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Assessment of Long Distance Chasing Photometer (NAG-ADF-300-2) by Estimating the Drug Atenolol with Povidone Iodine Via CFIA

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Abstarct

Atenolol was used with povidone iodine 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 reaction between atenolol and povidone iodine in an aqueous medium. Optimum parameters was studied to increase the sensitivity development of method. Calibration graph was linear in the range of 2-19 mmol/L for cell A and 5-19 mmol/L for cell B. Limit of detection 146.4848 ng/55 µL and 2.6600 µg/200 µL respectively to cell A and cell B. Correlation coefficient (r) 0.9957 for cell A and 0.9974 for cell. Relative standard deviation (RSD %) was lower than 1%, (n=8) for the determination of atenolol at concentration (5, 9 and 17) mmol/L for cell A and (5, 13 and 17) mmol/L for cell B respectively. The results were compared with classical method UV-spectrophotometric at λ max=270 nm by using method standard addition via t-test, at 95% confidence level. The comparison of data explain that long distance chasing photometer (NAG-ADF-300-2) is the choice with excellent extended detection and wide application.

Keyword: Atenolol, Attenuation of light, Continuous flow injection analysis, Turbidity, Fluorescence.

1. Introduction

Atenolol is a beta (□1) selective (cardio selective) adrenoceptor blocking agent. The main uses of atenolol are in the treatment of hypertension and coronary heart disease. Atenolol is also used to lower the risk of death after a heart attack. The chemical name of atenolol is 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzene acetamide or called Tenormin, white powder, molecular formula C14 H22 N2O3, molecular Weight 266.336 g/mol, structure of atenolol is shown in **Figure 1.** Atenolol are mostly hydrophobic compounds therefore their limited aqueous solubility is the most challenging problem in atenolol development that causes their poor bioavailability. Many published works could be found regarding the improvement of the low bioavailability of poorly soluble atenolol including solubilization techniques. Among the

broad variety of methods proposed for enhancing atenolol solubility, the addition of pharmaceutic cosolvents is the most widely used technique for atenolol in aqueous media [1-4]. The solubility enhancement of poorly soluble atenolol can be achieved by the changes of temperature [5]. Solubility enhancement by using alkaline medium [6], stability of atenolol in acidic environment depending on diversified polarity [7]. Previous studies applied spectrofluorometry to estimation of atenolol in aqueous solution and samples human urine because of its sensitivity, selectivity and low cost instrumentation [8]. Photoluminescence of metal nanoparticles that offer emission of light without requiring conjugation with luminous dyes is the basis for the new method [9]. Determination of atenolol by using high performance liquid chromatographic (HPLC) [10-14], Uv-vis spectrophotometry [3, 15-19]. Chromatographic densitometry [7]. Reflectance spectroscopy [20]. Spectrofluorometry method [2].Potentiometric titration [21]. And GC-Mas [4].

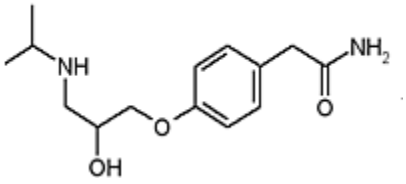


Figure 1. Structure of atenolol.

Flow Injection Analysis (FIA) as "technique based on the sequential injection of a discrete liquid sample into a moving, no segmented continuous carrier stream, several methods depend on continuous flow injection analysis [22-33].

Infection can slow down the wound healing process. However, the infection can be effectively prevented using an antiseptics. Povidone iodine is the most effective antiseptic to prevent infection .It has been widely used on 10% concentration. However, either in vivo or in vitro studies indicated that the povidone iodine, in 10% concentration, is toxic to fibroblast, povidone iodine brown powder, molecular formula $(C_6H_9NO)_n$. x I [34, 35].

The aim of this work was to assessment of a new long distance chasing photometer (NAG-ADF-300-2) by determination of atenolol with povidone iodine. The method is based on the quenching of the povidone iodine by using drug atenolol via 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 $0\text{-}180^0$ via two flow cells of 110 mm and 60 mm length (NAG-ADF-300-2 analyzer) [36].

2. Chemicals and Apparatus

2.1 Reagents and chemicals

All analytical chemicals reagent were used for all solutions and dissolved by distilled water. A standard solution of 50 mmol/L of atenolol, was prepared by dissolving 1.3317 g in a 100 ml. A series of povidone iodine solutions were prepared from the dilution of standard solution 50 mmol/L with distilled water.

2.2 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 $0\text{-}180^0$ and diverged or fluorescence light at $0\text{-}90^0$ 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 ($0\text{-}180^0$and $0\text{-}90^0$), cell (cell number 2) which is covered by 6 WSLED and a single photo cell (solar) of 60 mm length at each side. Using peristaltic pump (Ismatec, Switzerland)

and six-port medium pressure IDEX Corporation USA injection valve with sample loop (1 mm i.e. Teflon, variable length). Potentiometric was recorder to estimate the output signals (Siemens, Germany). UV-spectrophotometric (RF-1501, shimadzu, Japan) was use for classical methods.

3. Methodology

Two manifold designs were investigated to choose the optimum manifold system for the determination of atenolol by using povidone iodine system. The first is shown in **Figure 2.A.** The manifold reaction system is consists of one line represents the carrier stream povidone iodine (5 mmol/L) which passes through injection valve in which sample segment of atenolol (5 mmol/L) was used at 2.9 mL/min flow rate. 100 µL as a sample volume and that passes through the measuring cell (A and B) to quench of povidone iodine molecule is shown in **Figure 2.C**. The second manifold design of flow injection analysis is shown in **Figure 2.B.** The manifold reaction system is consists of two lines: first line is carrier stream (distilled water) leading to the injection valve, which allows the use of 100 µL of atenolol sample segment (5 mmol/L) and flow rate 3.2 ml/min, while the second line supply the povidone iodine molecule solution (5 mmol/L) and a flow rate of 4.6 ml/min. The two lines mixes together at a Y-junction and leading to measuring cell (A and B). The response obtained is shown in **Figure 2.D.**

