Determination of Atenolol in pharmaceutical formulations by continuous flow injection analysis via turbidimetric (T_{180}^{o}) and scattered light effect at two opposite position ($2N_{90}^{o}$) using Ayah $4S_W$-3D-T_{180} -$2N_{90}$ -Solar - CFI Analyser

Nagam S. Turkey Al-Awadie[1]*, Ahmed F. Khudhair[2]

[1]Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq
[2]Department of Chemistry, College of Science, University of kerbala, kerbala, Iraq

Abstract

A simple, rapid and sensitive method for the analysis of Atenolol in pure and pharmaceutical preparation as an alternative analytical procedure were developed by continuous flow injection analysis via turbidimetric (T_{180}^{o}) and scattered light effect at two opposite position ($2N_{90}^{o}$). The method is based upon the formation of white precipitate for the ion pair compound by phosphomolybidic acid with Atenolol in aqueous medium. The precipitate is measured via the attenuation of incident light and scattering of the incident light in two opposite direction namely $+90^{o}$ and -90^{o} angle were measured. Chemical and physical parameters were investigated. The linearity of Atenolol is ranged from (0.1-11) $mmol.L^{-1}$, with correlation coefficient r=0.9938, lower limit of detection (LOD) 0.05 $mmol.L^{-1}(3S_B)$(S/N=3) for n=13 and the relative standard deviation for 7 $mmol.L^{-1}$ Atenolol solution is lower than 3% (n=7). The method was applied successfully for the determination of atenolol in three pharmaceutical drugs. A comparisons were made between the newly developed method of analysis with the classical method (uv-spectrophotometry at wave length 274nm) of analysis using the standard addition method via the use of t-test. It shows that there was no significant difference at α=0.05(95% confidence) between the two methods. Therefore the newly developed method can be accepted as an alternative method for the analysis of Atenolol, in addition to comparison between the official value and the calculated value for both methods

Key word: Atenolol, Spectrophotometry, Turbidity & Nephelometry, Flow injection analysis

تقدير الأتينولول في مستحضراته الدوائيه باستخدام تحليل الحقن الجرياني المستمر عن طريق قياس التعكريه (T_{180o}) وتأثير استطاره الضوء عند اتجاهين متعاكسين ($2N_{90o}$) باستخدام المحلل Ayah $4S_W-3D-T_{180}-2N_{90}-Solar-CFI$

نغم شاكر تركي العوادي[1*]، أحمد فاضل خضير[2]

قسم الكيمياء ، كلية العلوم ، جامعة بغداد ، بغداد ، العراق. قسم الكيمياء ، كلية العلوم ، جامعة كربلاء ، كربلاء ، العراق

الخلاصة

استحدثت طريقة بسيطة وسريعة لتقدير أتينولول بشكله النقي او على هيئة مستحضرات دوائية من خلال اقتران تقنية الحقن الجرياني المستمر مع قياس التعكريه والاستطاره. تستند الطريقة على تكوين راسب ابيض لمزدوج ايوني بين الاتنولول وحامض مولبدات الفسفوريك في الوسط المائي ، تم قياس التعكرية للراسب عن طريق توهين الضوء الساقط وكذلك استطارته عند زاوية قائمه وبأتجاهين متعاكسين +90 و – 90 . تم دراسة المتغيرات الكيميائية والفيزيائية للحصول على الضروف المثلى لتعين الأتينولول، مدى الخطية للأتينولول بين

*Email: nagamturkey2013@Gmail.com

(0.1−11) مللي مول.لتر$^{-1}$ مع معامل الارتباط r=0.9938 وحدود الكشف (L.O.D) 0.05 ملي مول.لتر$^{-1}$ ($3S_B$) (S/N = 3) ولـ n = 13 ,الانحراف القياسي المئوي اقل 3% لتركيز 7 مللي مول.لتر$^{-1}$ أتينولول ل(n = 7). طبقت هذه الطريقة بنجاح لتعين الأتينولول في ثلاثة عقاقير دوائية. اجريت مقارنة بين الطريقة المستحدثة مع الطريقة التقليدية للقياس الطيفي (مطيافية الأشعة فوق البنفسجية عند طول موجي 274نانومتر) باستخدام طريقة الاضافات القياسية وذلك من خلال استخدام اختبار t – ولوحظ عدم وجود فرق جوهري عندα =0.05 وحدود ثقه 95% بين الطريقتين وبالامكان استخدام الطريقه المستحدثه كطريقة بديلة لتقدير أتينولول ، بالاضافه الى المقارنه بين القيمه الرسميه والقيمه المحسوبه لكلا الطريقتين .

Introduction

Atenolol (ATL) figure 1-,[1], chemically known as 4-(2-hydroxy-3-[(1- methylethyl) amino] propoxy) benzeneacetamide .Atenolol belongs to beta blocker group. This is effective in the management of cardiovascular diseases such as hypertension, chronic stable angina pectoris and cardiac arrhythmias. It is also used to reduce the risk of mortality and non-fatal re-infarction in survivors of acute myocardial infarction. ATL is used in conjunction with cardiac glycosides, diuretics and angiotensin converting enzyme inhibitors (ACE-1) in the management of mild to moderate severe heart failure of ischemic or cardiomyopathic origin, to reduce manifestations of the disease progression, including cardiovascular death and hospitalization, and improved clinical status of patient [1,2].

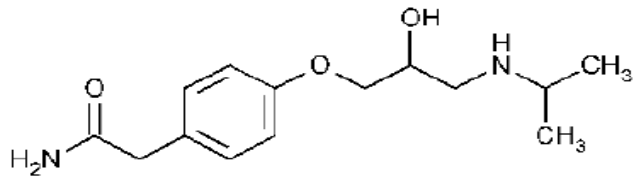

Figure 1- Structure of atenolol

Turbidimetry and nephelometry are closely related analytical techniques based on the scattering of radiation by a solution containing dispersed particulate matter. When a radiation passes through a transparent medium in which solid particles are dispersed, part of the radiation is scattered in all directions, giving a turbid appearance to the mixture. Turbidimetry is based on the measuring of the intensity of the radiation transmitted by the solution whereas nephelometry is based on the scattered radiation at an angle, usually at the right angle. The intensity of radiation appearing at any angle depends upon the number of particles, their size and shape, as well as the wavelength of the radiation [3]. The development of the first analytical turbidimeter was in the 1960s and the fundamental optical technology remained unchanged until the mid-1980s. Since then, instrument design technology has advanced dramatically and many new designs have resulted. These new designs have evolved to address many of the traditional interferences associated with turbidity. Because different technologies (such as light sources and detector design) have been used to compensate or eliminate interferences such as color, bubbles, stray light, absorption, and path length, it is often difficult or impossible to compare measurements[4].The first report in using the turbidimetry in the flow-injection system for determination of sulfate by monitoring the barium sulfate suspension. In spite of the routine use of flow-injection system with turbidimetric detection for the determination of inorganic species in plants and water [5] . Applications to pharmaceutical products were limited. [6, 7]

Literature survey reveals that various analytical methods have been reported for determination of Atenolol in pure form and in pharmaceutical formulations which include high performance liquid chromatography (HPLC) for determination of Atenolol in tablets [8-15] , Other methods reported in the literature for determination of atenolol : uv-vis spectrophotometry. [16-20] high-performance thin-layer chromatographic [21] , ultra performance liquid chromatography (UPLC) [22], gas chromatography (GC) [23-26], nonsuppressed ion chromatography [27] , UV derivative spectrophotometry [28-31], potentiometry [32,33], capillary electrophoresis [34-37], voltametry [38-41] atomic absorption spectrometry (AAS), [42] flourometry[43,44] , differential scanning calorimetry (DSC) ,thermogravimetry (TG) [45] , Chemiluminescence.[2,46],diffuse reflectance spectroscopy[47],Biosensor [48] and phosphorimetry [49].

