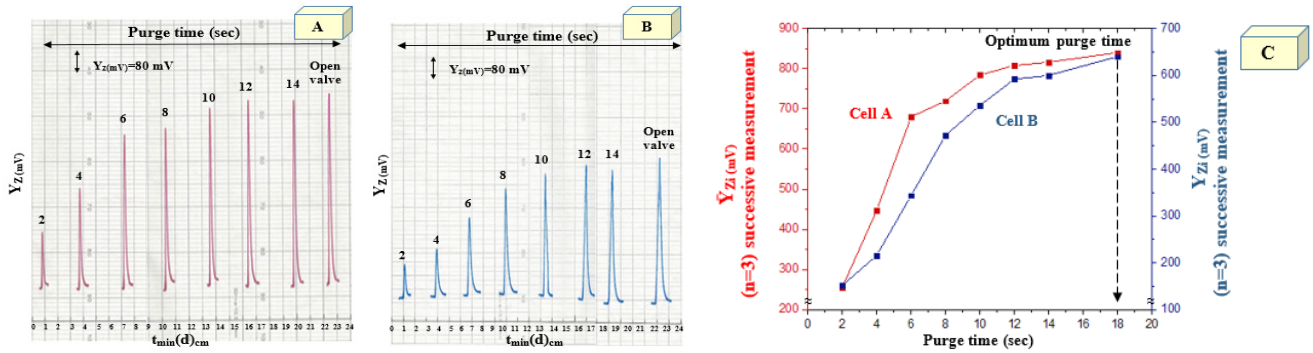



**Figure 4:** Effect of purge time on: A, B: S/N energy transducer response versus $t_{min}$ (d) $_{cm.}$
**C:** Attenuation of incident light expressed as an average peak height in mV ($\bar{Y}_{Z\ i\ (mV)}$)
For cell A and cell B by propranolol (10 mmol/L) - Bi (III) (10, 12 mmol/L for cell A and Cell B respectively) system at flow rate of the carrier stream 3.6, 2 ml/min for cell A and Cell B, I=3 for cell A, I=2 for cell B and 200 µL of sample volume.

So, it was found that using different length of reaction coil (variable volume) will be introduced leading to dilution of sample segment reaction product which that ladies to an even distribution when used of a very high volume, will lead to a highly dispersed of precipitate which cause a weaker signal (or even undetected signal). Therefore a compromise of using a convenient reaction coil length and in watching the results of measurement shows that a direct measurement with no coils in the manifold system will the way of the system works i.e., better with very good excellent output, in addition to trust ability of S/N response and $Y_{Z\,(mV)} - t_{min}\,(d)_{\,cm}$ response profile. From the results are tabulated in Table 4, conducting linear regression for the five chosen $\Delta\, t_{\,sec}$, which reflect the use of various variable reaction coils (mainly 0, 157, 235, 314, and 392.5 µL). The correlation coefficient (r) shows that cell A with capital $R^2$ % (97.31%) indicates that a robust linear equation of the kind:

$$\hat{Y}_{\Delta\, t\,(sec)} = Y_{\Delta\, t\,(sec)} + (\Delta\, t_{\,sec}/\Delta V_{\mu L(R\,c)}\,[V_{(R\,c)}]\ \mu L$$

$\hat{Y}_{\Delta\, t\,(sec)}$: Intercept (Sec units); $Y_{\Delta\, t\,(sec)}$: slope=$y_{axis}/x_{axis}$

The chosen equation in case of cell A shows a value of r=0.9865 and $R^2$% =97.31% which an excellent linearity with little variation of slope i.e.; -62.0 sec for every

$10^3$sec (-0.0620 ×1000 /1000 sec = -62.0/1000 sec)

The $10^3$sec represent a change in width $\Delta\, t_{\,sec}$.

Returning to data of cell B, the % of capital $R^2$=0.9999 a good representation which tells us; the used manifold system is chosen is quite good enough.