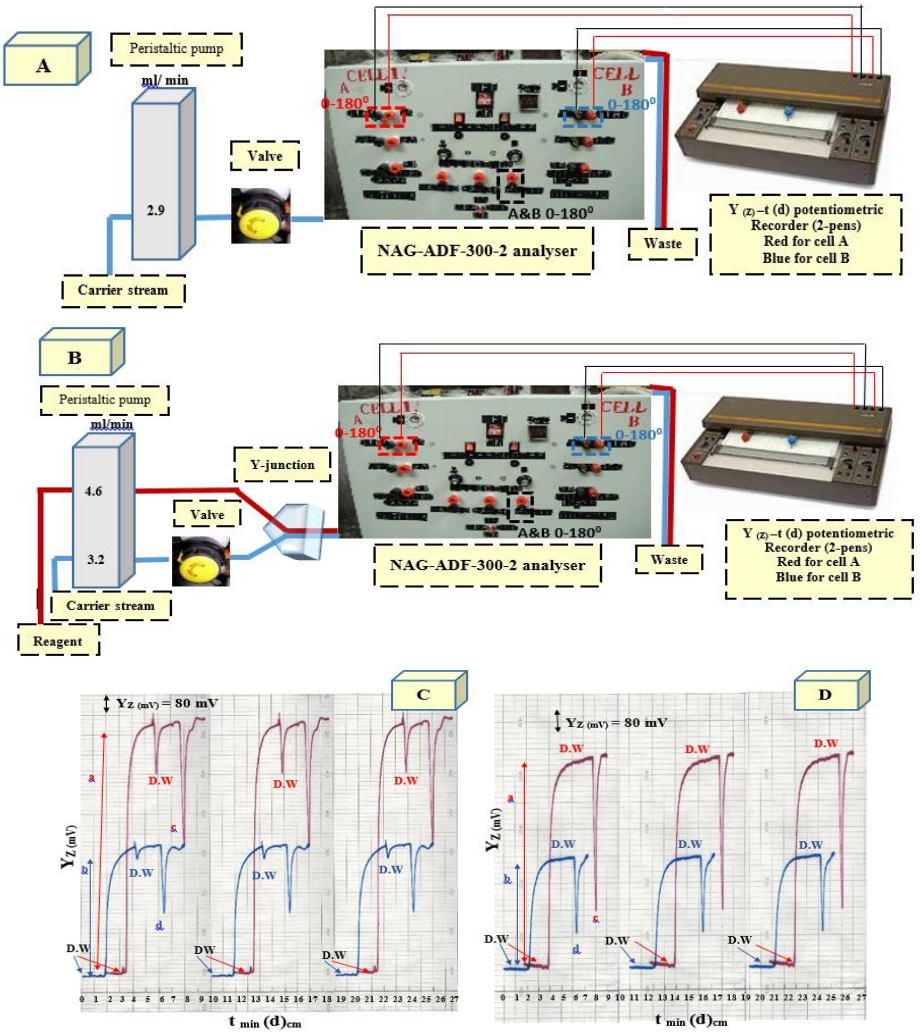
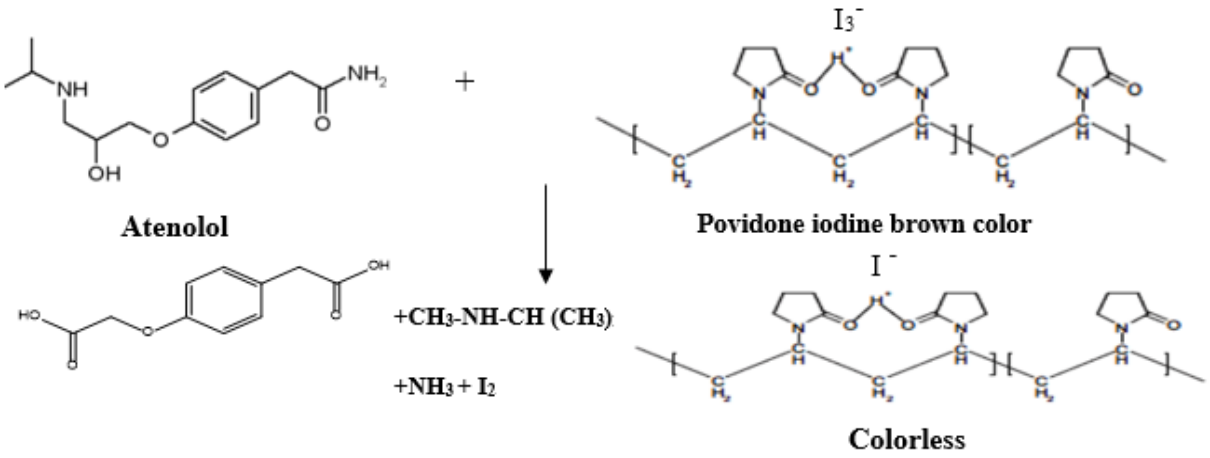


Figure 2.A, B. Schematic diagram of manifold flow injection analysis using one and two manifold design.
C, D: Profile of $Y_{Z\,(mV)} - t_{\,min}\,(d)_{\,cm}$ energy transducer response output using a, b total continuous Response of povidone iodine 5 mmol / L for cell A, cell B c, d total quenched of povidone Iodine for cell A, cell B by using sample volume 100 µL for one line and two line.

Scheme 1. Shows a proposed expected mechanism for the reaction of atenolol with povidone iodine [37-39].



Scheme 1. Proposed mechanism of reaction between atenolol and povidone iodine.

4. Results and Discussion

4.1. Study of the optimum intensity used for cell A and cell B

Choosing the optimum intensity for either cells (cell no.1-cell A and cell no.2-cell B with in between distance of 100 mm. An arbitrary selected intensity was put into work (an intensity of indication approximate of selector switch for cell A was on no.3, while it was on no.2 for cell B which was based on preliminary experiment).These numbers reflect the 0-1-2-3-4 intensities which were varied a according to nature and type of reaction carried out. On the basis of obtained responses profile **Figure 2.A , B.** other necessary chemical and physical parameters were carried out, which describe the full detailed study supported by the recorded of $Y_{Z\ (mV)} - t_{\ min}$ (d) $_{cm}$ response.

Table 1. Shows the intensity (I) of the response for either cells from I=1 to I=4.The optimum intensity of the measuring cell A, I=3 and I=2 for cell B which was adopted in subsequent studies.

Table 1. Effect of intensity on Response profile, total continuous response of povidone iodine, quenched Of povidone iodine, remained of povidone iodine for cell A and cell B by using 100 µL sample Volume concentration of the atenolol (5 mmol/L]) with povidone iodine (5 mmol/L), speed of Recorder = 60 cm/hr.