This paper describes a flow injection (FI) turbidimetric and nephelometric method for determination of atenolol in pharmaceutical formulations. The method is based upon the formation of a white precipitate for the ion pair compound by phosphomolybidic acid with Atenolol in aqueous medium. The precipitate is measured via the attenuation of incident light .Also scattering of the incident light in $+90^{o}$ and -90^{o} angle were measured. The output signal was recorded as an analytical response via time for each signal concentration level. The purposed method is simple and rapid . Each determination can at least three characteristic factors or property of formed precipitate (i.e. attenuation and reflection of light at two opposite reverse direction). The proposed method for the available drug samples in the local market were applies.

Experimental

Reagents and chemicals

All reagents were of analytical reagent grade and distilled water was used to prepare the solutions. A standard solution of Atenolol ($C_{14}H_{22}N_2O_3$, 266.3 g.mol-1,SDI, 0.1mol.L-1) was prepared by dissolving 2.6630 g of Atenolol in 5ml of 1mol.L-1 HCl and diluting to the mark with distilled water in a 100ml calibrated flask. Farther dilution was made whenever it was necessary. A stock solution of phosphomolybdic acid (PMA, $H_3PMo_{12}O_{40}.xH_2O$, 1825.25 g.mol-1 , BDH, 0.1mol.L-1) was prepared by dissolving 18.2525 g of PMA in 100 ml of distilled water and further dilution was made to prepare solution changing variable concentration.

A 1mole.L-1Hydrochloric acid solution was prepared by diluted 88.25 ml of 35% HCl (1.18 g.ml-1, BDH) with distilled water in 1L calibrated flask. Aqueous solutions of sulfuric acid and nitric acid were prepared by diluting 55.5ml from H_2SO_4 (96%,1.84g.ml-1, BDH, 1mol.L-1) and 64ml from HNO_3 (70%,1.42g.ml-1, BDH, 1mol.L-1) to 1L with distilled water.

Sample preparation

The procedure that was adopted for commercially available tablet by selecting thirteen tablets from three different manufacturer .The names of the different suppliers and dose of atenolol tablets (100mg) was recorded. The tablets were weighted, crashed, and grinded by pestle and mortar until fine powder +200 mesh. A 0.05 mmol.L-1 solution was prepared by weighing an amount equivalent to (0.666g) active ingredient for each pharmaceutical preparation. The powder was dissolved in deionized water followed by filtration to remove any undissolved residue affecting the response. The filtrate was completed to 50ml. Further dilution was necessary to allocate the concentration within the linearity of the calibration graph.

Apparatus

Peristaltic pump – 4 channels (Switzerland) an Ismatic type ISM796, A rotary 6- port injection valve (Teflon) ,(IDEX corporation, USA).The response was measured by a homemade Ayah 4SW-3D-T180 - 2N90 - Solar - CFI Analyser [50].Which uses four snow white LED for irradiation of the flow cell at 2mm path length. . Three solar cell used as a detector for collecting signals via sample travel for 40mm length. The readout of the system composed of x-t potentiometric recorder (KOMPENSO GRAPH C-1032) SIEMENS(Germany) or digital AVO-meter(auto range)(0.00-2000mV)(China).Spectrophotometric readings under batch conditions were made by means of a Shimadzu (Japan) UV-1800 double-beam spectrophotometer and quartz cuvette with an optical path length of 10 mm. Figure.2- is shown the flow diagram for atenolol determination.

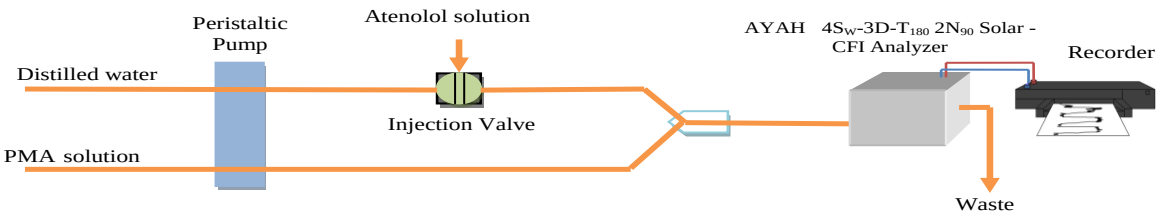

Figure 2- Flow gram for atenolol determination.

Methodology

Atenolol flow injection system, figure.2- for the reaction of ATL-PMA to form white precipitate as an ion pair is composed of two lines : The first line is the carrier stream (distilled water) at 2.2 ml.min-1Flowrate which leads to the injection valve to carry atenolol sample of 60 µl; while the second line supplies phosphomolybdic acid solution (3.5 mmol.L-1) at 2.3 ml.min-1. Both line meet at a junction (Y- junction), with an outlet for reactants product of ion pair. Using Ayah 4SW-3D-T_{180} - $2N_{90}$ - Solar - CFI Analyser four successive instantly signals can be recorded through where the four signals represent turbidity (i .e attenuation of incident light) and reflection of incident light at two opposite direction at +90o, and -90o. While the fourth response represent the summation of both reflected light at two opposite direction. A proposed mechanism of ion pair for system ATL-PMA in aqueous medium is presented in figure.3.[51]

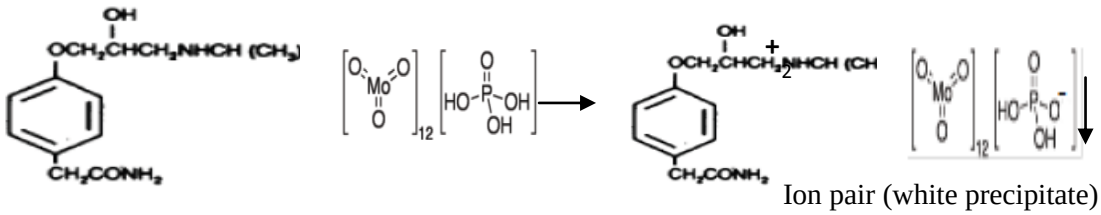

Figure 3- Probable proposed mechanism of reaction between atenolol and phosphomolybdic acid

Results and Discussion

Variable optimization

Chemical parameters(mainly concentration and pH of the reaction medium) as well as physical parameters (intensity of incident light p^o ,volume of coil , flow rate, sample volume) were studied

Effect of Phosphomolybidic acid (PMA) concentration

The effect of the precipitating reagent (PMA) concentration on the sensitivity in general was studied. A series of solutions (1- 4 $mmol.L^{-1}$) were prepared. 10 $mmol.L^{-1}$ from ATL was used as the injected concentration using 60 µl sample volume at 2.9$ml.min^{-1}$ flow rate. The intensity of incident light of total four LEDs 1400 mV. Table 1- summarizes the total results obtained; recoded via four x-t potentiometric recorder. It can be seen that an increase in PMA causes an increase in the incident light due to the attenuation of the light on particle surface. While there is an increase of at N_L followed by a nearly constant response .The same behavior is observed in N_R as shown in figure.4-A. . 3.5 $mmol.L^{-1}$ PMA concentration was regarded as the optimum response that used for further work. figure.4-B shows response profile of the variable PMA concentration on $T_{0\text{-}180}$, scattering light at angles ±90^o.