The designed unit shows clearly that the choice of any part within the unit is carefully selected as there was no much difference from 0, 157 and 235 µL for both cells (see table no 4, the first three rows of $t_{\,sec}$ (arrival time) and $\Delta\, t_{\,sec}$ (base width)). While increase $\Delta\, t_{\,sec}$ caused by increased volume of reaction coil at volume of 392.5 µL, 24 sec for cell A and 85.5 sec for cell B. This explains an increase of 30% ((85.5--24/85.5) × 100) in width caused by delayed detection (after traveling 210 mm (110 mm+ 100 mm) until detector of cell B can see the aggregated particulate). The value of **(a)** in cell B is less (**a** = 78.0016 sec) due to decreased attenuation; due to the allowance of conglomerates of precipitated particulate, which lead to allowing more light to pass through. While in cell A starting the precipitation process, i.e., suspension like precipitate, which attenuates more of the incident light.

### Estimating the linear dynamic range from scatter plot for the variation of propranolol versus S/N energy transducer response

Variable concentration of propranolol solutions (0.005-35 mmol/L) for cell A and (0.01-50 mmol /L) for cell B, respectively, using the optimum chemical and physical parameters were prepared. This will represent the x-axis (Independent variable). The attenuation of incident light that was measured gave the following S/N energy transducer responses as Y here represent the dependent variable; in which the height of response increased when the analyte of concentration is increased.

The directly proportional up to 25 mmol/L and 45 mmol/L for cell A and cell B respectively between variation precipitate particulate formation and concentration might be attributed to increase of many such as: internal reflection, refraction, absorbance and diverged light from within the precipitated particles when the beam of light diffused inside of particles. Also, obstruction of light by the precipitated particulate, while gives at the end for all these factors combined all together, the measurement due was only at 0-180° angle. Figure 5. A, B explains the variance ranges for each cell. (i.e.; scatter plot at range (0.005-35) mmol/L, dynamic range (0.005-27) mmol/L, working range (0.005-25) mmol/L and linear dynamic range (0.1-25) mmol/L for cell A and scatter plot at range (0.01-50)

**Table 4:** Effect volume of reaction coil on the attenuation of incident light expressed as an average Peak heights (mV) for determination of propranolol (10 mmol/L) using Bi (III) (10, 12 mmol/L for cell A and cell B respectively), speed of recorder 60 cm/hr., the flow rate of Carrier stream 3.6 ml/min for cell A and 2 ml/min for cell B, 200 µL volume of Sample, I = 3 for cell A and I = 2 for cell B.

| *Reaction coil length (cm) r = 0.5 mm* | *Volume of reaction coil (µL) $V_{R\,c}$* | *Attenuation of incident light expressed as an average peak heights (n = 3) $\bar{Y}_{Z\,i\,(mV)}$* | *RSD%* | *Confidence interval at (95%) $\bar{Y}_{Z\,i\,(mV)} \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$* | *t (sec)* | *Base width Δ t (sec)* | *$V_{add}$ (mL) at flow cell* | *Concentration mmol/L at flow cell* | *D f at flow cell* |
|---|---|---|---|---|---|---|---|---|---|
| *Cell A* | $\hat{Y}_{\Delta\, t\,(sec)}$=49.6177- 0.0620 ($V_{R\,c}$) µL r=0.9865, $r^2$=0.9731 , $R^2$%=97.31 | | | | | | | | |
| 0* | 0* | 840 | 0.2369 | 840 ± 4.9438 | 18.0 | 48.0 | 7.7200 | 0.2591 | 38.60 |
| 20 | 157.0 | 728 | 0.1813 | 728 ± 3.2793 | 19.5 | 42.0 | 6.7800 | 0.2950 | 33.90 |
| 30 | 235.0 | 280 | 0.5107 | 280 ± 3.5526 | 21.0 | 36.0 | 5.8400 | 0.3425 | 29.20 |
| 40 | 314.0 | 256 | 0.6328 | 256 ± 4.0246 | 22.5 | 30.0 | 4.9000 | 0.4082 | 24.50 |
| 50 | 392.5 | 208 | 0.8269 | 208 ± 4.2731 | 24.0 | 24.0 | 3.9600 | 0.5051 | 19.80 |
| *Cell B* | $\hat{Y}_{\Delta\, t\,(sec)}$=78.0016+0.0191 ($V_{R\,c}$) µL r=0.9999 , $r^2$=0.9999 , $R^2$%=99.99 | | | | | | | | |
| 0* | 0* | 640 | 0.3109 | 640 ± 4.9438 | 30.0 | 78.0 | 6.8300 | 0.2928 | 34.15 |
| 20 | 157.0 | 600 | 0.2467 | 600 ± 3.6768 | 31.5 | 81.0 | 7.0850 | 0.2823 | 35.42 |
| 30 | 235.0 | 544 | 0.2794 | 544 ± 3.7762 | 33.0 | 82.5 | 7.2125 | 0.2773 | 36.06 |
| 40 | 314.0 | 536 | 0.3022 | 536 ± 4.0246 | 34.5 | 84.0 | 7.3400 | 0.2725 | 36.70 |
| 50 | 392.5 | 560 | 0.3000 | 560 ± 4.1737 | 36.0 | 85.5 | 7.4675 | 0.2678 | 37.34 |