Intensity (I)	Total response of povidone iodine $\bar{Y}_{Z\ i\ (mV)}$	RSD%	Confidence interval at (95%) $\bar{Y}_{Z\ i\ (mV)} \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$	Quenched of povidone iodine mV	Remained of povidone iodine m V
Cell A					
1	368	0.1060	368±0.9689	272	96
2	544	0.1195	544±1.6148	384	160
3	816	0.1201	816±2.4346	592	224
4	200	0.6050	200±3.0060	180	20
Cell B					
1	336	0.1339	336±1.1179	216	120
2	440	0.1318	440±1.4409	296	144
3	96	0.5938	96±1.4160	80	16
4	80	1.2250	80±2.4346	60	20

I=3 (cell A) and I=2 (cell B)	776 (cell A) and 408 (cell B)	0.1559 (cell A) and 0.3554 (cell B)	776±3.0060(cell A) and 408±3.6022(cell B)	576 (cell A) and 280 (cell B)	200 (cell A) and 128 (cell B)

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

One line system reduction of povidone iodine is low and the response intensity is high, so that the same concentration of atenolol could not suppress the povidone iodine response significantly compared with the atenolol which echoes the povidone in the two line system. Atenolol in a two line system was able to quell the povidone almost entirely because the povidone were less intense due to the dilution and dispersion of the two line system.

4.2. Optimization of variables for one line manifold system

All chemicals parameters mainly reagent concentration and kind of carrier stream for the atenolol with povidone iodine system as well as physical parameters volume of sample, flow rate were studied using one lines manifold system **Figure 2.A.**

4.2.1. Chemical variable effect

Using the optimum concentration of povidone iodine for the determination of atenolol using one line manifold system.

4.2.1.1. Povidone iodine concentration

A series of the povidone iodine solutions 1-10 mmol/L at 2.9 ml/min flow rate were prepared. Atenolol 5 mmol/L used with 100 µL volume of sample. Each measurement was sequential for three times. The increase of the concentration povidone iodine and quenching.increases of povidone iodine response is shown in **Figure 3 A, B, C.** (8 mmol/L) was chosen as optimal quenching intensity with low of reagent concentration.

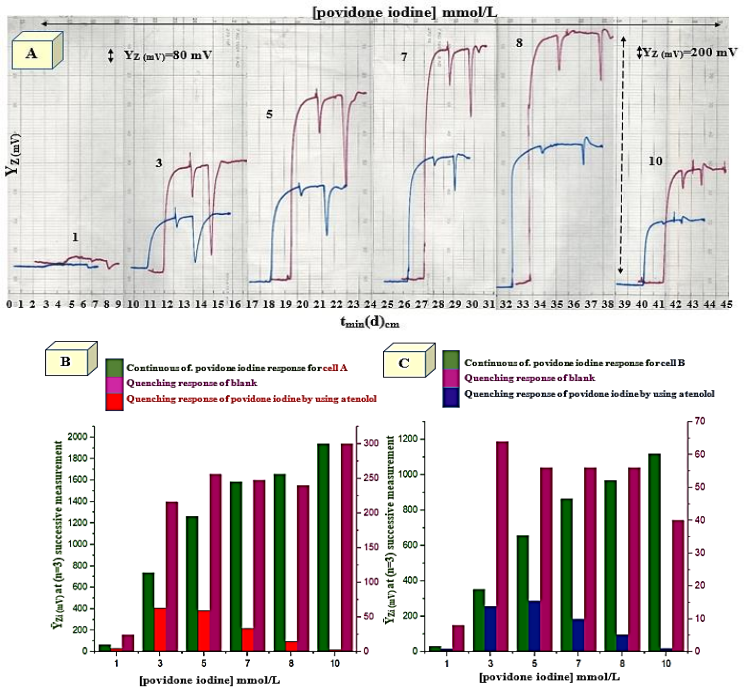


Figure 3.A. Effect of concentration variable of povidone iodine on S/N energy transducer response.

B, C: Attenuation of incident light expressed as an average peak heights versus povidone iodine concentration, quenching of povidone iodine by D.W and atenolol for cell A and cell B.

4.2.1.2. Effect of Different Medium

Atenolol (5 mmol/L) - povidone iodine (5 mmol/L) system was studied in solution in different media (sodium carbonate and sodium hydroxide) at 10 mmol/L concentration in addition to aqueous medium as a carrier stream. The results explain using the salt solutions lead to decrease sharpness of response and

change of the response format therefore, the water medium of the reaction was selected. The results were summarized in **Table 2.**

Table 2. Effect of different medium on the measurement of energy transducer response for Determination of atenolol.

Type of medium 10 mmol/L	Povidone iodine response $\bar{Y}_{Z\ i\ (mV)}$	Response of blank $\bar{Y}_{Z\ i(mV)}$	Total quenched povidone iodine $\bar{Y}_{Z\ i\ (mV)}$	Quenched povidone iodine $\bar{Y}_{Z\ i(mV)}$	Remained povidone iodine $\bar{Y}_{Z\ i(mV)}$
	$\bar{Y}_{Z\ i\ (mV)} \pm t_{0.05/2,\ 2}\ \sigma_{n-1}/\sqrt{n}$				
Cell A					
H_2O	1656±4.7698	240±4.6953	336±2.7824	96	1320
Na_2CO_3	1616±3.8009	240±4.5214	176±3.6022	-64	1440
NaOH	1616±4.9188	240±2.7824	344±4.7450	104	1272
Cell B					
H_2O	968±2.9066	56±2.4346	152±1.6645	96	816
Na_2CO_3	968±3.7761	56±2.7327	168±3.2792	112	800
NaOH	968±3.6767	56±3.5277	184±3.7761	128	784

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

4.2.2. Physical Variables

4.2.2.1. Flow rate

Figure 2. A which shows that a one lines manifold system were used a variable flow rates. Different variable responses were obtained even different profile were described. **Figure 4.** A shows the different types of responses characterization by response of attenuated signal versus flow rate. While **Figure 4 B, C.** Shows quenched in mV, Remained of povidone in mv, Peak base width in mV and addition volume in ml for cell A and cell B, flow rate of The carrier stream 4.4 ml/min utilizations the optimum choice even it is at the edge of the curve. From the figure we notice the increase of the sharp peak and quenched povidone iodine by increasing the flow rate and decreasing the width base **Δ t** of the response.