Table no. 1: Result of PMA on the measurement of attenuation of incident light (turbidity) as well as reflection of light at two opposite position also algebraic sum of them.

[PMA] $mmol.L^{-1}$	Type of measurement $\bar{y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0\text{-}180)}$	Scattering of light (L) N_L (+90)	Scattering of light (R) N_R (-90)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90)
1.0	40±1.067	16±1.023	8±1.033	12±2.012
2.0	264±1.186	124±0.000	28±1.282	84±0.000
2.5	488±1.520	180±2.344	56±1.677	132±2.232
3.0	680±0.000	188±1.233	68±1.871	144±1.562
3.5	736±1.190	200±1.201	64±2.198	152±0.000
4.0	736±1.651	192±2.012	68±2.554	150±1.664

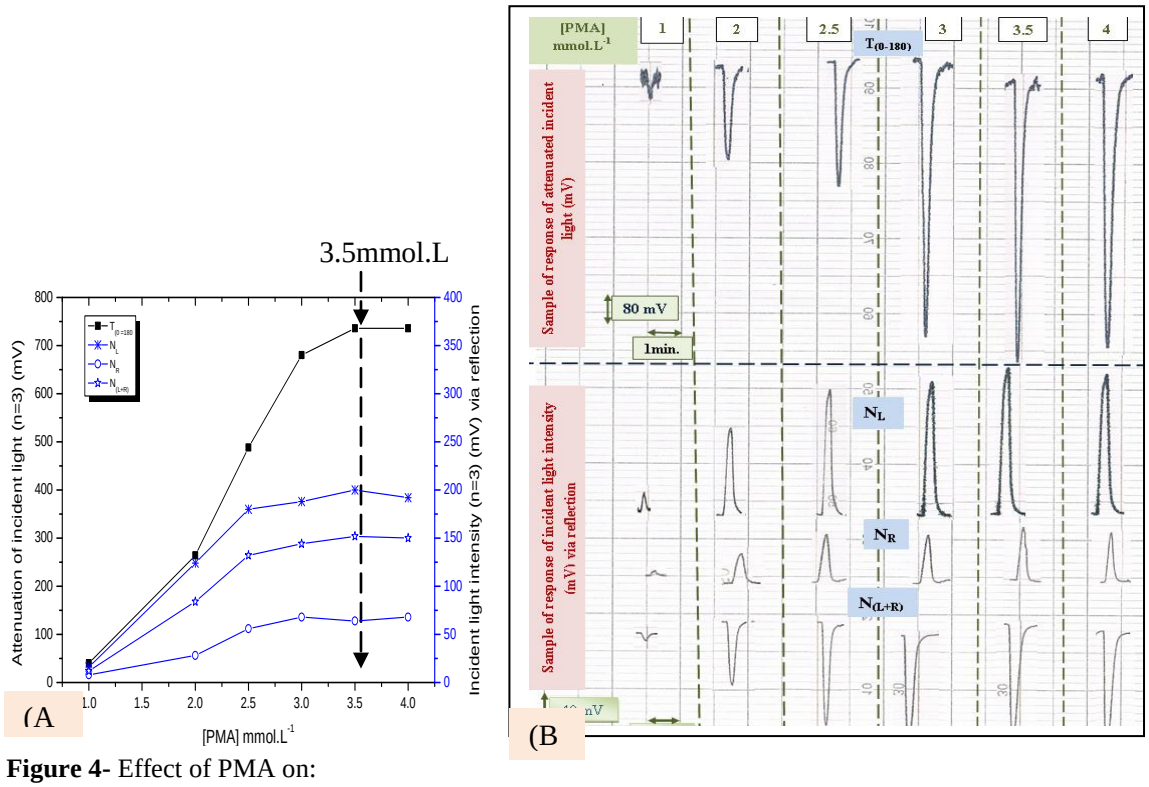

Figure 4- Effect of PMA on:
(A) Attenuation of incident light, scattering of light at two opposite directions and their total outcome.
(B) Response profile.

Effect of pH for the reaction medium

The effect of pH of the reaction medium on the sensitivity in general was studied. Different acid solutions (0.1mol.L^{-1} for each of HCl, H_2SO_4 and HNO_3) were prepared. It can be seen that an increase in sensitivity of response in aqueous medium of carrier stream causes an increase in the incident light due to the attenuation of the light on particle surface. Also an increase of at N_L and The same behavior is observed in N_R as shown in figure.5. . Aqueous medium (i. e. Distilled water) was regarded as the optimum medium for the use in this research.

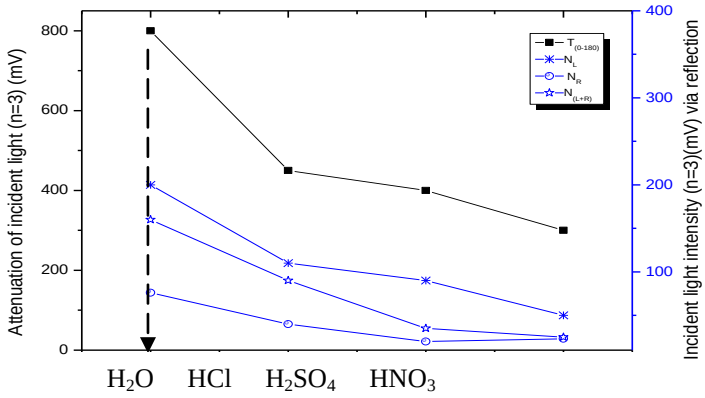

Figure 5- Effect of pH of medium on the measurements of turbidity as well as reflection of light at two opposite position and algebraic sum of them.

Incident light intensity

Intensity of light source was studied using 3.5mmol.L^{-1} of PMA; while maintaining other variable as in previous experiment. Variable intensity of light source was used 0.66 – 1.85 volt by variation of light intensity channel in AYAH $4S_W$-3D-T_{180} -$2N_{90}$-Solar GFI Analyzer operation where read by AVO-meter. The results tabulated in table 2- which shows that an increase on the attenuation of

incident light, scattering of light in two way and outcome of scattering light (electrical outcome) (±90) with increased intensity of source light. The intensity of (1.45 volt) was selected as the optimum voltage that can be supplied to give a better reproducible outcome. figure 6- shows the effect of variation of light intensity on attenuation of light, scattering light (±90) and the algebraic sum of them.

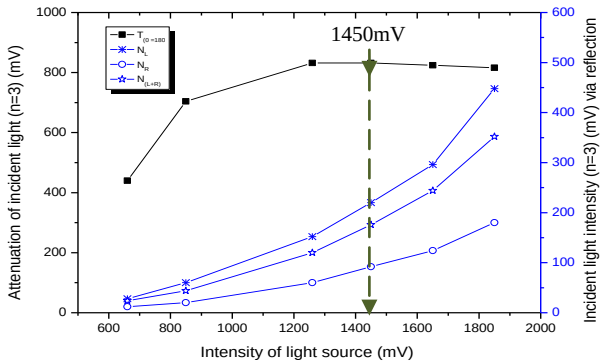

Figure 6- Influence of variation of intensity of light source on the measurements of turbidity as well as reflection of light at two opposite position .Also algebraic sum of them is shown.