0* without coil, t: Arrival time from injection valve reaching to measuring cell (sec),Δ t: Base width of peak(sec), $t_{0.05/2,2}$ =4.303,Df : Dilution factor at flow cell, linear equation expressed as

$\hat{Y}_{\Delta\, t\,(sec)} = Y_{\Delta\, t\,(sec)} + (\Delta\, t_{\,sec}/\Delta V_{\mu L})\,[V_{(R\,c)}]\ \mu L$, $V_{(R\,c)}$ volume of reaction coil.

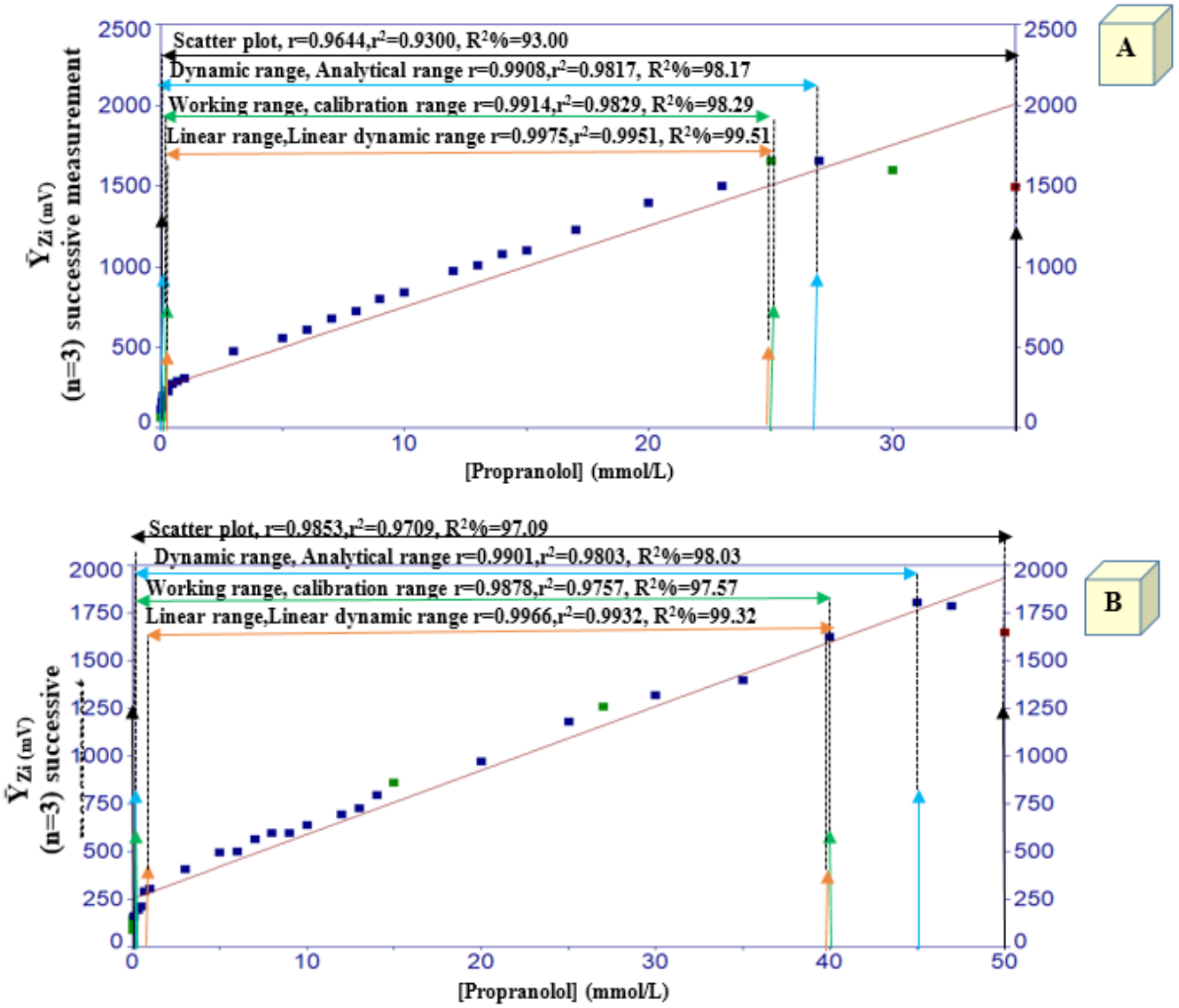



**Figure 5:** Different range for the effect of propranolol concentration on the attenuation of incident Light using NAG-ADF-300-2 analyzer.
**A:** for cell A
**B:** for cell B

mmol/L, dynamic range (0.01-45) mmol/L, working range (0.01-40) mmol/L and linear dynamic range (1-40) mmol/L for cell B ).

While increasing the concentration more than 25 and 45 mmol/L for cell A and cell B respectively causing that the signal (S/N) energy transducer independent on concentration, It is might be attributed to an agglomeration of particulate and increase of inter-spatial distances which lead to increase of transmitted light toward the detector. The results obtained tabulated in Table 5.

**Limit of Detection**

The L.O.D of an analyte may be characterization as that: concentration, which gives an instrument signal y significantly different from the blank or background signal. This description provides the analyst with a good deal of freedom to decide the exact definition of L.O.D. There is an increasing trend to define the L.O.D. as the analyte concentration giving a signal equal to the blank signal, $y_B$ plus three standard deviations of the blank $S_B$.

$L.O.D = y_B + 3S_B$

We have been using three approaches for the expression of L.O.D

*Gradual dilution*

Practically based on successive dilution of the lowest concentration used in the calibration graph, this should be regarded as the real and trustable value of D.L. i.e., Reliable D.L. for the proposed method.

