CI&CEQ

Available on line at
Association of the Chemical Engineers of Serbia *AChE*
www.ache.org.rs/CICEQ

Chemical Industry & Chemical Engineering Quarterly 18 (1) 43–52 (2012) CI&CEQ

KUDIGE NAGARAJ PRASHANTH
KANAKAPURA BASAVAIAH SAMEER A. M.
ABDULRAHMAN NAGARAJU RAJENDRAPRASAD
KANAKAPURA BASAVAIAH VINAY

Department of Chemistry, University of Mysore, Manasagangotri, Mysore, Karnataka, India

SCIENTIFIC PAPER

UDC 615.22:661.12

DOI 10.2298/CICEQ110721045P

APPLICATION OF BROMATE-BROMIDE MIXTURE AS A GREEN BROMINATING AGENT FOR THE SPECTROPHOTOMETRIC DETERMINATION OF ATENOLOL IN PHARMACEUTICALS

Two highly sensitive spectrophotometric methods are proposed for the quantification of atenolol (ATN) in pure drug as well as in pharmaceutical formulations. The methods are based on the bromination reaction of ATN with a known excess of bromate-bromide mixture in acid medium followed by the determination of unreacted bromine. The residual bromine is determined by its reaction with excess iodide and the liberated iodine (I_3^-) is either measured at 360 nm (method A) or reacted with starch followed by the measurement of the starch-iodine chromogen at 570 nm (method B). Under the optimum conditions, ATN could be assayed in the concentration ranges of 0.5-9.0 and 0.3-6.0 μg mL^{-1} for methods A and B, respectively, with corresponding molar absorptivity values of 2.36×10^4 and 2.89×10^4 L mol^{-1} cm^{-1}. Sandell's sensitivity values are found to be 0.0113 and 0.0092 μg/cm^2 for methods A and B, respectively. The proposed methods were successfully applied to the analysis of different commercial brands of pharmaceutical formulations and the results obtained by the proposed methods were in good agreement with those obtained using the reference method. The reliability of the methods was further ascertained by recovery studies using standard- addition method.

Keywords: atenolol, assay; bromate-bromide mixture; spectrophotometry; pharmaceuticals.

Atenolol (ATN), chemically known as 4-{2-hydroxy-3-[(1-methylethyl)amino]propoxy}benzeneacetamide [1] is a β1-selective (cardioselective) adrenoreceptor antagonist drug commonly used for the treatment of myocardial infarction, arrhythmias, angina, disorders arising from decreased circulation and vascular constriction, including migraine [2]. Indian Pharmacopoeia [3] describes a UV-spectrophotometric method as an official method for the assay of ATN in tablets whereas high performance liquid chromategraphic (HPLC) method is the official method in British Pharmacopoeia [4]. Various techniques have been developed for the determination of ATN in pharmaceuticals which include diffuse reflectance spectroscopy [5], HPLC [6-26], high performance thin layer chromatographic (HPTLC) [27,28], ultra performance liquid chromatography (UPLC) [29], gas chromategraphy (GC) [30,31], non-suppressed ion- chromategraphy [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], visible spectrophotometry [51-62] and titrimetry [60-62].

To the best of our knowledge, there are twelve reports on the use of visible spectrophotometry for the determination of ATN in pharmaceutical formulations. Agrawal *et al*. [51] have reported the measurement of a red-violet colored ferric hydroxamate complex obtained by the reaction of ATN with hydroxylamine hydrochloride in NaOH medium and subsequent reaction of the resultant hydroxamic acid derivative with

Correspondening author: K. Basavaiah, Department of Chemistry, University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India.
E-mail: basavaiahk@yahoo.co.in
Paper received: 21 July, 2011
Paper revised: 28 September, 2011
Paper accepted: 5 October, 2011