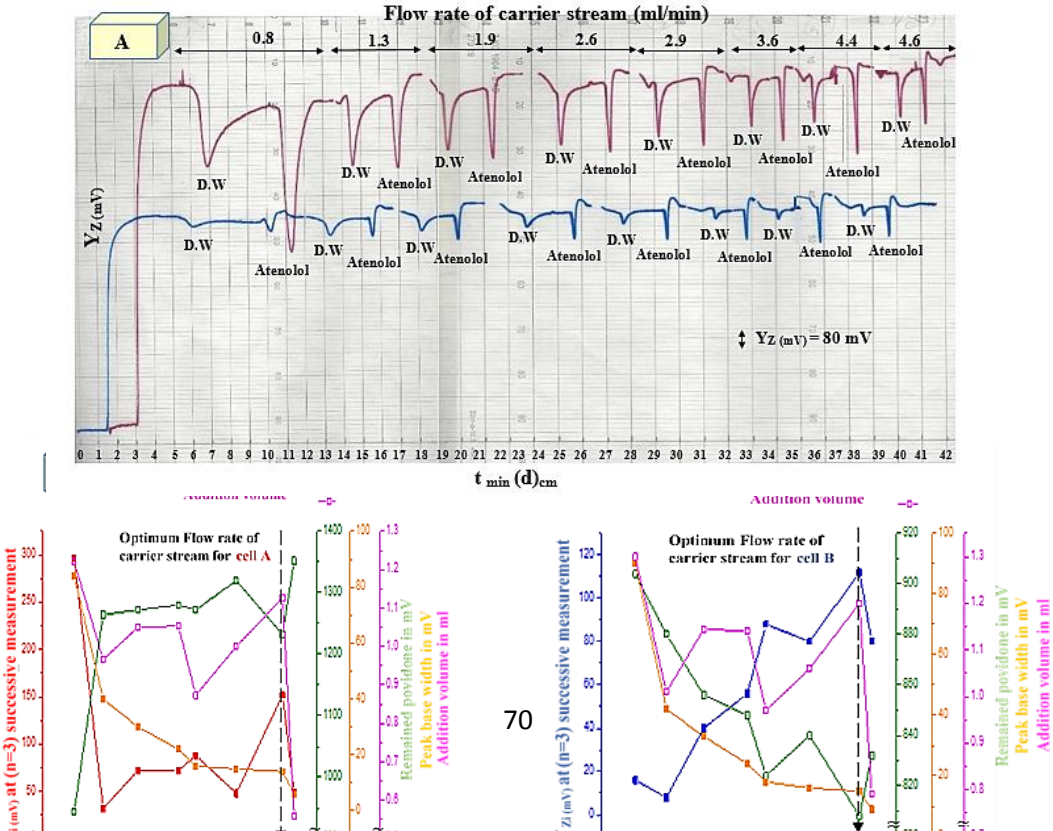


Figure 4. Effect of the variation of flow rate on: A: S/N energy transducer response versus t_{min} (d) $_{cm}$ B, C: Attenuation of incident light expressed as an average peak heights in mV ($\bar{Y}_{Z\,i\,(mV)}$) for cell A, cell B by using atenolol (5 mmol/L) – povidone iodine (8 mmol/L) system, 100 µl volume of sample, I=3for cell A and I=2 for cell B.

4.2.2.2. Sample Volume

Using atenolol (5 mmol/L) – povidone iodine (8 mmol/L) system and variable length of Teflon tube ranging (2.6-25.5) cm of diameter (D) 1 mm, that is equivalent to (20-200) µL of sample volume.

It was noticed that an increase volume of sample cause the increase of response for cell B and decrease of response for cell A expressed as a quenching of povidone iodine. A compromise was made in this study to choose 55 µL for cell A and 200 µL for cell B as a suitable most convenient of sample size level. All results were tabulate in **Table 3.**

Table 3. Effect of the variation volume of sample on the total quenched, quenched, remained of povidone Iodine by using (5 mmol/L) concentration for atenolol and (8 mmol/L) concentration of povidone Iodine, speed of recorder 60 cm/hr. and flow rate of carrier stream 4.4 ml/min.

Loop length (cm) r=0.5 (mm)	Sample volume (µL)	Total quenched povidone iodine $\bar{Y}_{Z\,i\,(mV)}$	RSD%	Confidence interval at (95%)	Quenched povidone iodine $\bar{Y}_{Z\,i\,(mV)}$	Remained povidone iodine $\bar{Y}_{Z\,i(mV)}$	Δ t (sec)	t (sec)	V_{add} (ml) at flow cell	Concentration mmol/L at flow cell	D f at flow cell
Cell A											
2.60	20	88	0.2614	88±0.5714	0	1512	5	12	0.3867	0.2586	19.34
3.10	24	216	0.5602	216±3.0060	88	1368	7	15	0.5373	0.2233	22.39
4.10	32	280	0.4000	280±2.7824	136	1296	9	18	0.6920	0.2312	21.63
7.00	55	416	0.2668	416±2.7575	208	1264	10	22	0.7883	0.3489	14.33
9.00	71	416	0.2452	416±2.5340	176	1296	12	25	0.9510	0.3733	13.39
12 74	100	368	0.2853	368±2.6085	152	1232	14	28	1.1267	0.4438	11.27
20.40	160	520	0.2923	520±3.7761	176	1104	20	35	1.6267	0.4918	10.17
25.50	200	560	0.3250	560±4.5214	200	1/040	30	40	2.4000	0.4167	12.00
Cell B											
2.60	20	48	1.1667	48±1.3912	24	928	7	17	0.5333	0.1875	26.67
3.10	24	104	0.7885	104±2.0371	72	880	8	20	0.6107	0.1965	25.45
4.10	32	120	0.3750	120±1.1179	88	872	10	23	0.7653	0.2091	23.91
7.00	55	184	0.1141	184±0.5217	136	792	11	26	0.8617	0.3191	15.67
9.00	71	184	0.1033	184±0.4720	120	784	13	28	1.0243	0.3466	14.43
12.74	100	160	0.0750	160±0.2981	112	808	15	30	1.2000	0.4167	12.00
20.40	160	240	0.5042	240±3.0060	160	712	28	40	2.2133	0.3615	13.83
25.50	200	392	0.2602	392±2.5340	296	584	35	45	2.7667	0.3614	13.84

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

4.2.2.3. Effect of Reaction Loop Length

Variable coil lengths 0, 20, 30, 40 and 50 cm were studied. These length comprises a volume (0-392.5 µl) which connected after valve directly in flow system. While keeping all other changeable constant (i.e.; atenolol (5 mmol/L), - povidone iodine (8 mmol/L) system, 4.4 ml/min flow rate for carrier stream (distilled water) and sample volume 55, 200 µl for cell A and cell B respectively**. Figure 5.** Shows the manifold design system for determination of atenolol in the presence of reaction coil. 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 6. A, B, C and D shows that the increase of coil volume will lead to a highly dispersed which cause a weaker signal or even undetected signal. Therefore a compromise of using a convenient reaction coil length was is not using of reaction coil.