Table 2- Effect of Intensity of light source (LED) (P^o) on attenuation of incident light, scattering of light at two opposite position and the algebraic sum of them.

Intensity of source (Volt)	Type of measurement $\bar{y}_i \pm t_{0.05/2}\, \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0-180)}$	Scattering of light (L) N_L (+90)	Scattering of light (R) N_R (-90)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90)
0.66	440±1.820	28±1.344	12±2.034	24±1.342
0.85	704±1.784	60±1.578	20±1.900	44±1.004
1.26	832±0.897	152±0.000	60±0.000	120±2.056
1.45	832±1.556	220±1.034	92±2.895	176±1.442
1.65	824±1.443	296±0.000	124±1.988	244±1.268
1.85	816±1.262	448±1.563	180±2.666	352±1.342

Reaction coil length

Variable coil length 0 - 50cm was studied this range of length comprises a volume of 0 -1572 µl which connected after Y-junction directly in flow system, figure.2-. Optimum PMA concentration, and light intensity of 1450mV were used. Table 3- shows all the results obtained for turbidimtry and the reflection of light at two opposite direction with the algebraic sum of both opposite signals. The table shows clearly that no reaction coil will serve as a more reproducible and more sensitive measurements. figure.7- shows the effect of reaction coil length on attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them.

Table 3- Effect of coil volume on attenuation of incident light, scattering of light at two opposite directions and total outcome of them via reflection .

Length of coil (cm) Diameter (2mm)	Volume of coil (µl)	Arrival time of injected sample to nubble of the measure (Sec.)	Dilution factor (D.F)	Type of measurement $\bar{y}_i \pm t_{0.05/2}\, \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
				Attenuation of incident light $T_{(0-180)}$	Scattering of light (L) N_L (+90)	Scattering of light (R) N_R (-90)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90)
0	0	12	1.00	864±1.224	236±1.987	108±1.666	180±1.522
10	314	18	6.23	840±1.003	280±1.055	112±1.898	220±2.562
20	628	20	11.47	800±0.000	292±1.676	104±2.305	228±2.567
30	942	22	16.70	752±0.563	260±0.000	88±1.532	200±2.432
40	1252	24	21.87	720±1.546	264±1.906	76±1.268	192±1.278
50	1572	26	27.17	728±1.980	248±1.576	80±1.034	184±1.264

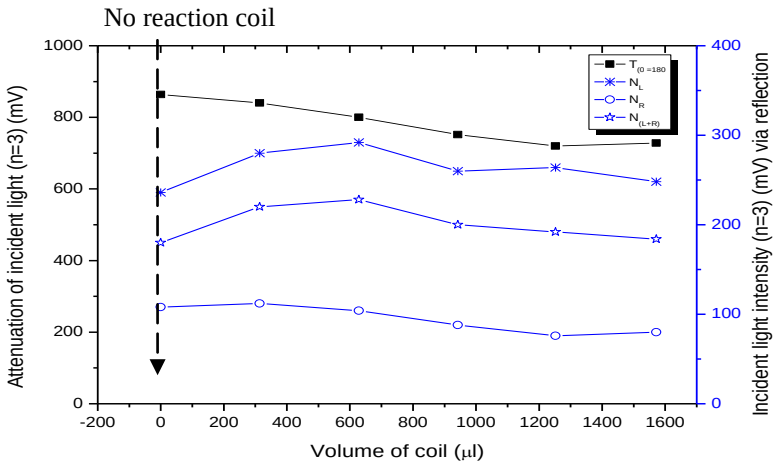

Figure 7- Effect of reaction coil length on attenuation of incident Light (turbidity), scattering of light at two opposite directions, the algebraic sum of them via reflection.

Flow rate

Using optimum parameters achieved in previous sections. A variable flow rate (0.8-3.63 ml.min^{-1}) was studied. Table 4- and figure 8- were notice that at low flow rate there is an increase in dispersion and dilution. A 2.2 ml. min^{-1} carrier stream flow rate was chosen on the basis of the best sensitivity and repeatability.

Table 4- Effect of peristaltic pump speed on attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of the scattering light.

Speed of peristaltic pump (indication approximate)	Flow rate of PMA ml.min^{-1}	Flow rate of carrier ml.min^{-1}	Type of measurement $\bar{y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
			Attenuation of incident light $T_{(0-180)}$	Scattering of light (L) N_L (+90)	Scattering of light (R) N_R (-90)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90)
10	0.80	0.73	752±1.023	200±1.743	64±2.034	140±1.045
20	1.55	1.50	816±1.982	192±1.044	64±1.965	144±1.034
30	2.30	2.20	856±1.547	184±0.000	72±1.034	148±1.653
30	3.00	2.90	840±1.870	180±0.000	68±2.589	140±1.054
40	3.63	3.50	754±0.000	172±0.000	64±0.000	142±0.000

Sample volume

Under the conditions already selected; the injection volume was varied from 20 to 70 µl using open valve mode i.e. allowance for continuous purge of sample from the sample loop in injection valve. An increasing in the injection volume led to a significant increase in sensitivity, more perceptible than low volumes as shown in figure.(9-A) which shows that the optimum sample volume 60 µl gave regular responses to the attenuation of incident light and scattering of light in two way (±90). Using larger volume > 60µl even though it gave a slight response but it was characterized with the width of possible attribution to a long time duration of the precipitate ion pair formed as illustrated in figure. (9-B).

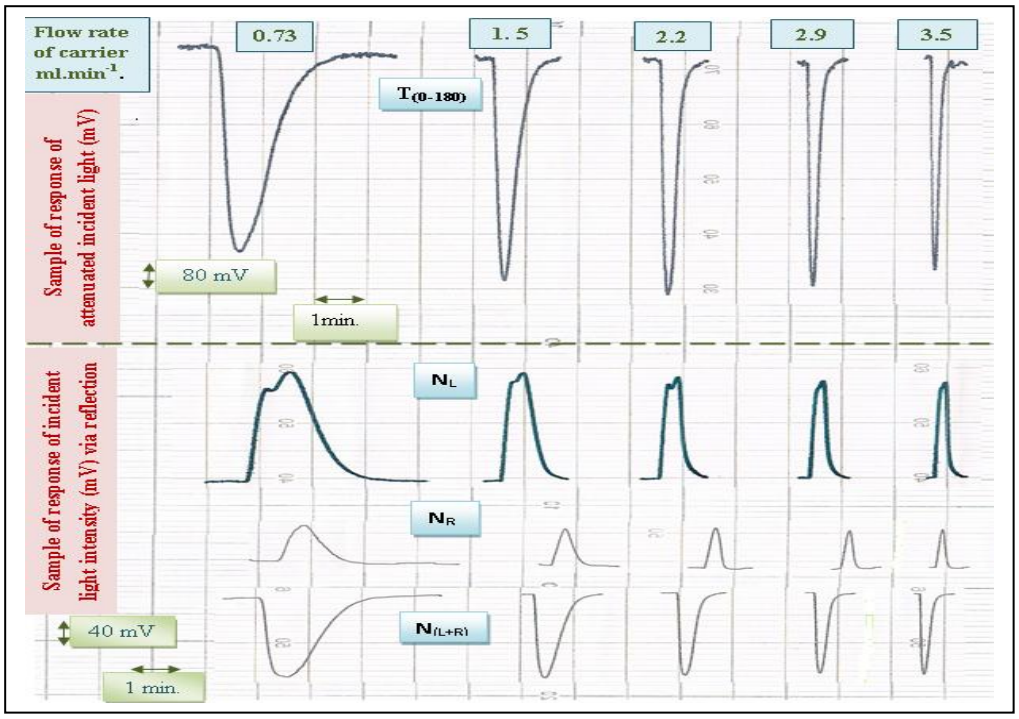

Figure 8- Response profile of flow rate vs. attenuation of incident Light (turbidity), scattering of light at two opposite directions and the algebraic sum of them via reflection.