*Theoretically (method of slope)*

$L.O.D. = 3S_B/slope$

$S_B = \sigma_{n-1}$ B standard deviation of blank n = 13

**Table 5:** Summary of result for linear regression for the variation of S/N energy transducer Response with propranolol concentration using the first-degree equation of the form Ŷ=a+ b x at optimum conditions.

| *Type of mode* | *Range of [Propranolol] mmol/L(n)* | *$\hat{Y}_{Z\,i\,(mV)}=a_{mV}\pm S_a t+ b(\Delta y_{mV}/\Delta x_{mmol/L)} \pm S_b t$ [Propranolol] mmol/L at confidence level 95%,n-2* | *r, $r^2$, $R^2$%* | *$t_{tab}$ at 95%, n-2* | *Calculated t-value $t_{cal}=/r/\sqrt{n-2}/\sqrt{1-r^2}$* |
|---|---|---|---|---|---|
| Cell A | | | | | |
| Cell B | | | | | |
| Scatter plot | 0.005-35 (29) | 249.3756±76.9038+50.2076±5.4394 [Propranolol] mmol/L | 0.9644,0.9300,93.00 | 2.052 << 18.9404 | |
| | 0.01-50 (29) | 254.8339±49.1885+33.5728±2.2958 [Propranolol] mmol/L | 0.9853,0.9709,97.09 | 2.052 << 30.0126 | |
| Dynamic range or analytical range | 0.005-27 (27) | 202.5907±38.7960+59.1084±3.3267 [Propranolol] mmol/L | 0.9908,0.9817,98.17 | 2.060 << 36.6210 | |
| | 0.01-45 (27) | 235.0683±37.7246+36.2044±2.1134 [Propranolol] mmol/L | 0.9901,0.9803,99.03 | 2.060 << 35.2709 | |
| Working range or calibration range | 0.005-25 (26) | 195.0237±36.0050+60.8856±3.3843 [Propranolol] mmol/L | 0.9914,0.9829,98.29 | 2.064 << 37.1412 | |
| | 0.01-40 (26) | 231.4567±38.8207+36.7303±2.4405 [Propranolol] mmol/L | 0.9878,0.9757,97.57 | 2.064 << 31.0436 | |
| Linear range or linear dynamic range | 0.1-25 (20) | 261.6682±23.7854+56.5978±1.9609 [Propranolol] mmol/L | 0.9975,0.9951,99.51 | 2.101 << 60.4576 | |
| | 1-40 (18) | 318.1497±27.7635+33.0439±1.4524 [Propranolol] mmol/L | 0.9966,0.9932,99.32 | 2.120 << 48.3422 | |