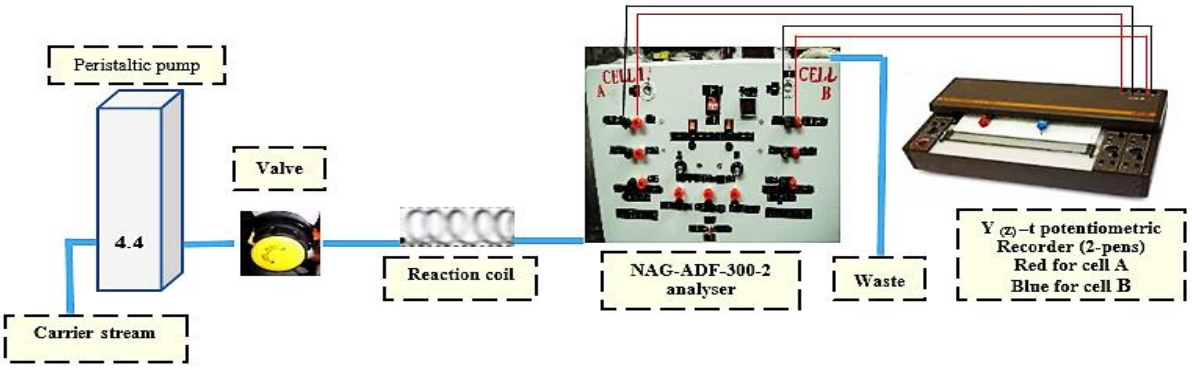
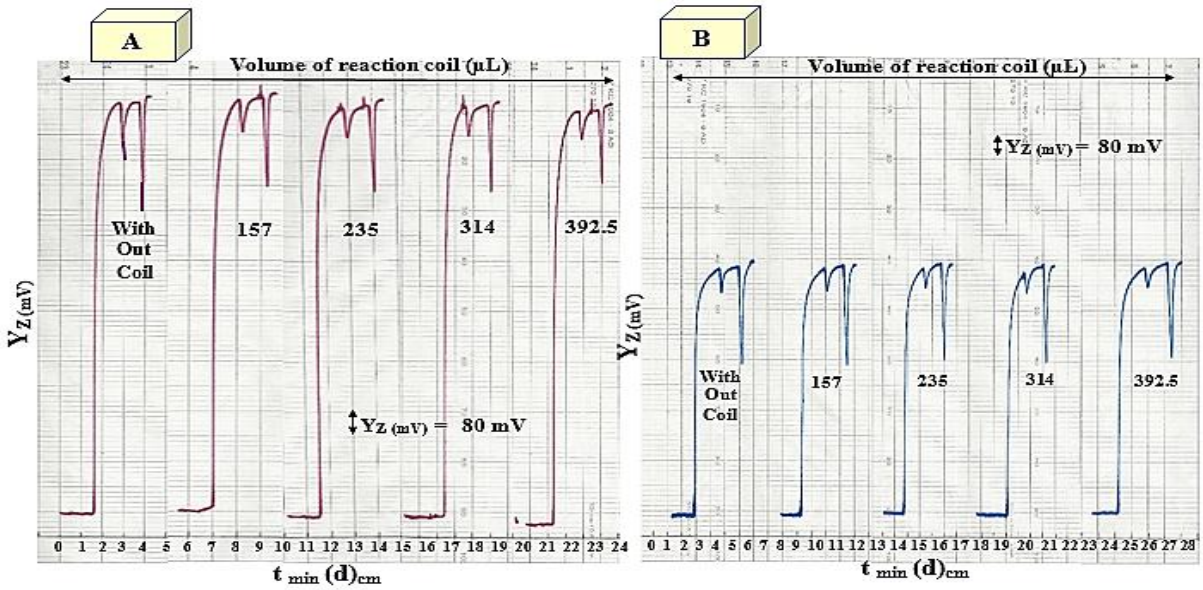


Figure 5. Manifold design system for evaluation of atenolol in the presence of reaction coil



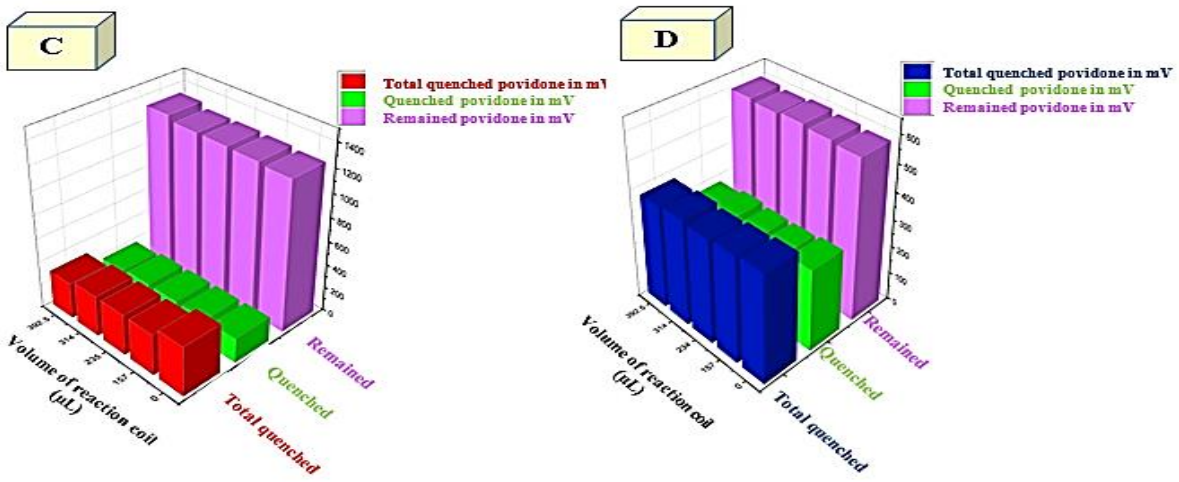


Figure 6. Effect of reaction coil on: A, B: S/N- energy transducer response versus t_{min} (d) $_{cm}$ for cell A and cell B respectively C, D: Total quenched, quenched and remained of povidone in mv for cell A, cell B by using atenolol (5 mmol/L) - povidone iodine (8 mmol/L) system, intensity I=3 for cell A and I=2 for cell B, flow rate 4.4 ml/min , sample volume 55,200 µL for cell A and cell B respectively.

5. Study of the Optimum Intensity Used for White Snow Light Emitting Diodes (WSLEDs) in NAG-ADF-300-2 analyser for one line

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 front panel diagram of NAG-ADF-300-2 **Figure 2 A.**The selector switch gives 0-1-2-3-4 i.e.; four choices plus the off position for both cell individually controlled.

It was noticed that a selection of 3 position (i.e.; I=3) was 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 a convenient intensity .It was same intensity chosen in first of atenolol study of assessment. The higher intensity (I=3) for cell A, while it was not necessary to use high light intensity for cell B due to quenching of povidone iodine. Therefore, a low intensity of height is required for cell B. The results summed up in **Table 4.**

Table 4. Effect of intensity on attenuation of incident light expressed as an average peak heights (mV) For total quenched, quenched and remained of povidone in mV by using atenolol (5 mmol/L) – Povidone iodine (8 mmol/L) system, speed of recorder 60 cm/hr., flow rate carrier stream 4.4 ml / min and sample volume 55,200 µL for cell A and cell B respectively .