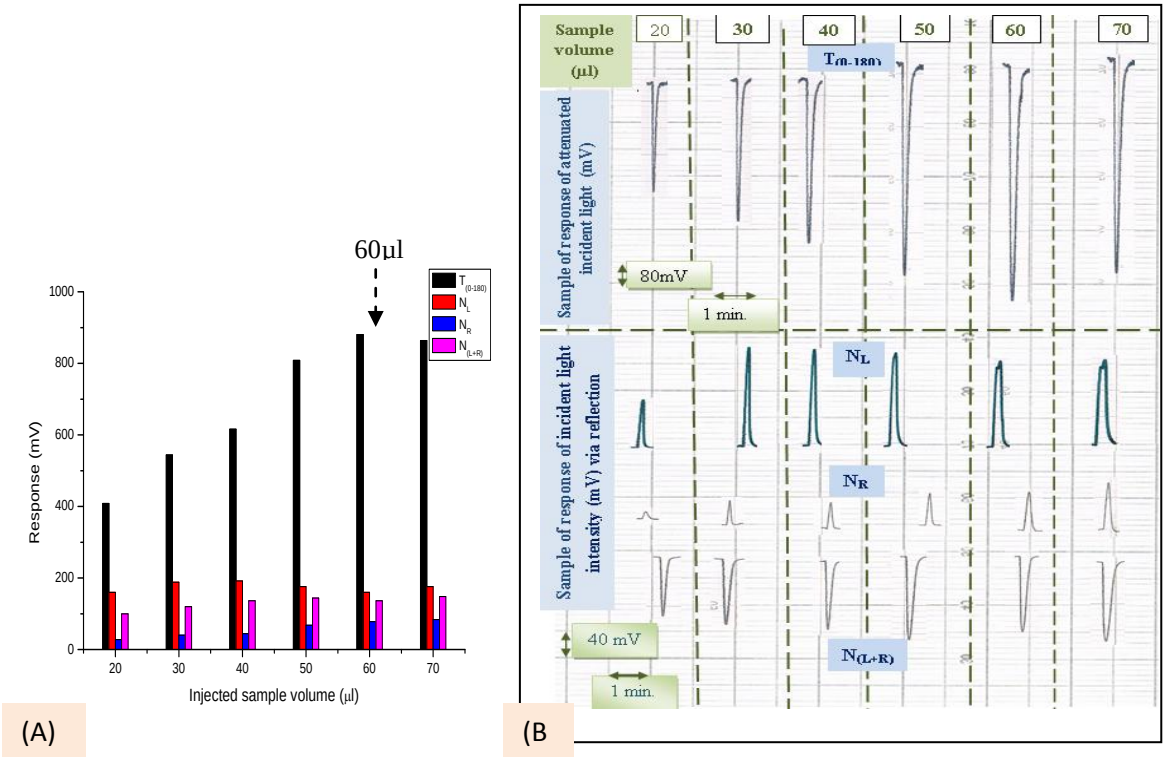

Figure 9- Effect of of sample volume on
(A) Attenuation of incident Light, scattering of light at two opposite directions and the algebraic sum of them via reflection. (B) Response profile.

Calibration curves and statistical parameters

After optimizing the measurement parameters, the calibration curves of continuous flow injection analysis via turbidimetric ($T_{180}{}^{o}$) and scattered light effect at two opposite position ($2N_{90}{}^{o}$) method were evaluated. A series 0.1-11 mmol.L^{-1} Atenolol solution were prepared .Table 5- tabulate the results obtained.

Table 5- Summary of linear regression equation[52,53] for estimate of atenolol by FIA method

Type of measured	Range of [ATL] mmol.L^{-1} n=13	y^(mV)=a± S_at+ b± S_bt[x] at confidence interval 95%, n-2	r r^2%	t_{tab} at 95% confidence interval, n-2 $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
$T_{(0-180)}$	0.1-11	16.77±37.86+82.31±6.07[x]	0.9938 98.78	2.201<<26.992
N_L	0.1-11	10.69±9.35+17.54±3.28[x]	0.9918 98.37	2.201<<23.305
N_R	0.1-11	-4.26±6.07+7.723±0.97[x]	0.9823 96.51	2.201<<15.774
$N_{(L+R)}$	0.1-11	2.53±10.74+15.3±1.72[x]	0.9859 97.20	2.201<<17.676

y^= Estimated response (mV) for (n=3) , [x] = Atenolol conc. (mmol.L^{-1}), r = correlation coefficient, r^2%= linearity percentage

Limit of detection for ATL was conducted through three methods as tabulated in table 6- at injected sample volume of (60µl) . Also L.O.Q was reported.

The value of RSD% for some selected concentration of ATL tabulated in table 7. This low percentage of relative standard deviation (less than 3%) indicate a reliable measurement can be achieved using this method . The response profile is illustrated in figure 10.

Table 6- Limit of detection of Atenolol at optimum parameters depend on $T_{(0-180)}$.

Theoretical based on the value of slope $X=3S_B/slope$	Theoretical based on the linear equation $\acute{Y}=Y_B+3S_B$	Practically based on gradual dilution for the minimum concentration	L.O.Q $\acute{Y}=Y_B+10S_B$
0.1005 mmol.L^{-1}	0.1008 mmol.L^{-1}	0.0500 mmol.L^{-1}	0.3360mmol.L^{-1}

X= value of L.O.D based on slope, S_B=standard deviation of blank, Y_B=Average response for blank, L.O.D =limit of detection, L.O.Q. =limit of quantitate

Table 7- Repeatability of Atenolol at optimum parameters.

[ATL] mmol.L^{-1}	Type of measurement	Average response $\bar{Y}_i$(mV) (n=7)	σ_{n-1}	R.S.D%	Confidence interval of the average response (95% confidence) $\acute{Y}_i \pm t_{0.05/2}\,\sigma_{n-1}/\sqrt{n}$
3	$T_{(0-180)}$	232	1.56	0.67	232± 1.637
	N_L	74	0.52	0.70	74±0.546
	N_R	18	0.89	4.90	18±0.934
	$N_{(L+R)}$	50	0.72	1.44	50±0.756
7	$T_{(0-180)}$	640	1.33	0.21	640±1.396
	N_L	140	0.22	0.16	140±0.231
	N_R	50	1.26	2.52	50±1.323
	$N_{(L+R)}$	116	1.19	1.03	116±1.290