**n:** no. of measurement

*Theoretically (Linear equation method)*

$\hat{Y} = y_B + 3S_B$

$Y_B$: average response for the blank solution, this is equivalent to intercept (a) in straight line equation

y= a + b x

The final two methods are an output of a linear regression graph treatments where the obtained (fact) results are subjected to statistical treatments, these methods can be used as an approximate indication but should not unless otherwise defined.

A study was carried out to calculate the limit of detection of propranolol-Bi (III) (10, 12 mmol/L) respectively to for cell A and cell B system through three methods, as tabulated in Table 6.

## Repeatability

The expressed relative standard deviation as a percentage which is equal to the repeatability of the measurement. Frequent measurements for eight consecutive injections were measured at fixed concentrations of propranolol for three concentrations were used 0.5,10 and 25 mmol/L respectively in optimum parameters. The obtained results are tabulated in Table 7. In addition to the study of repeatability with a minimum of the RSD%, which equal to 1%.

## Classical method

The estimate of new developed methodology (i.e., NAG-ADF-300-2 analyzer) for the evaluation of propranolol utilization propranolol – Bi (III) (10, 12 mmol/L for cell A and cell B respectively) system was compared with the available

**Table 6:** Limit of detection for propranolol at optimum parameters using 200 µl as an injection Sample, 3.6, 2 ml/min flow rate for cell A and cell B respectively, [Bi (III)] = 10 mmol/L For cell A and 12 mmol/L for cell B.

| *Type of cell* | *Practically based on the gradual dilution for the minimum concentration in scatter plot (0.005 mmol/L) for cell A (n*) (0.01 mmol/L) for cell B (n*)* | *Theoretical based on the value of slope x = $3S_B$/slope* | *Theoretical based on the linear equation $\hat{Y} = Y_b + 3Sb$* | *n* |
|---|---|---|---|---|
| Cell A | 0.001 mmol/L (40 mV) 51.8698 ng/200 µL | 709.3425 ng/200 µL | 86.9813 µg/200 µL | 20 |
| Cell B | 0.007 mmol/L (50 mV) 363.0886 ng /200 µL | 1.4834 µg /200 µL | 152.0860 µg/200 µL | 18 |

X=L.O.D based on slope and $S_B$= standard deviation of blank repeated for 13 times. : $Y_b$ average response for blank= intercept (a), $S_b$: standard deviation equal to S y/x (residual), Ŷ estimated response (mV), n: number of injection, n* number of measurement for scatter plot

**Table 7:** Repeatability of propranolol at optimum parameters with 200 µL sample volume

| *[propranolol] mmol/L* | *Attenuation of incident light expressed as an average peak heights (n = 8) $\bar{Y}_{Z\,i\,(mV)}$* | *RSD%* | *Confidence interval at (95%) $\bar{Y}_{Z\,i\,(mV)} \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$* |
|---|---|---|---|
| Cell A | | | |
| 0.5 | 272 | 0.7279 | 272 ± 1.6556 |
| 10 | 840 | 0.2357 | 840 ± 1.6556 |
| 25 | 1656 | 0.1184 | 1656 ± 1.6389 |
| Cell B | | | |
| 0.5 | 216 | 0.8426 | 216 ± 1.5218 |
| 10 | 640 | 0.3078 | 640 ± 1.6472 |
| 25 | 1184 | 0.1681 | 1184 ± 1.6639 |

(n= 8) number of injection, $t_{0.05/2,\,7} = 2.365$

literature methods, namely UV-spectrophotometric method and turbidimetric method which was based on:

- Spectrophotometric method: based on the measurements of absorbance for the range of concentration 0.01-0.5 mmol/L at $\lambda_{max}$ = 289 nm[25] using quartz cell.
  Table 8 shows the variable data treatments. The detection limit was 0.005 mmol/L (5 µmol/L) equivalent to 1.2967 µg/sample.
- Turbidometry measurement: which is based on the reaction of propranolol-Bi (III) (10 and 12 mmol/L for cell A and cell B) system, which already it used after established as can be seen in table 8 shows the best linear range extend from 0.1-11 mmol/L with correlation coefficient of 0.9980 and % capital R square = 99.61 %, n = 13 (no. of measurements).