Intensity (I)	Total quenched povidone iodine $\bar{Y}_{Z\,i\,(mV)}$	RSD%	Confidence interval at (95%)	Quenched of povidone iodine mV	Remained of povidone iodine mV
Cell A					
1	160	0.6125	160±2.4346	80	528
2	208	0.4760	208±2.4594	96	776
3	416	0.2668	416±2.7575	208	1264
Cell B					
1	256	0.3711	256±2.3601	200	440
2	392	0.2602	392±2.5340	296	584
3	376	0.3511	376±3.2792	224	272
4	96	1.5833	96±3.7761	56	72

The intensity of output response is varied for cell A and cell B

6. Estimating the Linear Dynamic Range From Scatter Plot For The Variation Of Atenolol Versus S/N Energy Transducer Response

A series of atenolol solutions (0.03-40, 0.07-40 mmol/L) using the optimum chemical and physical parameters; for cell A and cell B respectively were prepared and this will represent the x-axis (Independent variable).The attenuation of incident light was measured and gave the following S/N energy transducer responses as Y here represent the dependent variable as shown in Figure 7 A, B. In which, the height of response increased for cell A and B when the analyte of concentration is increased. It can be seen from the **Figure 8 A, B.** Explains the variance ranges for each cells. (i.e.; scatter plot at range (0.03-40) mmol/L, dynamic range (0.03-30) mmol/L, working range (0.03-25) mmol/L and linear dynamic range (2-19) mmol/L for cell A and scatter plot at range (0.07-40) mmol/L, dynamic range (0.07-30) mmol/L, working range (0.07-25) mmol/L and linear dynamic range (5-19) mmol/L for cell B).

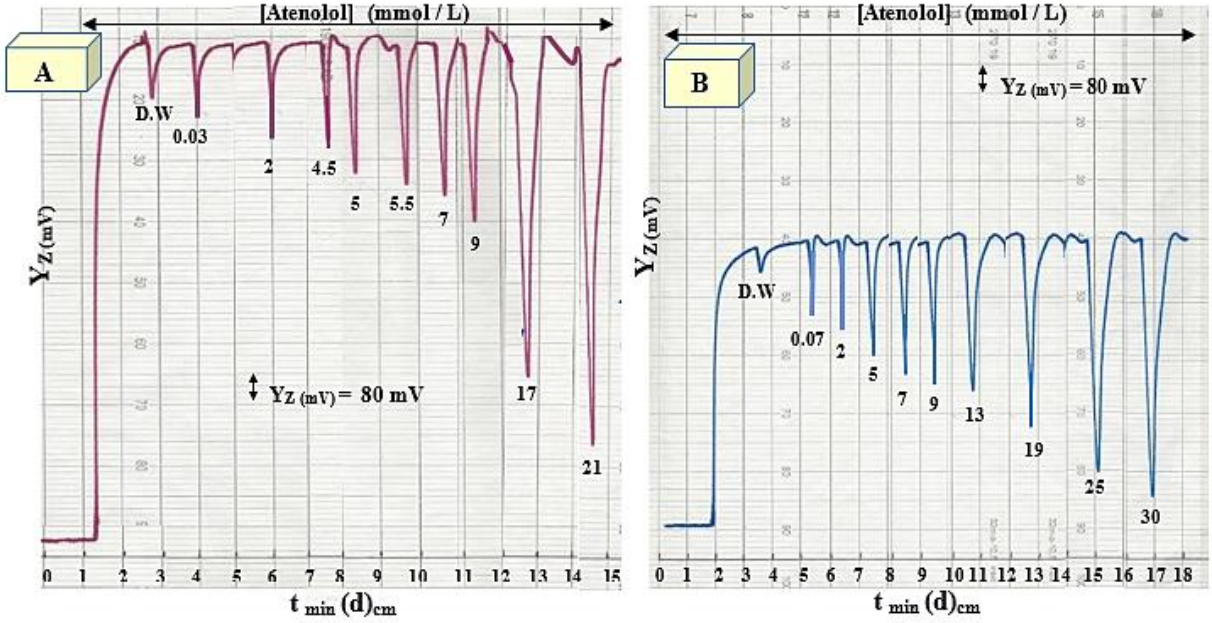


Figure 7. Some of response profile versus time using povidone iodine (8 mmol/L), sample volume 55,200 µL respectively to cell A and cell B .I=3 for cell A, I=2 for cell B.
A: for cell A, B: for cell B.

6.1. Limit of Detection

In general terms, the LOD of an analyte may be characterization as that: concentration which gives an instrument signal y9 significantly different from the blank or back ground signal. This characterization gives the analyst a perfect 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 deviation of the blank S_B.

$$LOD = y_B + 3S_B$$

1. Gradual dilution

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

2. Theoretically (method of slope)

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

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

3. Theoretically (equation of linear 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 except if otherwise defined.

A study was done to calculate the detection limit of atenolol- povidone iodine (8 mmol/L) system through three methods as tabulated in **Table 5**.

Table 5. Limit of detection for atenolol at using optimum parameters 55,200 µl for cell A and cell BRespectively as an injection sample, 4.4 ml/min flow rate of carrier stream, [povidone iodine]8mmol/L.

Type of cell	Practically based on the gradual dilution (0.03 mmol/L) (n*) (0.07 mmol/L)(n*)	Theoretical based on the value of slope	Theoretical based on the linear equation	n
Cell A	0.01 mmol/L (25 mV) 146.4848 ng/55 µL	216.7975 ng/55 µL	23.9635 µg/55 µL	20
Cell B	0.05 mmol/L (104 mV) 2.6600 µg/200 µL	2.8400 µg/200 µL	62.4600 µg/200 µL	18

X=LOD based on slope and S_B= standard deviation of blank repeated for 12 times. : Y_b average response for blank= intercept (a), S_b: standard deviation equal to S y/x (residual): $\hat{Y}$ estimated response (mV), n: number of injection, n* number of measurement for scatter plot

6.2. Repeatability

The relative standard deviation expressed as a percentage which is equally to the repeatability of the measurement. A repeated measurements for eight repeated injections were measured at steady concentrations of atenolol for three concentrations were used 5, 9, 17 mmol/L for cell A and 5, 13, 17 for cell B in optimum parameters. The obtained results is listed in **Table 6** is which showed the repeatability at 5, 9, 17 mmol/L for cell A and 5, 13, 17 for cell B respectively. In addition to study of repeatability with minimum of the RSD% which equal to 1%.

