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Determination of Atenolol and its preparations by Acid-Base Titration in Non-aqueous Medium

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ABSTRACT

Two simple, rapid, reliable, precise and accurate and cost-effective non-aqueous titrimetric procedures have been developed for the determination of atenolol (ATN) in bulk drug and its pharmaceutical formulations. The methods are based on the titration of the drug in glacial acetic acid with acetous perchloric acid to the visual end point using crystal violet as indicator or to the potentiometric end point using a modified glass electrode-SCE system. The methods were applicable over the range of 1.5-15 mg ATN and the calculations are based on a 1:1 reaction stoichiometry. The procedures were also applied for the determination of ATN in its dosage forms and the results were found to be in a good agreement with those obtained by the reference method. The precision results, expressed by intra-day and inter-day relative standard deviation values, were satisfactory (RSD < 2.35%).The accuracy was satisfactory as well (RE ≤ 1.92%). Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures as shown by the recovery study via standard addition technique with percentage recoveries in the range 99.56-101.2% with a standard deviation of ≤ 0.31 %.

Keywords: *Atenolol, Non-aqueous titrimetry, Pharmaceuticals, Crystal violet, Potentiometry.*

INTRODUCTION

Atenolol (ATN), chemically known as 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy) benzeneacetamide[1] (Fig.1), is a β1-selective (cardio selective) adrenoreceptor antagonist drug used for anti-angina treatment to relieve symptoms, improve tolerance and as an anti-arrhythmic to help regulate heartbeat and infections. It is also used in management of alcohol withdrawal, in anxiety states, migraine prophylaxis, hyperthyroidism and tremors.[2] The drug is official in Indian Pharmacopoeia[3] which describes a UV-spectrophotometric method and also in British Pharmacopoeia[4] which recommends high performance liquid chromatographic (HPLC) method for its determination. Several methods have been reported for the determination of ATN in pharmaceutical dosage forms and include diffuse reflectance spectroscopy,[5] HPLC,[6-26] high performance thin layer chromatographic (HPTLC),[27,28] ultra performance liquid chromatography (UPLC),[29] gas chromatography (GC),[30,31] non-suppressed ion-chromatography,[32] flourimetry,[33,34] differential scanning calorimetry (DSC) and thermogravimetry (TG),[35] electrophoresis,[36-38] voltammetry,[39] ion-selective electrode (ISE) based potentiometry,[40] atomic absorption spectrometry (AAS),[41] UV-spectrophotometry,[42-50] and visible spectrophotometry.[51-62]

Some titrimetric procedures were also found in the literature for the assay of ATN. Basavaiah et al (60) have reported a titrimetric method involving the oxidation of ATN by a measured excess of CAT in acid medium followed by the determination of the residual oxidant by iodometric back titration. Bromination reaction of ATN

with a known excess of bromate-bromide mixture in acid medium followed by the determination of unreacted bromine by its reaction with excess iodide and the liberated iodine is titrated to the starch end point using sodium thiosulphate have been reported by the same authors (61) which involves multi step procedure. Another method reported by Basavaiah et al (62) involves the reaction of weakly basic ATN with a measured excess of acid, the residual acid being back titrated with sodium hydroxide, which requires 20 minutes contact time.

In the present investigation, the basic property of the drug molecule is exploited for developing two simple, accurate, precise and inexpensive titrimetric procedures for the assay in which ATN in acitic acid is titrated with acetous perchloric acid to a visual end point employing crystal violet as indicator or potentiometric end point using modified glass electrode-SCE system. These methods, in addition to being rapid and sensitive gave satisfactory results when applied to formulations containing ATN.

MATERIALS AND METHODS

Apparatus

An Elico 120 digital pH meter provided with a combined glass-SCE electrode system was used for potentiometric titration. The KCl of the salt bridge was replaced with saturated solution of KCl in glacial acetic acid.

Reagents and Solutions

All chemicals used were of analytical reagent grade. All solutions are made in glacial acetic acid (S. D. Fine Chem, Mumbai, India) unless mentioned otherwise.

Perchloric Acid (0.005 M):

The stock solution of (~0.1 M) perchloric acid (S. D. Fine Chem, Mumbai, India) was standardized with pure potassium hydrogen phthalate and crystal violet as indicator [63], and then diluted appropriately with glacial acetic acid to get a working solution of 0.005 M perchloric acid.