**Assessment of NAG-ADF-300-2 analyser using two cell and Multi Solar Cells for the Determination of Propranolol in drugs**

The newly developed methodology (NAG-ADF-300-2) was used for the determination of propranolol in three different samples of drugs from three different of companies (Becardin, S.D.I, Iraq, 40 mg), (Indicardin, APM, Jordan, 10 mg) and (Propranolol, Actavis, UK, 10 mg).

The continuous flow injection analysis used for homemade NAG-ADF-300-2, which means a long distance chasing photometer for 300 mm length with 2 mm path length to chase and accumulate output response from attenuation of incident light at 0-180° via the use of two cells of 110 mm (cell A) and 60 mm length (cell B). And it was compared with two methods which include UV-spectrophotometric via the measurement of absorbance at $\lambda_{max}$ =289 nm and turbidimetric method, the measurement of scattered light at 0- 180° for yellow precipitate particles of propranolol – Bi(III) (10,12 mmol/L for cell A and cell B respectively ) system. A series of solution were prepared of each drug (10 mmol/L) by transferring of 1 mL to each of the five volumetric flask (10 mL) followed by the addition of 0, 0.2, 0.6, 1, 1.4 mL from 100 mmol/L of standard solution to obtain 0,2,6,10,14 mmol/L for developed method ,while UV-spectrophotometric classical method by transferring of 0.025 mL to each of the five volumetric flask (10 mL) followed by the addition of 0, 0.02, 0.04, 0.06, 0.08 mL from 5 mmol/L of standard solution of propranolol to obtain 0,0.01,0.02,0.03,0.04 mmol/L and turbidimetric method by transferring of (10 mmol/L) by transferring of 2.5 mL to each of the five volumetric flask (10 mL) followed by the addition of 0, 0.2, 0.4, 0.6, 0.8 mL from 50 mmol/L of standard solution of propranolol to obtain 0,1,2,3,4 mmol/L ,Taking into a consideration that the first flask is for the sample .Three methods conducted the measurements. Results were mathematically treated for the standard addition method. A calibration curve for the three samples by three methods using NAG-ADF-300-2 Analyzer as a developed method, spectrophotometer for absorbance, and turbidimetric method. Table 9. A and B were shown a practical content of active ingredient at 95% confidence level and efficiency of determination in addition to paired t-test, which shows a comparison at two difference paths.[26]

First test: Comparison of Newly developed method (NAG-ADF-300-2) analyzer with official quoted value B.P[27] (40, 10 mg), as shown in table 9. B (column 5) by calculated t-values of each company and these comparisons with tabulated t-value.

A hypothesis can be estimated as follow.

Null hypothesis: There is no significant difference between the means obtained from three sources of three different companies ($\bar{w}_i$) and a quoted value (µ)

i.e.; $H_o: \bar{w}_i = \mu$

For: Becardin (S.D.I., 40 mg, Iraq), Indicardin (APM, 10 mg, Jordan), and Propranolol (Actavis, 10 mg, UK) companies.

Against:

Alternative hypothesis: there is a significant difference between the means and quoted value

i.e., $\bar{w}_o \neq \mu$ for three different companies

Since some value obtained $t_{cal} > t_{tab}$ (4.303) at confidence level 95% and degree of freedom =2; Null hypothesis will be reject and accepting the alternative hypothesis; these mean that there is a significant difference between the quoted active ingredient value and the measured value. One this base; the newly developed method can be used equally well as standard reference methods. Another obtained $t_{cal}$ –value indicates that there was no significant different between the newly developed method and claimed method by the company as calculated t – value is less than tabulated t – value.