Table 6. Repeatability of atenolol at optimum parameters with 55 µl volume of sample for cell A and 200 µl sample volume for cell B.

[Atenolol] mmol/L	Average of total quenched povidone $\bar{Y}_{Z\,i\,(mV)}$	Quenched povidone ($\bar{Y}_{Z\,i\,(mV)}$	RSD%	Confidence interval at (95%)
Cell A				
5	416	208	0.2668	416±0.9281
9	584	376	0.2072	584±1.0117
17	1040	832	0.2029	1280±1.7643
Cell B				
5	**392**	**296**	**0.2602**	**392±0.8529**
13	**544**	**448**	**0.1195**	**544±0.5435**
17	**620**	**524**	**0.2129**	**620±1.1037**

Response of continuous povidone iodine =1680 mV for cell A, =976 mV for cell B, response of blank = 208 mV for cell A, =96 mV for cell B, $t_{0.05/2,\,7}$= 2.365, number of injection =8.

7. Classical method of UV- Spectrophotometric

The assessment evaluation of new developed methodology (i.e.; NAG-ADF-300-2 analyser) for the determination of atenolol using atenolol - povidone iodine (8 mmol/L) system. A new developed method was compared with the available literature method, namely UV-spectrophotometric method [40], which was based on the measurements of absorbance. Concentration range of method 0.01-6 mmol/L at λ_{max}= 270 nm using quartz cell. **Table 7.** Shows the variable data treatments. The detection limit was 0.005 mmol/L (5 µmol/L) equivalent to 1.3317 µg / sample.

Table 7. Different ranges for the atenolol concentration versus absorbance using spectrophotometer (Classical method).

Type of mode	Range of [atenolol] mmol/L(n)	$\hat{Y}_{Z\,i} = a \pm S_a\, t + b\,(\Delta y_i / \Delta x_{mmol/L}) \pm S_b\, t$ [Atenolol] mmol/L at confidence level 95%,n-2	r, r^2, R^2%	t_{tab} at 95% ,n-2	Calculated t-value $t_{cal}=/r/\sqrt{n-2}\,/\sqrt{1-r^2}$
Scatter plot	0.01-6 (18)	0.3291 ±0.2033+0.3706±0.0856 [Atenolol] mmol/L	0.9167,0.8404,84.04	2.120 < 9.1785	
Dynamic range or analytical range	0.01-5 (17)	0.2632±0.1750+0.4449±0.0891 [Atenolol] mmol/L	0.9396,0.8829,88.29	2.131 < 10.6344	
Working range or calibration range	0.01-4 (16)	0.1926±0.1386+0.5371 ± 0.0871 [Atenolol] mmol/L	0.9623,0.9260,92.60	2.145 << 13.2375	
Linear range or linear dynamic range	0.05-2.5 (13)	0.0864±0.0599+0.7157± 0.0548 [Atenolol] mmol/L	0.9934,0.9869,98.69	2.201 28.7747	

8. Assessment of NAG -ADF-300–2 Analyser Using Two Cell And Multi Solar Cells For The Determination of Atenolol In Drugs

The newly developed methodology (NAG-ADF-300-2) was used for the determination of atenolol in three different samples of drugs from three different of companies (Atenolol, Bristol, UK, 100 mg),(Vascoten, medochemie, Cyprus, 100 mg) and (Novaten, Ajanta, India, 100 mg).

The continuous flow injection analysis used of homemade NAG-ADF-300-2. Which that mean a long distance chasing photometer for 300 mm length with 2mm path length to chase and accumulate output response from attenuation of incident light at $0\text{-}180^0$ via the use of two cells of 110 mm (cell A) and 60 mm length (cell B). The newly developed methodology comparison with UV-Spectrophotometric method via measurement at λ_{max} =270 nm.

A series of solution were provided of each drug (20 mmol/L) by transferring of 0.5 mL to each of the five volumetric flask (10 mL) followed by the addition of 0.0, 0.2, 0.4, 0.6, 0.8 mL from 50 mmol/L of standard solution to obtain 0,1,2,3,4 mmol/L for developed method ,while classical method (20 mmol/L) by transferring of 0.5 mL to each of five volumetric flask (10 mL) followed by addition of 0.0, 0.04, 0.06, 0.08, 0.1 mL from 50 mmol/L of standard solution of atenolol to obtain 0,0.2,0.3,0.4,0.5 mmol/L. Taking into a consideration that the first flask is for the sample. The measurements were conducted by both methods. Results were mathematically treated for method of standard addition. **Table 8 A, B.** have shown a practical consist of active ingredient at 95% confidence level & efficiency of evaluation in addition to paired t-test which shows a comparison at two difference paths [41, 42].First test: Comparison of newly developed method (NAG-ADF-300-2) analyser with official quoted value B.P [43]. (100 mg) as shown in **Table 8 B.** (column 5) by calculated t-values of each individual company and these compare with tabulated t-value. A hypothesis can be estimated as follow null hypothesis: There is no important

difference between the means obtained from three source of three different companies ($\bar{w}_i$) and quoted value (μ)

i.e.; $H_{o: \bar{w} i} = \mu$

For: Atenolol (Bristol, 100 mg, UK), Vascoten (Medochemie, 100 mg, Cyprus) and Nova ten (Ajanta, 100 mg, India) companies. Against: Alternative hypothesis: there is an important difference between the means and quoted value

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

Some value obtained $t_{cal} > t_{tab}$ (4.303) confidence level at 95% and degree of freedom =2; Null hypothesis will be reject and accepting the alternative hypothesis; these mean that there is an important 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 indicated 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. So, the newly method capable was used as an alternative analysis method for the evaluation of atenolol in different drugs.

Second test: Using paired t – test at $\alpha = 0.05$ (2-tailed) for using developed method NAG-ADF-300-2 analyser and the compare with classical method using shimadzu (UV-1800 double beam) spectrophotometer as shown in table 8 B (column 6). Taking into the 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: $\mu_{\text{NAG-ADF-300-2 analyser}} = \mu_{\text{UV-SP.}}$

There is no significant difference between the mean of different two methods. An alternative hypothesis: There is an important difference between the mean of classical method and NAG-ADF-300-2 analyser i.e.; Alternative H_1: $\mu_{\text{NAG-ADF-300-2 analyser}} \neq \mu_{\text{UV-SP.}}$

The obtained results indicated clearly that there was no significant differences between newly developed method and UV-spectrophotometric (classical method) at 95% ($\alpha = 0.05$) confidence level as the calculated t_{cal} (0.2107 and /-0.3551/) is less than t_{tab} (4.303) for each cell (i.e.; cell A & cell B) for the evaluation of atenolol in pharmaceutical drugs as shown in **Table 8 B.** (column 6).