$FeCl_3$. The determination of atenolol through charge transfer complex formation reaction using choranilic acid has been reported by Agarwal *et al.* [52] and Yu *et al.* [53]. The method developed by Korang *et al.* [54] was based on the treatment of a $CHCl_3$ extract of powdered tablets of atenolol with acetaldehyde, a halogenated benzoquinone reagent (chloranil, 2,5-dichlorobenzoquinone or 2,6-dibromobenzoquinone chlorimine) and propan-2-ol. Two kinetic spectrophotometric methods (fixed-concentration method and fixed-time method) were developed exploiting the slow reaction between ATN and ammonium vanadate in sulphuric acid medium [55]. Al-Ghannam and Belal [56] have reported the kinetic spectrophotometric assay of drug based on the reaction between ATN and 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole in borate buffer of pH 8 at the boiling temperature. Hiremath *et al.* [57] developed a method which involves the oxidation of atenolol by a known excess of permanganate in alkaline medium and determination of unreacted permanganate spectrophotometrically at 526 nm. Determination of ATN in basic medium, following the addition of sodium nitroprusside to generate a colored complex has been reported by Bashir *et al.* [58]. Basavaiah *et al.* [59] have reported a method based on the oxidation of ATN by a measured excess of chloramine-T followed by determination of the unreacted oxidant by a charge-transfer complexation reaction involving metol and sulphanilic acid. The same authors [60] reported another method in which the oxidation of ATN by a known excess of chloramine-T in acid medium followed by determination of the unreacted oxidant by reacting with a fixed amount of either metanil yellow or indigo carmine. A similar method employed bromate-bromide mixture, methyl orange as reagents in acid medium has also been reported by the same authors [61]. An acid-base reaction employing phenol red for the assay of ATN was reported by Basavaiah *et al.* [62]. However, many of the above methods suffered from one or other disadvantage like poor sensitivity, narrow linear dynamic range, measurements done at shorter wavelengths, heating or cooling step, use of organic solvents, use of expensive chemical and/or complicated experimental setup, as can be seen from Table 1.

The aim of the present work was to develop two sensitive, cost effective spectrophotometric methods for the determination of ATN in pure drug as well as in pharmaceuticals. The proposed methods utilize bromate-bromide mixture in acid medium as the eco-friendly and green brominating agent, and potassium iodide and starch as auxiliary reagents. The reaction conditions were thoroughly studied, and under optimum conditions, the procedures provide highly sensitive and selective assays for ATN in commercial dosage forms.

EXPERIMENTAL

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) provided with 1 cm matched quartz cells was used for all absorbance measurements.

Reagents and materials

All reagents and chemicals used were of analytical or pharmaceutical grade and distilled water was used to prepare the solutions.

Bromate-bromide mixture

A standard stock solution of $KBrO_3$-KBr equivalent to 300 μg/mL $KBrO_3$ was prepared by dissolving accurately weighed 30 mg of $KBrO_3$ (S. D. Fine Chem. Ltd., Mumbai, India) and 0.3 g of KBr (Merck, Mumbai, India) in water and diluting to the mark in a 100 mL calibrated flask. The stock solution was diluted with water to get bromate-bromide mixture solutions containing 30.0 μg/mL in $KBrO_3$ for use in method A and 15 μg/mL in $KBrO_3$ for method B.

Potassium iodide

A 2% potassium iodide (Merck, Mumbai, India) solution was prepared by dissolving 2 g potassium iodide with water in a 100 mL calibrated flask. This solution was prepared a fresh daily.

Starch solution

One gram of starch (LOBA Chemie Ltd., Mumbai, India) was made in to paste with water and slowly poured with constant stirring into 100 mL boiling water, boiled for 5 min, cooled and used. This solution was prepared freshly every day.

Hydrochloric acid

Concentrated acid (Merck, Mumbai, India, Sp. gr. 1.18) was diluted appropriately with water to get 3 M HCl for use in both methods.

Sodium acetate

A 3 M aqueous solution of sodium acetate was prepared by dissolving suitable quantity of sodium acetate trihydrate crystals (Merck, Mumbai, India) in water for use in method A.

Standard solution of ATN

Pharmaceutical grade atenolol certified to be 99.89% pure was received from Cipla India Ltd., Mumbai, India, as gift and was used as received. A

Table 1. Comparison of the performance characteristics of the proposed methods with the existing visible spectrophotometric methods for atenolol