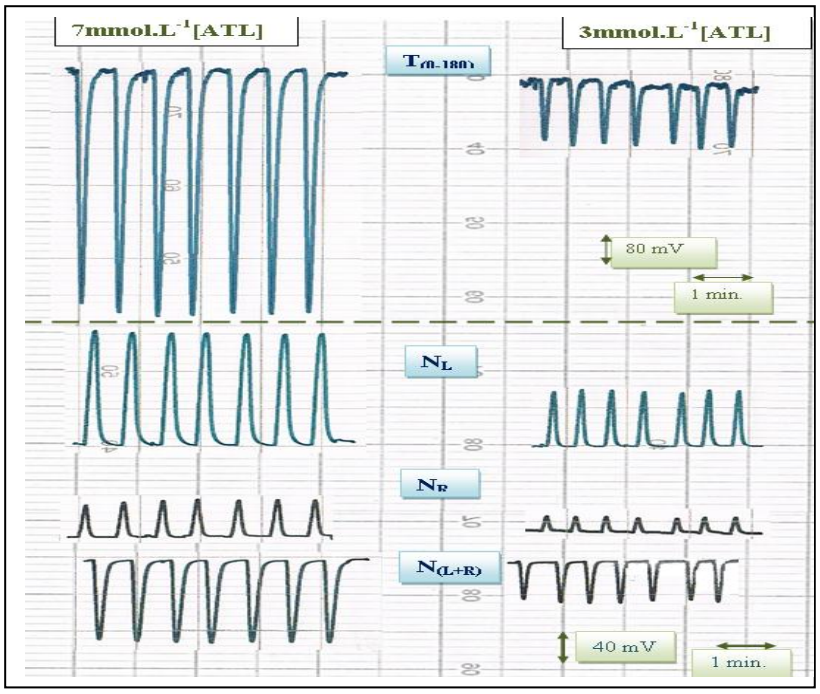

Figure 10- Response profile of repeatability to attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them via reflection.

Analysis of pharmaceutical preparation

The CFIA via turbidity (T_{0-180}) and scattered light at two opposite position ($2N\pm_{90}{}^{o}$) method using Ayah $4S_W$-3D-T_{180} - $2N_{90}$ - Solar - CFI Analyzer achieved in this work was used for the analysis of ATL in three different of pharmaceutical preparation (TENORMIN- Astrazeneea, Atenolol Taolecates the Ajenta, Atenolol - actavix) and was compared by UV-spectrophotometry method via the measurement of λ_{max} at 274nm, linear calibration curve was obtained for the concentration range of 0.01-2.5 mmol.L^{-1} ,correlation coefficient was 0.9981 and limit of detection was 0.006 mmol.L^{-1}. A series of solution were prepared of each pharmaceutical drug (50 mmol.L^{-1}) by transferring 1ml to each of the five volumetric flask (10 ml), followed by the addition of 0, 0.4 , 0.6 , 0.8 and 1ml from 50 mmol.L^{-1} standard solution of ATL in order to have the concentration range from 0 -5 mmol.L^{-1} for the preparation of standard addition calibration plot . The measurement were conducted by both methods.

Results were mathematically treated for standard addition method. The results were tabulated in table 8- at confidence interval 95%. t-test was used as shown in table 9- which shows a comparison .Treatment of data were subjected to two different paths:

First: taking into consideration that all sample from different companies are of the same population standard; i.e. neglecting individual differences between one manufacturer and another as in a strazeneea, Ajenta and activix , As they all Quoted value the same (100mg).

Table 9-. column. 12. shows the paired t-test applied i.e. both methods uv-spectrophotometric and adopted new method (FIA-T&N), there were no significant difference between the tabulated value and the practically found value for both methods.

Second: there were not drawn from the same standard population . Table 9- column 10, 11 shows the calculated t-values by individual t-test.

Column no.10 indicate three t-values, the tablet mad by actavix shows that there is a significant difference while the other two does no show that, while column no 11 shows the compare between Quoted value and calculate t-value that indicate that there were no significant difference between the Quoted value of each individual company with calculated t- value at 95% confidence interval as the calculated t-value is less than critical tabulated t-value.

In addition to: in the above treatment the two approaches that have been indicate that the adopted method can validate the minor differences in each tablets manufacturer by different company (taking in to consideration that individual additives that is used by different companies has no significant differences as mention earlier.

Table 8- Atenolol determination in pharmaceutical tablets by standard addition method using FI – T&N method (depend on $T_{(0-180)}$) & UV method

Sample No.	Pharmaceutical drug company And Claimed content of active ingredient	Weight of sample (g) that equivalence to (666 mg) of active ingredient to obtain 0.05mol.L^{-1} of atenolol in 50ml	Confidence interval of the mean $\overline{W} \pm t_{\frac{0.05}{2},(n-1)} \frac{\sigma_{n-1}}{\sqrt{n}}$ (g m)	y^(mV)=a± S_at+ b± S_bt[x] at confidence interval 95%, n-2	r r^2%	Theoretical calculated active material (mg)	[ATL]mmol.L^{-1} measured				[ATL] mmol.L^{-1} measured to $T_{(0-180)}$	Practically found content of active ingredient mg	Efficiency of determination
				Proposed method FI – T&N			$T_{(0-180)}$	N_L	N_R	$N_{(L+R)}$	Proposed method FI – T&N		
				uv- sp method							uv-sp method		
1	TENORMIN Astrazeneea U.Klimited U.K 100mg	2.8180	0.4233±0.00198	596.48±111.81+110.54±34.02 [x]	0.9862 97.26%	100±0.467	5.3	5.3	5.2	5.2	5.3	106 ±0.620	106%
				0.055±0.108+1.219±0.087 [x]	0.9981 99.64%						1.9	95± 0.444	95%
2	Atenolol Taolecates NOVATEN-100 Ajenta India 100mg	2.6273	0.3945±0.0039	437.30±53.52 +96.108±16.26[x]	0.9957 99.15%	100±0.9886	4.6	4.6	4.5	4.5	4.6	92±0.842	92%
				0.055±0.108+1.219±0.087 [x]	0.9981 99.64%						1.75	92.5± 0.92	92.5%
3	Atenolol tablets actavix U.K 100mg	2.9950	0.4497±0.0027	499.51±75.78+115.46±23.06[x]	0.9941 98.83%	100±0.600	4.4	4.5	4.2	4.2	4.4	88±1.363	88%
				0.055±0.108+1.219±0.087 [x]	0.9981 99.64%						1.77	93.5±0.555	93.5%

y^= Estimated response (mV) for (n=3) , [x] = Atenolol conc. (mmol.L^{-1}), r = correlation coefficient, r^2%: linearity percentage ,
FI – T&N= CFIA via turbidity (T_{0-180}) and scattered light at two opposite position ($2N\pm_{90}{}^{o}$) method ,
uv-sp = uv-spectrophotometry method.

Table 9- Results of t-test for the new adopted method (depend on $T_{(0-180)}$) and uv- spectrophotometric method for the determination of Atenolol in pharmaceutical drugs.