Crystal violet indicator (0.1 %):

Prepared by dissolving 50 mg of dye (S. D. Fine Chem, Mumbai, India) in 50 mL of glacial acetic acid.

ATN Standard drug solution (1.5mg ml^{-1}):

Stock standard solution containing 1.5 mg mL^{-1} drug was prepared by dissolving the required amount of ATN (Cipla India Ltd., Mumbai, India) in glacial acetic acid.

GENERAL PROCEDURES

Visual Titration

An aliquot of the drug solution containing 1.5-15.0 mg of ATN was measured accurately and transferred into a clean and dry 100 mL titration flask and the total volume was brought to 10 mL with glacial acetic acid. Two drops of crystal violet indicator were added and titrated with standard 0.005 M perchloric acid to a blue colour end point. An indicator blank titration was performed and corrections to the sample titration were applied. The amount of the drug in the measured aliquot was calculated from

$$Amount\ (mg) = VM_wR/n$$

where V = volume of perchloric acid consumed (mL); M_w = relative molecular mass of the drug; R = molarity of the perchloric acid and n = number of moles of perchloric acid reacting with each mole of ATN.

Potentiometric Titration

An aliquot of the standard drug solution equivalent to 1.5-15.0 mg of ATN was measured accurately and transferred into a clean and dry 100 mL beaker and the solution was diluted to 25 mL by adding glacial acetic acid. The combined glass-SCE (modified) system was dipped in the solution. The content was stirred magnetically and the titrant (0.005 M $HClO_4$) was added from a microburette. Near the equivalence point, titrant was added in 0.1 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential (e.m.f) was noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant observed. The equivalence point was determined by plotting the titration curves (volume of titrant

versus e.m.f; first derivative curve or second derivative curve). The amount of the drug in the measured aliquot was calculated as described under visual titration.

Procedure for tablets

Twenty tablets each containing 25, 50 or 100 mg of ATN were weighed accurately and pulverized. An amount of powdered tablet equivalent to 150 mg of ATN was transferred into a 100 ml calibrated flask and 60 mL of glacial acetic acid was added. The content was shaken thoroughly for about 15-20 min, diluted to the mark with glacial acetic acid, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 ml portion of the filtrate was discarded and a suitable aliquot was taken and assayed by following the general procedures described for visual and potentiometric end point detection.

RESULTS AND DISCUSSION

The present methods are based on the neutralization reaction involving the basic property of ATN and employ two techniques. The methods are based on the principle that substances, which are weakly basic in aqueous medium, when dissolved in non-aqueous solvents exhibit enhanced basicity thus allowing their easy determination. In the present titrimetric methods, the weakly basic property of ATN was enhanced due to the non-levelling effect of glacial acetic acid and titrated with perchloric acid with visual and potentiometric end point detection. Crystal violet gave satisfactory end point for the amounts of analyte and concentrations of titrant employed. A steep rise in the potential was observed at the equivalence point with potentiometric end point detection (Fig. 1). With both methods of equivalence point detection, a reaction stoichiometry of 1:1 (drug:titrant) was obtained which served as the basis for calculation. Using 0.005 M perchloric acid, 1.5-15.0 mg of ATN was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficients of 0.9989 and 0.9997 obtained by the method of least squares for visual and potentiometric methods, respectively. From this it is implied that the reaction between ATN and perchloric acid proceeds stoichiometrically in the ratio 1:1 in the range studied.

METHOD VALIDATION

Intra-day and inter-day accuracy and precision

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of ATN within the range of study in each method were analysed in seven and five replicates in method A and method B, respectively, during the same day (intra-day precision) and five consecutive days (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated. The RSD values of intra-day and inter-day studies for ATN showed that the precision of the methods was good (Table 1). The accuracy of the methods was determined by the percent mean deviation from known concentration, and results are presented in Table 1.

Ruggedness of the methods

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different burettes. The inter-analysts RSD were $\leq$ 0.72 % whereas the inter-burettes RSD for the same ATN amounts ranged from 0.38 – 0.75 % suggesting that the developed method was rugged. The results are shown in Table 2.