Sl. No.	Reagent(s) used	Methodology	λ_{max} nm	Linear range, μg/ml (ε / L mol^{-1} cm^{-1})	LOD μg/mL	Reaction time	Remarks	Ref.
1.	Hydroxylamine hydrochloride-iron (III)	Ferric hydroxamate complex measured	510	50-800 (5.3×10^2)	Not reported	20-30 min	Less sensitive, heating required	51
2.	Chloranilic acid	Charge transfer complex measured	534	25-250	Not reported	-	Less sensitive, use of organic solvents	52
3.	Chloranilic acid	Charge transfer complex measured	530	10-280	Not available	Not available	-	53
4.	Acetaldehyde-Chloranil	-	690	Not available	Not available	Not available	Use of organic solvents	54
5.	NH_4VO_3	Reaction rate measured	750	Not available	Not available	Not available	Heating required	55
6.	4-Chloro-7-nitrobenzo--2-oxa-1,3-diazole	Coupling product measured as a function of time	460	5-50	1.3	30 min	Heating required	56
7.	Potassium permanganate in alkaline medium	Unreacted $KMnO_4$ measured Rate-constant method Fixed-concentration method Fixed-time method	526	6.66-10.65 6.66-5.33 6.66-7.99	-	4 h	Time -consuming, involve judicial control of many experimental variables	57
8.	Sodium nitroprusside	Complex of ammonia and nitroprusside measured	495	0.5-30 (3.01×10^5)	0.01	5 min	Heating required	58
9.	Chloramine-T-metol-sulphanilic acid	Unreacted chloramine-T measured	520	2.5-25 (3.24×10^3)	2.34	20 min	Less sensitive	59
10.	Chloramine-T Metanil yellow Indigo carmine	Unreacted chloramine-T measured	530 610	1-12 (1.19×10^4) 2.5-20 (6.65×10^3)	0.32 0.04	10 min	-	60
11.	Bromate-bromide mixture Methyl orange	Unreacted bromine measured	520	0.5-4.0 (4.13×10^4)	0.07	15 min	-	61
12.	Phenol red	The change in the color of phenol red measured	430	3.0-30 (3.47×10^3)	4.61	-	Less sensitive	62
13.	Bromate-bromide mixture: Iodine Starch-iodine	Tri-iodide ion measured Starch-iodine complex measured	360 570	0.5-9.0 (2.36×10^4) 0.3 -6.0 (2.89×10^4)	0.10 0.08	15 min	Simple, sensitive and no heating step. No use of organic solvent. Use of a green brominating reagent.	Proposed methods

stock standard solution equivalent to 100 μg/mL ATN was prepared by dissolving accurately weighed 25 mg of pure drug with water in a 250 ml calibrated flask. This stock solution was diluted with water to get the working concentrations of 20 and 15 μg/mL for methods A and B, respectively.

The pharmaceutical preparations Atenex-25 (25 mg ATN per tablet) from Zydas Healthcare, East Sikkim, India; Atekind-50 (50 mg ATN per tablet) from Mankind Pharma Ltd., New Delhi, India, and Aten-100 (100 mg ATN per tablet) from Zydas Healthcare, East Sikkim, India, were purchased from commercial sources in the local market and subjected to analysis.

Procedures

Method A (based on the measurement of tri-iodide ion)

Varying aliquots (0.25-4.5 mL) of standard ATN solution (20 μg/mL) were accurately transferred into a

series of 10 mL calibrated flasks and the total volume was adjusted to 4.5 mL with water. One mL of 3 M HCl was added to each flask followed by the addition of 1 mL bromate-bromide mixture solution (30 μg/mL in $KBrO_3$). The content was mixed well and let stand for 15 min with occasional shaking. Then, 1.0 mL of 3 M sodium acetate solution was added to each flask followed by 1 mL of 2% potassium iodide. The volume was brought up to the mark with water and the absorbance of the resulting triiodide ion was measured at 360 nm after 5 min against the water.

Method B (based on the measurement of starch-iodine chromogen)

Into a series of 10 mL calibrated flasks, different aliquots (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mL) of standard ATN (15 μg/mL) solution were transferred using a micro burette. The total volume in each flask was brought to 4 ml by adding required quantity of water. The solution was acidified by adding 1.0 mL of 3 M HCl, after which 1.0 ml of bromate-bromide (15 μg/mL in $KBrO_3$) solution was added to each flask. The flasks were kept aside for 15 min with periodic shaking; 1.0 mL of 2% potassium iodide was added and the content was mixed well. After 5 min, 1 mL of 1% starch solution was added to each flask and the volume was made up to the mark with water and mixed well. The absorbance of the resulting blue chromogen was measured at 570 nm against water blank after 5 min.