**Table 8:** Different ranges for the propranolol concentration versus absorbance and scatter Light using spectrophotometer and turbidimeter (classical method).

| *Type of mode* | *Range of [propranolol] mmol/L(n)* | *$\hat{Y}_{Z\,i} = a \pm S_a\, t + b\,(\Delta y/\Delta x_{mmol/L}) \pm S_b\, t$ [Propranolol] mmol/L at confidence level 95%,n-2* | *r, $r^2$, $R^2$%* | *$t_{tab}$ at 95%, n-2* | *Calculated t-value $t_{cal}=/r/\sqrt{n-2}/\sqrt{1-r^2}$* |
|---|---|---|---|---|---|
| *UV- Spectrophotometer at $\lambda_{max}$= 289 nm.* | | | | | |
| *Turbidometer (FTU)* | | | | | |
| Scatter plot | 0.01-0.5 (26) | 0.2726 ±0.1569+3.5216±0.5938 [Propranolol] mmol/L | 0.9284,0.8619,86.19 | 2.064 < 12.2389 | |
| | 0.1-25 (17) | 67.0806 ±29.6533+5.9698±2.7891[Propranolol]mmol/L | 0.7623,0.5811,58.11 | 2.131 < 4.5616 | |
| Dynamic range or analytical range | 0.01-0.45 (24) | 0.2068 ±0.1309+4.0458±0.5533 [Propranolol] mmol/L | 0.9554,0.9127,91.27 | 2.074 < 15.1666 | |
| | 0.1-13 (14) | 32.5034±8.1880+13.2561±1.1821 [Propranolol]mmol/L | 0.9901,0.9803,98.03 | 2.179 << 24.4364 | |
| Working range or calibration range | 0.01-0.3 (18) | 0.0387 ±0.0358+5.7252±0.2330 [Propranolol] mmol/L | 0.9970,0.9941,99.41 | 2.120 << 51.9193 | |
| | 0.1-11 (13) | 29.1766±3.6788+14.2527±0.5916 [Propranolol]mmol/L | 0.9980,0.9961,99.61 | 2.201 << 53.0023 | |
| Linear range or linear dynamic range | 0.01-0.25 (16) | 0.0138 ±0.0099+6.0492±0.0766 [Propranolol] mmol/L | 0.9997,0.9995,99.95 | 2.145 << 167.2818 | |
| | 0.1-11 (13) | 29.1766±3.6788+14.2527±0.5916 [Propranolol]mmol/L | 0.9980,0.9961,99.61 | 2.201 << 53.0023 | |

n: number of injection

**Table 9:** B: Summary of results for practical content, efficiency (Rec %) for determination of propranolol in three samples of drugs, and t-test for comparison of three methods.

*Type of method*
*Newly developed methodology*
*Cell A*
*Cell B*
*UV- Sp. Classical method Absorbance measurement at* $\lambda_{max}$*=289 nm*
*Turbidometer (FTU)*