Sample No.	Pharmaceutical drug company And Claimed content of active ingredient	Practical Content(mg)		d (mg) FI– T&N with uv-Sp	$\bar{X}_d$ FI– T&N with uv-Sp	σ_{n-1} FI– T&N with uv-Sp	σ_{n-1} FI– T&N	σ_{n-1} uv-Sp	$(X-\mu)\sqrt{n}/$ FI– T&N With Quoted	$(X-\mu)\sqrt{n}/_S$ uv-Sp With Quoted	Paired t-test $\bar{X}_d\sqrt{n}/\sigma_{n-1}$ FI– T&N with uv-Sp	t_{tab} at 95% confidence interval, n-1
		Proposed method FI – T&N	UV-Sp method									
1	TENORMIN Astrazeneea U.Klimited U.K 100mg	106 ±0.620	95± 0.444	11.0			2.5	3.4	4.15	2.54		
2	Atenolol Taolecates NOVATEN-100 Ajenta India 100mg	92±0.842	92.5± 0.92	0.5	5.67	5.25	3.4	3.2	3.95	4.05	1.867 <<4.303	
3	Atenolol tablets actavix U.K 100mg	88±1.363	93.5±0.555	5.5			3.5	3.8	5.93	2.96		

FI– T&N= CFIA via turbidity (T_{0-180}) and scattered light at two opposite position ($2N\pm_{90}{}^{o}$) method , uv-Sp = UV-spectrophotometry method

Conclusion

A simple, accurate and sensitive FI turbidimetric and nephelometric method proposed. The new method can be used to determine of Atenolol in pure and pharmaceutical preparation. White precipitate formed as ion pair compound of ATL used phosphomolybidic acid. Which is used Ayah $4S_W$-3D-T_{180} -$2N_{90}$ -Solar - CFI Analyser to measure and did not show significant differences in analytical performance when compared with other methods as shown in table (8 and 9). Therefore. It can be regarded as an alternative reliable determination method for the drug discussed in this research.

Acknowledgement

I would like to express my deepest gratitude to Prof. Dr. Issam M.Shakir Al-Hashimi for his appreciable advice, important comments support and encouragement.

References

1. Vaikosen E. N.; Ebeshi1, B. U. and Joffa, P. P. K. **2012** .Simple, Sensitive and Reproducible Acetous Perchlorate and Spectrophotometric Determination of Atenolol in Tablet Dosage Form. *Journal of Pharmaceutical Science & Research*, 4(10), pp:1933 – 1938.
2. Dongdong, Li;, Jianxiu, Du and Jiuru, Lu. **2008** .Chemiluminescence determination of atenolol in biological fluids by a europium-sensitized permanganate-sulfite system. *Microchimica Acta* , 161,pp: 169–173.
3. Matthieu, T.and Carlos, A. R.. **2009**.Flow injection visible diffuse reflectance quantitative analysisof total sulfur in biodiesel, in plant leaves and in natural waters , *Ecl. Quím., São Paulo*, 34(2),pp: 29 – 36.
4. Mike, S. .**2002**. "Turbidity instrumentation an overview of today's available techenology" *Turbidity and Other Sediment Surrogates Workshop* , April 30 – May 2, Reno, NV .
5. Krug ,F. J.; Filho, H. B.; Zagatto ,E. A. G. and Jørgensen. S. S. Jørgensen. **1977**. Rapid determination of sulphate in natural water and plant digests by continues flow injection turbidimetry. *Analyst*, , 102, pp:503-508.
6. Cícero, O. C.; Airton V.P.; Clezio A. and Orlando F. **1999**. Flow injection turbidimetric determination of thiamine in pharmaceutical formulations using silicotungstic acid as precipitant reagent, *Talanta*, 48 (3),pp: *659-667* .
7. Calatayud ,J. M. ; Falcó, P. C. and Sanchez, A. . **1987**. Turbidimetric determination of chlorhexidine using flow injection analysis. *Analyst*, 112**,**pp:87-90
8. Thulasamma, P; and Venkateswarlu P .**2012**. RP-HPLC method for simultaneous determination of atenolol and indapamide in pharmaceutical dosage forms, human blood and milk. *European Journal of Chemistry*. 3 (2),pp :138-142.
9. Kumudhavalli , M. V. ; Anand Babu, Jayakar, K. and B.. **2011** .Development and validation of a RP-HPLC Method for Simultaneous Estimation of Atenolol and Nitrendipine in Tablet Dosage Form. *Der Pharma Chemica*, 3 (4),pp:63-68.

10. Orville, H. W.; Edwin, N. A. and William, D. M. **1978** .Rapid determination of atenolol in human plasma and urine by high-pressure liquid chromatography. *Journal of Pharmaceutical Sciences* , 67(7),pp: 1033–1035.

11. Claudio, G.;Anna T.; Sergio, C. and Giovanni, Z. **1997**. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography application to pharmacokinetic studies in man. *Journal of Chromatography B: Biomedical Sciences and Applications*, 698 (1–2),pp: 187–194.

12. Chiu, , J.N. ; Zhang, R.C. Li and Raymond, K. **1997** .Efficient assay for the determination of atenolol in human plasma and urine by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B: Biomedical Sciences and Applications* ,691(2),pp: 473–477.

13. Anil, K.; Erika R.M. and Maria Inês R.M. **2001** .Development and Validation of a Chiral Liquid Chromatographic Method for the Determination of Atenolol and Metoprolol Enantiomers in Tablet Preparations. *Journal of AOAC International*, 84(6),pp: 1724-1729.

14. Radulovic, D. ; Zivanovic, L. J. ; Velimirovic, G. and Stevanovic, D. **1991**. High-Performance Liquid Chromatographic Determination of Atenolol in Tablets. *Analytical Letters*, 24 (10),pp:1813-1823.

15. Magali, D.; Charlotte, D.; François P. and Philippe M. **2004** .Simultaneous determination of thirteen β-blockers and one metabolite by gradient high-performance liquid chromatography with photodiode-array UV detection. *Forensic Science International*, 141(1),pp: 23–31.

16. Kudige, N. P. and Kanakapura B. **2011**. Sensitive Spectrophotometric Determination of Atenolol in Pharmaceutical Formulations Using Bromate-BromideMixture as an Eco-Friendly Brominating Agent. *Journal of Analytical Methods in Chemistry*, 2012(27),pp:1-12.

17. Mónica, C. F. ; Patricia M. C.and Teodoro S. K. **2003**. Chemometrics-assisted simultaneous determination of atenolol and chlorthalidone in synthetic binary mixtures and pharmaceutical dosage forms. *Analytical and Bioanalytical Chemistry* , 377(7-8),pp: 1159-1164.

18. Agrawal ,Y. K. ; Raman, K. ; Rajput, S. and Menon, S. K. . **1992**. Spectrophotometry Determination of Atenolol via Hydroxamic acid Formation. *Analytical Letters* , 25(8),pp: 1503-1510.

19. Priyanka, R. P.; Sachin, U. R.; Dhabale ,P. N. and Burade, K.B .. **2009** .Simultaneous UV Spectrophotometric Method for Estimation of Losartan Potssium and Amlodipine Besylate in Tablet Dosage Form. *Asian J. Research Chem*, **2**(1),pp:183-187.

20. Burade, A.S. ; Ragab, G.H and Saleh, H . **2002**. Colorimetric determination of β-blockers in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis* , 30(4),pp:1347–1353.

21. Argekar, A.P. and Powar, S.G. . **2000**. Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography. *Journal of Pharmaceutical and Biomedical Analysis* ,21(6),pp: 1137–1142.

22. Lakshmi, N. Y. and Barhate, V. D. **2010** .Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms. *Journal of Chemistry Metrol*, 4(1),pp: 1-20.

23. Bryan, S. and Paul, B. C. **1975** .The gas chromatographic determination of atenolol in biological samples. *Journal of Pharmacy and Pharmacology*, 27(6),pp: 430–433.

24. Magnar, E.; Kerstin, K. and Per-Olof , L. **1980** .Electron capture gas chromatographic determination of atenolol in plasma and urine, using a simplified procedure with improved selectivity. *Journal of Chromatography B: Biomedical Sciences and Applications* . 182(3)13,pp: 341–347.