Procedure for the tablets

Twenty tablets containing ATN were accurately weighed and finely powdered. A portion of the powder equivalent to 10 mg of ATN was transferred into a 100 mL calibrated flask to which 60 mL of water was added. The content was shaken for 15-20 min; the volume was diluted to the mark with water, mixed well and filtered using a Whatmann No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and a suitable aliquot of the filtrate (100 μg/mL ATN) was diluted appropriately with water to get 20.0 and 15.0 μg/mL ATN for the assay by methods A and B, respectively.

Procedure for the analysis of placebo blank and synthetic mixture

A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was extracted with water and the solution was made as described under "Procedure for the tablets" and then subjected to analysis.

A synthetic mixture was prepared by adding 10 mg of ATN to the placebo blank prepared above, homogenized and the solution was prepared as done under "Procedure for the tablets". The filtrate was collected in a 100 mL flask. The synthetic mixture solution (100 μg/mL in ATN) was appropriately diluted with water to get 20.0 and 15.0 μg/mL ATN solutions, and appropriate aliquots were subjected to analysis by methods A and B, separately.

RESULTS AND DISCUSSION

Bromate-bromide mixture in acid medium behaves as an equivalent solution of bromine and has been used for the assay of several organic pharmaceutical compounds [63-66]. The proposed methods use the bromine generated *in situ* by the action of the acid on bromate-bromide mixture which can be considered as a green brominating agent and are based on the bromination reaction of ATN with a known excess of bromate-bromide mixture in acid medium through eletrophilic substitution reaction. The main advantages of this reagent are replacement of the highly toxic and hazardous liquid bromine, no formation of hazardous byproducts, eco-friendly and easily available. The unreacted bromine is made oxidizes iodide and liberated iodine which will form tri-iodide ion (I_3^-) in the presence of excess iodide. The amount of iodine liberated, by the reaction of unreacted bromine with potassium iodide, was either measured directly at 360 nm (method A) or reacted with starch and resulting blue colored chromogen of starch-iodine complex is measured at 570 nm (method B) (Figure 1). The reaction scheme of the proposed methods is illustrated in Figure 2.

Optimization of experimental variables

Effect of acid concentration

The effect of acid concentration on the measured species was investigated by following the assay procedures. The effect of 1 mL of HCl of different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 M) was studied by measuring the absorbance of the colored product using a fixed concentration of ATN (6.0 μg/mL in method A and 2.0 μg/mL ATN in method B) (Figure 3). From Figure 3, it is clear that the absorbance of the colored product remained constant with 1.0 mL of 3 to 10 M HCl. Therefore, 1.0 mL of 3.0 M HCl was selected as the optimum for both methods.

Reaction time and color stability

The effect of time on the reaction between ATN and bromate-bromide mixture in the presence of HCl was studied by keeping all other reaction conditions

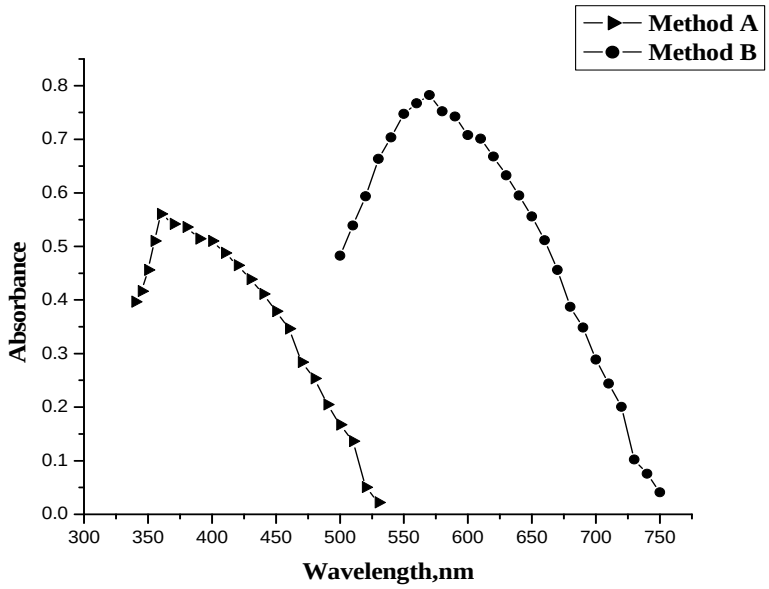

Figure 1. Absorption spectra of tri-iodide ion (method A) and starch-iodine complex (method B).