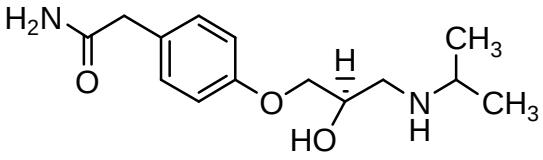
Application

The described titrimetric procedures were successfully applied to the determination of ATN in its pharmaceutical formulations (Atenex 25, Atekind 50 and Aten 100). The results obtained (Table 3) were statistically compared with the official IP method [3]. The official UV-spectrophotometric method involved the measurement of the absorbance of methanolic ATN tablet solution at 275 nm. The results obtained by the proposed methods agreed well with those of reference method and with the label claim. The results were also compared statistically by a Student's t-test for accuracy and by a variance F-test for precision with those of the reference method at 95 % confidence level as summarized in Table 3. The results showed that the calculated t-and F-values did not exceed the tabulated values inferring that proposed methods are as accurate and precise as the reference method.

Recovery Study

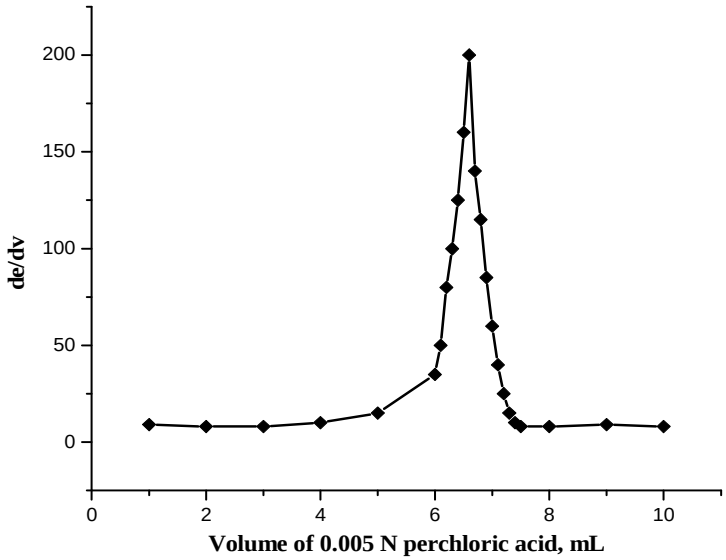
Accuracy and the reliability of the methods were further ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analysed): pure drug at three different levels was added, and the total was found

by the proposed methods. Each test was repeated three times. The results compiled in Table 4 show that recoveries were in the range from 99.56 to 101.2 % indicating that commonly added excipients to tablets did not interfere in the determination.



Atenolol (ATN)

Figure.1. Structure of atenolol



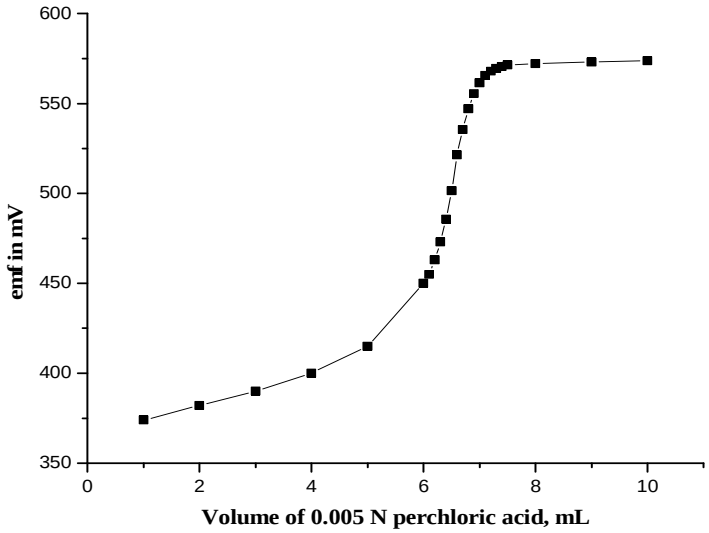


Figure.2. Potentiometric titration curves for 9 mg ATN Vs 0.005 M $HClO_4$.