25. Suk, H. W.; Robert, F. M. and Shaikh, B. M. **1978** .GLC determination of atenolol and β-blocking agents in biological fluids. *Journal of Pharmaceutical Sciences* , 67(9),pp: 1340–1342.

26. Bilal, Y. and Sakir A. **2009**. GC–MS Determination of Atenolol Plasma Concentration after Derivatization with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide. *Chromatographia*, 70(9-10), pp: 1399-1404.

27. Ghanem, R.; Bello, M. A.; Callejon, M., and Guirafim, A.. **1996**. Determination of beta-blocker drugs in pharmaceutical preparations by non-suppressed ion chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 15(3), pp: 383– 388.

28. Prasad, C. V.; Parihar, C.; T. Chowdhary, Purohit, R., S. and Parimo, P.. **1998** .Simultaneous Determination of Atenolol-Amlodipine and Haloperidol–Trihexyphenidyl in Combined Tablet Preparations by Derivative Spectroscopy. *Pharmacy and Pharmacology Communications* , 4(7),pp: 325–330.

29. Periasamy, U. **1994** . Determination of atenolol, nifedipine, aspirin and dipyridamole in tablet preparations by second-order derivative spectrophometry. *International Journal of Pharmaceutics* ,108(1), 25,pp : 11–19.

30. Vetuschi, C. and Ragno, G. . **1990** .Fourth UV derivative spectrophotometry for the simultaneous assay of atenolol and chlorthahdone in pharmaceuticals. *International Journal of Pharmaceutics* , 65(3),pp : 177–181.

31. Bilal, Y. **2008** "Determination of Atenolol in Pharmaceutical Preparation by Zero-, First-, Second- and Third Order Derivative Spectrophotometric Methods. *FABAD J. Pharm. Sci*.33,pp:119–129.

32. Arvand M. ; Vejdani, M. and Moghimi, M. **2008**. Construction and performance characterization of an ion selective electrode for potentiometric determination of atenolol in pharmaceutical preparations. *Desalination*, 225(1–3), pp: 176–184.

33. Mojtaba, S.; Fahimeh, J. and Soheila, H. **2005**. Preparation of an Atenolol Ion-Selective Electrode and its Application to Pharmaceutical Analysis" Analytical Letters , 38(3),pp: 401-410.

34. Jianshe, H.; Jinying, S.; Xiaoguang, Z. and Tianyan, Y. **2007**. Determination of Atenolol and Metoprolol by Capillary Electrophoresis with Tris(2,2′-bipyridyl)ruthenium(II) Electrochemiluminescence Detection. *The Japan Society for Analytical Chemistry*, 23,pp:183-188.

35. Khaldun, A.; Abdalla A ; Mohammed A. E.; Bahruddin S. and Shafida A. **2009**. Simultaneous Determination of Atenolol and Chlorthalidone in Pharmaceutical Preparations by Capillary-Zone Electrophoresis. *Analytical Letters*, 42(10), pp: 1458-1470.

36. Maguregui M. I. ; Alonso R. M. and Jiménez, R. M. . **1997**. Capillary Zone Electrophoretic Method for the Quantitative Determination of the β-Blocker Atenolol in Human Urine. *Journal of Liquid Chromatography & Related Technologies*, 20(20),pp:3377-3387.

37. Khaldun, M. A.; Bahruddin, S. and Hassan Y. A., **2010** .Simultaneous determination of atenolol and amiloride in pharmaceutical preparations by capillary zone electrophoresis with capacitively coupled contactless conductivity detection. *Biomedical Chromatography*, 24(9),pp: 948–953.

38. Rajendra, N. G.; Vinod, K. G.; Munetaka O. and Neeta B. **2006**. Differential pulse voltammetric determination of atenolol in pharmaceutical formulations and urine using nanogold modified indium tin oxide electrode. *Electrochemistry Communications* , 8(1),pp: 65–70.

39. Roopa, H. P. ;Rajesh N. H. and Nandibewoor, S. T. . **2009**. Voltammetric Oxidation and Determination of Atenolol Using a Carbon Paste Electrode. *Ind. Eng. Chem. Res.*, 48 (23),pp: 10206–10210.

40. Elen, R. S.; Roberta A. M.; Romeu C. R .and Orlando F. **2010** . Square-wave voltammetric determination of propranolol and atenolol in pharmaceuticals using a boron-doped diamond electrode. *Talanta* , 81 (4–5),pp: 1418–1424.

41. Mohsen, B.; Sayed, M. G. and Ebrahim, H. **2010** . A Gold Nanoparticle-Modified Carbon Paste Electrode as a Sensor for Simultaneous Determination of Acetaminophen and Atenolol. *Int. Journal of Electrochemistry Science*, 5**,**pp : 1922 – 1933.

42. El Ries, M. A. . **1995**. Indirect Atomic Absorption Spectrometric(AAS) Determination of Atenolol. *Analytical Letters* , 28 (9),pp: 1629-1639.

43. José, A M ; Aurelia A. M. and Pablo F. L. **1998**. Simultaneous determination of atenolol, propranolol, dipyridamole and amiloride by means of non-linear variable-angle synchronous fluorescence spectrometry. *Analytica Chimica Acta*, 370(1), pp: 9–18.

44. Patricia C. D. **2011** . Determination of atenolol in human urine by emission–excitation fluorescence matrices and unfolded partial least-squares with residual bilinearization. *Talanta*, 85(3), pp: 1526–1534.

45. George, P.; John W. R and S. W. Zito. **1995** .The combined use of DSC and TGA for the thermal analysis of atenolol tablets. *Journal of Pharmaceutical and Biomedical Analysis*, 13(2),pp: 103–110.

46. Jianxiu, D.; Dongdong L. and Jiuru L. **2010** .Chemiluminescence determination of naproxen based on europium(III)-sensitized KIO_4–H_2O_2 reaction. *Luminescence*, 25(1),pp: 76–80.
47. Gotardo, M. A.; Sequinel, R. ; Pezza, L. and Pezza, H. R.. **2008**. Determination of atenolol in pharmaceutical formulations by diffuse reflectance spectroscopy. *Ecl. Quím., São Paulo,* 33(4),pp: 7-12.
48. Dimitrios, P. N.; Syrago, E. P. and Maria, V. M. **2002** . A minisensor for the rapid screening of atenolol in pharmaceutical preparations based on surface-stabilized bilayer lipid membranes with incorporated DNA. *Bioelectrochemistry* , 58(1),pp: 107–112.
49. Marcela, A. C.; Luisina, O. G.; Germán P.; Héctor A. A. and Liliana B. **2007** . Determination of atenolol by the micelle-stabilized room-temperature phosphorescence methodology. *Luminescence*, 22(6),pp: 527–533.
50. PATENT Request 16/2013(22nd. Jan**.2013**).Present to *Central Organization for Standardization and Quality control* – Baghdad- IRAQ.
51. Kudige, N. P. and Kanakapura, B. **2012.** Simple, sensitive and selective spectropholtometric methods for the determination of atenolol in pharmaceuticals through charge transfer complex formation reaction.Acta Poloniae Pharmaceutica-Drug Rresearch, 69(2), pp:213-223.
52. Miler, J. C. and Miller, J.N., **1988**, Statistics for Analytical Chemistry, 2nd, Ed., John Wiley and N. y. Sons.
53. Bluman, A. G.,**1997**, *Elementary Statistics*, 3rd, Ed., WCB/MC Graw-Hill, New York.