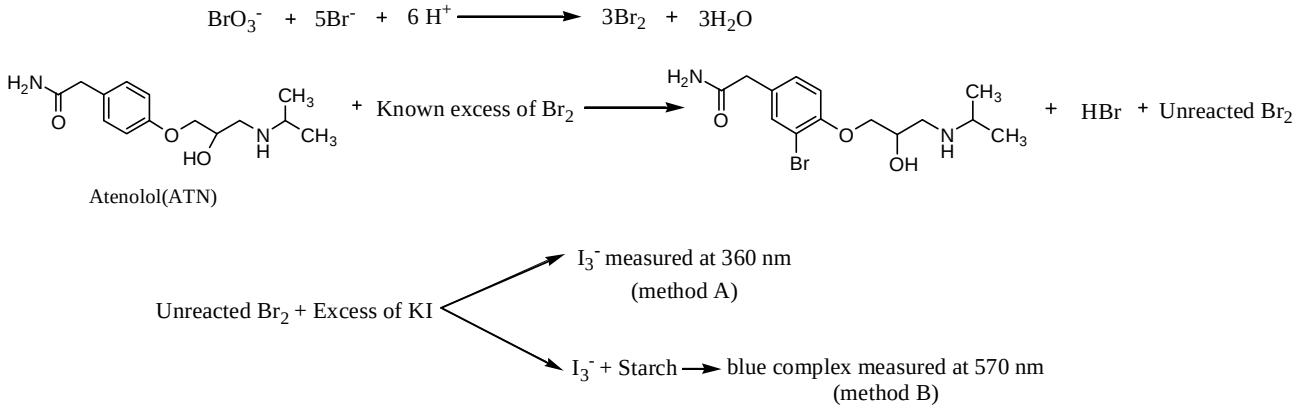

Figure 2. Reaction scheme of the proposed methods.

unchanged. The absorbance of the colored species was measured after different reaction times (5.0-45.0 min) and the results showed that the reaction was complete within 15 min in both methods. The stability of yellow tri-iodide ion in method A was up to 45 min, whereas the absorbance of the blue colored starch-iodine complex chromogen in method B remained stable for at least 1 h.

Effect of sodium acetate

The liberation of iodine did not stop even after 30 min under the specified acidic conditions, but upon adding sodium acetate the reaction ceased immediately. The amount of sodium acetate required was optimized and 1 mL of 3M sodium acetate in a total volume of 10 mL was found as the optimum.

Method validation

The proposed methods were validated in accordance with the current ICH guidelines [67].

Analytical parameters

The standard calibration curves under the optimum experimental conditions were constructed by plotting the absorbance vs. concentration. A linear correlation was found between absorbance at λ_{max} and concentration of ATN in the concentration ranges given in Table 2. The graphs are described by the regression equation:

$$Y = a + bX$$

(where Y = absorbance; a = intercept; b = slope and X = concentration in μg/mL). The regression parameters such as slope (b), intercept (a) and correlation coefficient (r) are presented in Table 2. The molar absorptivity (ε), Sandell's sensitivity, limits of detection (LOD) and quantitation (LOQ) of both methods are also given in Table 2. The high values of ε, low values of Sandell's sensitivity, LOD and LOQ values indicate the high sensitivity of the proposed methods.