| *No. of sample* | *Practical concentration ( mmol/L)in 10 mL* / *in 50 mL* | *Practical weight of propranolol* $\bar{w}$ *i (g) ±4.303* $\sigma_{n-1}/\sqrt{n}$ / *Weight of propranolol in tablet* $\bar{w}$ *i (mg) ±4.303* $\sigma_{n-1}/\sqrt{n}$ | *Efficiency of determination Rec.%* | *Individual t-test for compared between quoted value &practical value* $(\bar{w}\ i - \mu)\sqrt{n}/\sigma_{n-1}$ *Cell A or Cell B* | *Paired t-testt Compared between two methods* $t_{cal} = \bar{X}\ d\ \sqrt{n}/\sigma_{n-1}$ | $t_{tab}$ *at 95% confidence level(n-1)* |
|---|---|---|---|---|---|---|
| 1 | 1.0329 | 0.1339 ± 0.0325 | 103.2941 | 0.5654 < 4.303 | Cell A, UV | |
| | 10.3294 | 41.3176 ± 10.0285 | | | Cell A turbidity | |
| | 0.9324 | 0.1209 ± 0.0223 | 93.2380 | | $\bar{X}\ d$ = 0.7252 | |
| | 9.3238 | 37.2952 ± 6.8791 | | | $\sigma_{n-1}$ = 0.9243 | |
| | 0.0248 | 0.1285±0.0248 | 99.1019 | /-1.6919/< 4.303 | 1.3590 < 4.303 | |
| | 9.9102 | 39.6408 ± 7.6505 | | | $\bar{X}\ d$ = 1.0946 | |
| | 2.4223 | 0.1256±0.0284 | 96.8927 | | $\sigma_{n-1}$ = 1.2741 | |
| | 9.6892 | 38.7571±8.7635 | | | 1.4880 < 4.303 | |
| 2 | 0.9968 | 0.1293 ± 0.0123 | 99.6789 | /-0.1457/< 4.303 | | |
| | 9.9679 | 9.9679 ± 0.9482 | | | | |
| | 1.0764 | 0.1396 ± 0.0112 | 107.6413 | | | |
| | 10.7641 | 10.7641 ± 0.8636 | | | | |
| | 0.0253 | 0.1315 ± 0.0231 | 101.3701 | 3.8074 < 4.303 | Cell B, UV | |
| | 10.1370 | 10.1370 ± 1.7807 | | | Cell B, turbidity | |
| | 2.4286 | 0.1260 ± 0.0124 | 97.1431 | | $\bar{X}\ d$ = -0.2234 | |
| | 9.7143 | 9.7143 ± 0.9560 | | | $\sigma_{n-1}$=1.8499 | |
| 3 | 1.0264 | 0.1331 ± 0.0112 | 102.6418 | 1.3161 < 4.303 | /-0.2092/ < 4.303 | |
| | 10.2642 | 10.2642 ± 0.8637 | | | $\bar{X}\ d$ = 0.1460 | |
| | 1.0645 | 0.1380±0.0121 | 106.4458 | | $\sigma_{n-1}$ = 1.3961 | |
| | 10.6446 | 10.6446±0.9333 | | | 0.1811 < 4.303 | |
| | 0.0240 | 0.1244 ± 0.0221 | 95.9635 | 2.9717 < 4.303 | | |
| | 9.5963 | 9.5963 ± 1.7048 | | | | |
| | 2.4486 | 0.1270 ± 0.0238 | 97.9442 | | | |
| | 9.7944 | 9.7944 ± 1.8355 | | | | |

So, the newly method can be used as an alternative analysis method for the determination of propranolol in different drugs.

Second test: Using paired t-test at α = 0.05 (2-tailed) for the comparison of developed method using NAG-ADF-300-2 analyzer and classical method using shimadzu (UV-1800 double beam) spectrophotometer and turbidimetry via Turbidity-meter, HANNA , (Hungary). The measurement of scattered light at 0–180$^o$ had shown in table 9. B (column 6).

Taking into consideration that all drugs from different companies are the same population, i.e., neglecting individual differences between one manufacturer and another.

**Assumption**

Null hypothesis $H_o$: $\mu_{\text{NAG-ADF-300-2 analyzer}} = \mu_{\text{UV-SP}} = \mu_{\text{turbidimetry}}$

There is no significant difference between the mean of the different two methods.

An alternative hypothesis: There is a significant difference between the mean of classical method and6 NAG-ADF-300-2 analyzer

i.e., Alternative $H_1$: $\mu_{\text{NAG-ADF-300-2 analyzer}} \neq \mu_{\text{UV-SP}} \neq \mu_{\text{turbidimetry}}$

The obtained results indicate clearly that there were no significant differences between newly developed method,

UV- spectrophotometric and turbidimetric method (classical method) at 95% ($\alpha = 0.05$) confidence level as the calculated $t_{cal}$ (1.3590, 1.4880 and /-0.2092/, 0.1811) is less than $t_{tab}$ (4.303) for each cell (i.e., cell A and cell B) for the determination of propranolol in pharmaceutical drugs as shown in table 9. B (column 6).

## CONCLUSION

The estimate of long-distance chasing photometer (NAG-ADF-300-2) through this research work was applied utilization comparison between NAG-ADF-300-2 analyzer with classical method UV-Spectrophotometric method and turbidimetric method. It was recognized that a small range is obtained with UV-Spectrophotometric and turbidimetric method, while a large range was the characteristic of NAG-ADF-300-2 analyzer. A long-distance chasing photometer (NAG-ADF-300-2) is the choice with excellent extended detection and a wider applicability.

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