Table 8. A: Standard addition results for the determination of atenolol in three samples of drugs using NAG-ADF-300-2 analyser for cell A, cell B and classical methods.

No. of sample	Commercial name , Company Content Country	Type of method: Newly developed methodology; Cell A / Cell B; UV-Sp. Classical method Absorbance measurement at λ_{max} =270 nm: Confidence interval for the average Weight of Tablet $\bar{w}_i \pm 1.96\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Weight of sample equivalent to 1.33168 g(20 mmol/L)of the active ingredient Wi (g)	Theoretical content for the active ingredient at 95% (mg) $Wi \pm 1.96\sigma_{n-1}/\sqrt{n}$	Atenolol mmol/L					Equation of standard addition at 95% for n-2	r , r^2 , R^2%
					0	0.20ml	0.40ml	0.60ml	0.80ml	$\hat{Y}_{Z\,i\,(mV)}=a_{mV} \pm S_a t + b(\Delta y_{mV}/\Delta x_{mmol/}) \pm S_b t$[atenolol]mmol/L	
					0	1.00	2.00	3.00	4.00		
					0	0.04ml	0.06ml	0.08ml	0.10ml	$\hat{Y}_{Z\,i}=a \pm S_a t + b\,(\Delta y / \Delta x_{mmol/L}) \pm S_b t$[atenolol]mmol/L	
					0	0.20	0.30	0.40	0.50		
1	Atenolol Bristol 100 mg UK	0.4190±0.00204	5.5801	100±0.4868	90	130	240	310	379	78.2±41.3514+75.8±16.8815 [Atenolol]mmol/L	0.9927,0.9855,98.55
					65	120	190	240	309	63.2±13.0732+60.8±5.3372 [Atenolol]mmol/L	0.9988,0.9977,99.77
					0.392	0.511	0.518	0.551	0.612	0.4024±0.0528+0.4086±0.1604[Atenolol]mmol/L	0.9780,0.9564,95.64
2	Vascoten Medochemie 100 mg Cyprus	0.4030±0.0015	5.3661	100±0.3723	95	210	308	398	489	104.8±22.7863+97.6±9.3026[Atenolol]mmol/L	0.9986,0.9973,99.73
					85	150	235	320	400	78±16.2250+80±6.6240 [Atenolol] mmol/L	0.9990,0.9980,99.80
					0.592	0.681	0.751	0.812	0.856	0.5860±0.0274+0.5442±0.0834 [Atenolol]mmol/L	0.9965,0.9931,99.31
3	Novaten Ajanta 100 mg India	0.4028±0.0031	5.3643	100±0.7696	110	220	340	450	580	106±14.9249+117±6.0932[Atenolol]mmol/L	0.9996,0.9992,99.92
					78	166	250	330	420	79.2±6.7350+84.8±2.7496 [Atenolol] mmol/L	0.9998,0.9997,99.97
					0.351	0.423	0.459	0.511	0.532	0.3504±0.0204+0.3745±0.0620 [Atenolol]mmol/L	0.9960,0.9920,99.20

Ŷ: Estimated response in mV for developed method and absorbance for UV-Sp. method, r: correlation coefficient, r^2: coefficient of determination, R^2%: percentage capital R square, UV –Sp.: UV –Spectrophotometric method, $t_{0.05/2,}$□=1.96 at 95%,$t_{0.05/2,3}$=3.182 for n=5.

Table 8. B: Summary of results for practical content, efficiency (Rec %) for determination of atenolol in three samples of drugs and t-test for comparison two methods.

No. of sample	Type of method: Newly developed methodology — Cell A / Cell B; UV-Sp. Classical method Absorbance measurement at λ_{max} =270 nm				
	Practical concentration (mmol/L) in 10 ml / in 250 ml	Practical weight of atenolol $\bar{w}$ i(g) ±4.303 $\sigma_{n-1}/\sqrt{n}$ / Weight of atenolol 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 and cell B	Paired t –test Compared between two methods: $t_{cal} = \bar{X}\ d\ \sqrt{n}/\sigma_{n-1}$ / t_{tab} at 95% confidence level(n-1)
1	1.0317 / 20.6332	1.3738±0.6112 / 103.1645 ±45.8976	103.1645%	0.2967 < 4.303	cellA: $\bar{X}\ d$=0.4725, σ_{n-1}=3.8841, 0.2107 < 4.303
	1.0395/ 20.7895	1.3842 ±0.3928 / 103.9469 ±29.4974	103.9469%	0.5757 < 4.303	cell B: $\bar{X}\ d$= - 1.6243, σ_{n-1}=7.9219, /-0.3551/ < 4.303
	0.9848 / 19.6960	1.3114 ±0.0270 / 98.4788 ±2.0275	98.4788%		
2	1.0738 / 21.4754	1.4299 ±0.3982 / 107.3764 ±29.9023	107.3764%	1.0615 < 4.303	
	0.9750 / 19.5000	1.2984 ±0.2341 / 97.4995 ±17.5790	97.4995%	/-0.6121/ < 4.303	
	1.0768 / 21.5360	1.4340 ±0.0780 / 107.6792 ±5.8570	107.6792%		
3	0.9060 / 18.1196	1.2065 ±0.3582 / 90.5978 ±26.8977	90.5978%	/-1.5041/ < 4.303	
	0.9340 / 18.6792	1.2437 ±0.1982 / 93.3958 ±14.8839	93.3958%	/-1.9093/ < 4.303	
	0.9356 / 18.7128	1.2460 ±0.083 / 93.5633 ±6.2325	93.5633%		

μ: quoted value, x d: average of difference between two type of method (developed& classical), n (no. of sample) = 3, σ_{n-1}: standard deviation of different, $\bar{w}$ i: practically weight in mg, $t_{0.05/2,2}$=4.303.

9. Conclusion

The assessment of long distance chasing photometer (NAG-ADF-300-2) through this research work was applied using comparison between NAG-ADF-300-2 analyser with classical UV-spectrophotometric method using atenolol with povidone iodine in aqueous medium. Chemical and physical parameters were studied in this research work. It was recognized that a narrower range is obtained with UV-spectrophotometric, while a wider range was the characteristic of NAG-ADF-300-2 analyser. A long distance chasing photometer (NAG-ADF-300-2) is the choice with excellent extended detection and a wider applicability. In the future using a new 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 and the Diverged or Fluorescence light at 0-90° via two flow cells of 110 mm and 60 mm length (NAG-ADF-300-2) for study and determination of some selected drugs.

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