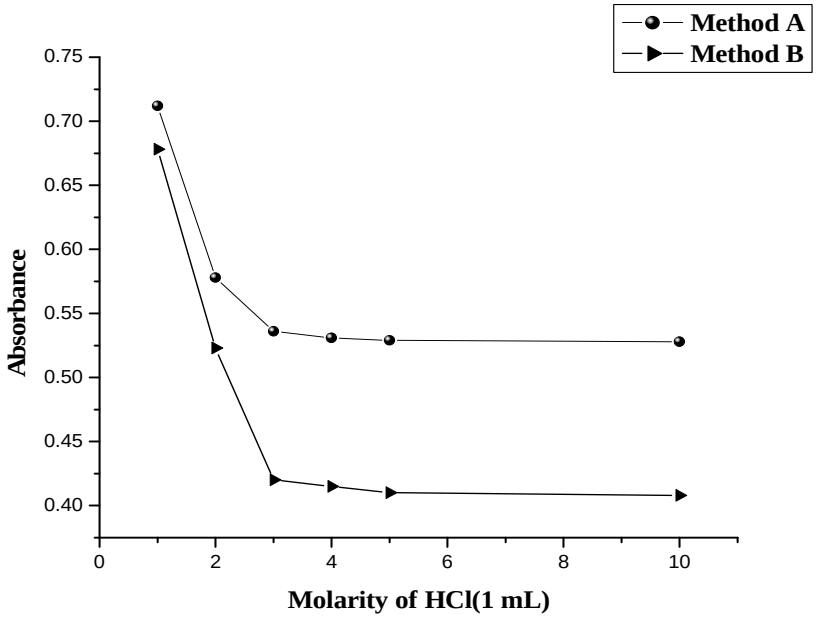

Figure 3. Effect of acid concentration on the formation of colored products.

Table 2. Regression and analytical parameters

Parameter	Method A	Method B
λ_{max} / nm	360	570
Beer's law limits (μg/mL)	0.5-9.0	0.3-6.0
Molar absorptivity (L/mol cm^{-1})	2.36×10^4	2.89×10^4
Sandell sensitivity[a] (μg/cm^2)	0.0113	0.0092
Limit of detection (μg/mL)	0.10	0.08
Limit of quantification (μg/mL)	0.32	0.25
Regression equation, Y[b]		
Intercept (a)	0.9111	0.7485
Slope (b)	-0.0835	-0.1035
Correlation coefficient (r)	-0.9991	-0.9992
Standard deviation of intercept (S_a)	0.08487	0.08826
Standard deviation of slope (S_b)	0.01688	0.02829

[a]Limit of determination as the weight in μg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. [b]$Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in μg ml^{-1}

Accuracy and precision

To compute the accuracy and precision of the proposed methods, the assay procedures described above were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision). These assays were performed for three concentration levels of ATN and the results of this study are summarized in Table 3. The percentage relative standard deviation (*%RSD*) values were ≤2.25 (intra-day) and ≤2.74 (inter-day), indicating high precision of the methods. Accuracy was evaluated as percentage relative error (*%RE*) between the measured mean concentrations and taken concentrations of ATN and the results are also presented in Table 3. The *%RE* values were ≤1.57 and demonstrate the high accuracy of the proposed methods.

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of acid and reaction time, and the effect of the changes on the absorbance of the measured species was studied. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as *%RSD* (≤2.26). Method ruggedness was expressed as the *%RSD* of the same procedure applied by four different analysts as well as using three different cuvettes. The inter-analysts *RSD*

Table 3. Intra-day and inter-day precision and accuracy evaluation

Method	ATN taken µg/mL	Intra-day (n = 7)			Inter-day (n = 5)		
		ATN found[a], µg/mL	*%RSD*[b]	*%RE*[c]	ATN found[a], µg/mL	*%RSD*[b]	*%RE*[c]
Method A	2.0	2.02	2.25	1.06	2.01	2.74	1.28
	4.0	4.05	0.92	1.18	4.03	1.26	1.57
	6.0	6.02	0.50	0.28	6.01	1.04	1.03
Method B	1.5	1.49	1.85	0.58	1.48	2.14	1.37
	3.0	3.01	1.34	0.47	2.98	2.06	1.15
	4.5	4.46	0.29	0.81	4.48	1.42	1.08

[a]Mean value of five determinations; [b]relative standard deviation (%); [c]relative error (%)

were within 1.6 whereas the inter-cuvettes RSD for the same ATN concentrations ranged from 1.92-2.77 suggesting that the developed methods were rugged. The results of this study are shown in Table 4.

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A convenient aliquot of placebo blank was extracted with water and was subjected to analysis following the recommended procedures. In both the methods, there was no interference by the inactive ingredients as shown by the near absorbance of the respective reagent blanks. A separate test was performed by applying the proposed methods to the determination of ATN in a synthetic mixture. The synthetic mixture solution was prepared as described under "Procedure for the tablets" and a suitable aliquot was subjected to analysis by methods A and B, separately. The percentage recovery of ATN was 101.02±1.21 for method A and 102.0±1.67 for method B. This confirms the selectivity of the proposed methods in the presence of commonly employed tablet excipients.