Table 1. Intra-day and inter-day accuracy and precision data

Method	ATN taken, mg	Intra-day accuracy and precision			Inter-day accuracy and precision		
		ATN found, mg	RE, %	RSD, %	ATN found, mg	RE, %	RSD, %
Visual titrimetry, (n=7)	6.0	6.07	1.13	1.52	6.08	1.36	1.76
	9.0	9.06	0.63	1.29	9.16	1.82	2.26
	12.0	12.11	0.92	0. 90	12.14	1.14	1.52
Potentiometric titrimetry (n=5)	6.0	5.99	0.17	1.19	6.10	1.64	2.24
	9.0	8.95	0.52	0.80	9.12	1.38	1.56
	12.0	11.91	0.71	0.49	12.23	1.92	2.35

RE.relative error, RSD. relative standard deviation.

Table 2 Method ruggedness expressed as intermediate precision (% RSD)

Method	ATN taken, mg	Ruggedness	
		Inter-analysts (% RSD): (n=4)	Inter-burettes (% RSD): (n=4)
Visual end point detection	6.0	0.58	0.75
	9.0	0.43	0.46
	12.0	0.72	0.38
Potentiometric end point detection	6.0	0.32	0.46
	9.0	0.56	0.72
	12.0	0.28	0.67

Table 3. Results of assay in tablets and comparison with the reference method.

Brand name	Label claim, mg/tablet	Found* (Percent of label claim ± SD)		
		Official method	Proposed methods	
			Visual titrimetry	Potentiometric titrimetry
Atenex 25	25	100.3±0.58	99.3±1.08 t=1.82 F=3.47	101.1±1.05 t=1.49 F=3.28
Atekind 50	50	99.67±0.67	98.89±0.94 t=1.51 F=1.97	100.2±0.78 t=1.17 F=1.36
Aten 100	100	100.6±0.82	100.4±1.11 t=0.32 F=1.83	101.3±1.28 t=1.03 F=2.44

**Average of five determinations. Tabulated t value at the 95% confidence level is 2.77. Tabulated F value at the 95% confidence level is 6.39.*

Table 4. Results of recovery study using standard addition method

	Visual titrimetry				Potentiometric titrimetry			
Tablet studied	ATN in tablet extract, mg	Pure ATN added, mg	Total ATN found, mg	Pure ATN recovered* %	ATN in tablet extract, mg	Pure ATN added, mg	Total ATN found, mg	Pure ATN recovered* %
Atenex 25	2.98	3.0	6.01	101.0±0.21	3.03	3.0	6.04	100.3±0.11
	2.98	6.0	9.05	101.2±0.21	3.03	6.0	9.09	101.0±0.15
	2.98	9.0	12.04	100.7±0.15	3.03	9.0	11.99	99.56±0.10
Atekind 50	2.97	3.0	5.98	100.3±0.14	3.0	3.0	6.01	100.3±0.10
	2.97	6.0	8.96	99.83±0.19	3.0	6.0	9.05	100.8±0.12
	2.97	9.0	12.00	100.3±0.16	3.0	9.0	11.98	99.78±0.09
Aten 100	3.01	3.0	6.03	100.7±0.11	3.04	3.0	6.07	101.0±0.21
	3.01	6.0	9.07	101.0±0.12	3.04	6.0	9.07	100.5±0.31
	3.01	9.0	12.00	99.89±0.11	3.04	9.0	12.15	101.2±0.21

**Mean value of three determinations.*

CONCLUSION

Many of the reported methods suffer from such drawbacks as high cost, multiple steps and also several clean-up steps (HPLC). They are time consuming and often poorly reproducible, some require toxic organic solvents. Any method chosen for routine analysis should be reasonably simple, used materials should readily available in the laboratory or readily obtainable, and require a minimum amount of equipment. These objectives have been fulfilled by the two titrimetric procedures developed. The methods provide two simple procedures for the determination of ATN in pharmaceuticals and its dosage forms. The reported potentiometric procedure has the distinct advantages over the previously reported methods in terms of simplicity of technique and ease of performance and does not need expensive and highly sophisticated equipment or high-cost organic solvents which are required for HPLC technique. Therefore, the proposed method can be used in laboratories where modern and expensive instruments are not available.

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