Application to formulations

The proposed methods were applied to the determination of ATN in three representative tablets: Atenex-25, Atekind-50 and Aten-100 purchased from local stores. The results presented in Table 5 showed that the methods are successful to the determination of ATN in pharmaceutical formulations without any detectable interference from the excipients present in the dosage forms. The obtained results (Table 5) were statistically compared with the reference method [3] which describes a UV-spectrophotometric method for the determination of ATN based on the measurements of the absorbance of the methanolic tablet solution at 275 nm. The results obtained by the proposed methods agreed well with those of the reference method. When the results were statistically compared with those of the reference method by applying the Student's *t*-test for accuracy and *F*-test for precision, the calculated Student's *t*-value and *F*-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively. Hence, no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision.

Recovery study

To assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. To a fixed and known amount of ATN in tablet powder (pre-analyzed), pure ATN was added at three concentration levels (50, 100 and 125% of the level present in the tablet) and the total was measured by the proposed methods. The determination with each concentration was repeated three times. In all the cases, the recovery percentage

*Table 4. Robustness and ruggedness (*RSD */ % (n = 3))*

Method	ATN taken µg/mL	Method robustness		Method ruggedness	
		Parameters altered			
		Volume of acid[a]	Reaction time[b]	Inter-analysts	Inter-cuvettes
A	2.0	0.78	1.68	0.85	2.06
	4.0	1.18	2.03	1.24	2.12
	6.0	0.84	1.85	1.26	2.28
B	1.5	1.26	2.26	1.28	2.36
	3.0	0.94	1.78	1.63	2.77
	4.5	1.52	1.34	1.09	1.92

[a]In methods A and B, the volume of 3 M HCl was 0.8, 1.0 and 1.2 mL; [b]The reaction time was 13, 15 and 17 min in methods A and B

Table 5. Results of analysis of tablets by the reference and proposed methods

Tablet brand name	Label claim, mg/tablet	Found (percent of label claim±*SD*)[a]		
		Reference method	Proposed methods	
			Method A	Method B
Atenex-25	25	100.3±0.58	101.1±0.94	100.6±0.63
			t = 1.62	*t* = 0.78
			F = 2.63	*F* = 1.18
Atekind-50	50	99.67±0.67	100.8±1.07	100.3±0.89
			t = 2.00	*t* = 1.12
			F = 2.55	*F* = 1.76
Aten-100	100	100.6±0.82	101.2±1.15	101.4 ±1.33
			t = 0.95	*t* = 0.64
			F = 1.97	*F* = 2.63

[a]Mean value of five determinations; tabulated *t*-value at the 95% confidence level is 2.78; tabulated *F*-value at the 95% confidence level is 6.39

Table 6. Results of recovery study by standard addition method

Tablets studied	Method A				Method B			
	ATN in tablets µg/mL	Pure ATN added µg/mL	Total found µg/mL	Pure ATN recovered[a] %±*SD*	ATN in tablets µg/mL	Pure ATN added µg/mL	Total found µg/mL	Pure ATN recovered %±*SD*
Atenex 25	4.04	2.0	6.13	104.5±1.36	2.01	1.00	3.02	101.0±1.90
	4.04	4.0	8.18	103.5±0.56	2.01	2.00	4.05	102.0±0.84
	4.04	5.0	9.21	103.4±0.53	2.01	3.00	5.08	102.3±0.49
Atekind 50	4.03	2.0	6.06	101.5±2.37	2.0	1.00	2.99	99.00±3.29
	4.03	4.0	8.08	101.3±0.98	2.0	2.00	3.98	99.00±0.85
	4.03	5.0	9.07	100.8±0.31	2.0	3.00	5.02	100.7±0.50
Aten 100	4.05	2.0	6.12	103.5±2.36	2.03	1.00	3.06	103.0±3.28
	4.05	4.0	8.16	102.8±1.49	2.03	2.00	4.07	102.0±0.84
	4.05	5.0	9.14	101.8±0.54	2.03	3.00	5.06	101.0±0.86

[a]Mean value of three determinations

values ranged between 99.0 and 104.5 with relative standard deviation in the range 0.31-3.37 %. The results of this study presented in Table 6 indicated that the various excipients present in the formulations did not interfere in the assay.

CONCLUSIONS

Two useful methods for the micro determination of ATN in bulk drug as well as in pharmaceutical formulations have been developed and validated as per the current ICH guidelines [67]. The methods use bromate-bromide mixture as a green brominating reagent instead of hazardous liquid bromine. The proposed methods are superior to most of the visible spectrophotometric methods reported so far [51--53,59,60,62] in terms of sensitivity as can be seen from the molar absorptivity values (Table 1). Some of the reported methods [58], even though more sensitive, suffer from the disadvantages of a heating step. The proposed methods have the advantages of selectivity and easily adaptable to routine analysis. Moreover, they are free from complicated analytical procedures such as heating or extraction steps and are cost effective when compared to several non-spectrophotometric methods [5-41].

REFERENCES

[1] The Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals, 11th ed., Merck & Co., Inc., Whitehouse Station, NJ, 1989, p. 136

[2] A.N. Wadworth, D. Murdoch, R.N. Brogden, Drugs **42** (1991) 468-510

[3] The Indian Pharmacopeia, 1996, 4th ed., Ministry of Health and Family Welfare, Government of India, New Delhi, p. 72

[4] The British Pharmacopoeia, 2003, London, Her Majesty's Stationery office

[5] M.A. Gotardo, R. Sequinel, L. Pezza, H.R. Pezza, Eclet. Quim. **33** (2008) 7-12

[6] P. Modamio, C.F. Lastra, E.L. Marino, J. Pharm. Biomed. Anal. **17** (1998) 507-513

[7] S.S. Zarapkar, S.S. Kolte, S.H. Rane, Indian Drugs **34** (1997) 350-353

[8] S.V. Erram, H.P. Tipnis, Indian Drugs **29** (1992) 436-438

[9] R. Jain, C.L. Jain, Microchem. J. **44** (1991) 187

[10] D. Radulovic, L.J. Zivanovic, G. Velimirovic, D. Stevanovic, Anal. Lett. **24** (1991) 1813-1823

[11] R.T. Sane, V.R. Bhate, V.G. Nayak, K.D. Ladge, Indian Drugs **28** (1991) 322-325

[12] Z. Pawlak, B.J. Clark, J. Pharm. Biomed. Anal. **10** (1992) 329-334

[13] L. Gong, Yaowu Fenxi Zazhi **9** (1989) 175-176

[14] S.I. Sasa, I.M. Jalal, H.S. Khalil, J. Liq. Chromatogr. Related Technol. **11** (1988) 1673-1696

[15] R. Ficarra, P. Ficarra, A. Tommasini, M.L. Calabro, C. Guarniera Fenech, Farmaco Ed. Prat. **40** (1985) 307-312

[16] I. Rapado-Martinez, M.C. Garcia-Alvarez-Coque, R.M. Villanueva-Camanas, J. Chromatogr., A **765** (1997) 221--223

[17] M.I.R.M. Santoro, H.S. Cho, E.R.M. Kedor-Hackmann, Drug Dev. Ind. Pharm. **26** (2000) 1107-1110

[18] R. Ceresole, M.A. Moyano, M.T. Pizzorno, A.I. Segall, J. Liq. Chromatogr. Related Technol. **29** (2006) 3009-3019

[19] L. Bing, D.F. Hu, F. Liu, Yaowu Fenxi Zazhi **24** (2004) 485-486

[20] A.K. Singh, E.R.M. Kedor-Hackmann, M.I.R.M. Santoro, J. AOAC Int. **84** (2001) 1724-1729

[21] R. Bhushan, S. Tanwar, Biomed. Chromatogr. **23** (2009) 1291-1299

[22] R.K. Barman, M.A.U. Islam, M. Ahmed, M.I.I. Wahed, R. Islam, A. Khan, M.B. Hossain, B.M. Rahman, Pak. J. Pharm. Sci. **20** (2007) 274-279

[23] A. Holbrook, A.M. Krstulovic, J.H. Miller, J. Rysaluk, Pharmeuropa **3** (1991) 218-225

[24] R. Mehvar, J. Chromatogr., B **85** (1989) 402-408

[25] P. Parrilla Vazquez, M. Martinez Galera, A. Serrano Guirado, M.M. Parrilla Vazquez, Anal. Chim. Acta **666** (2010) 38-44

[26] S. El Deeb, S. Deeb, Chromatographia **71** (2010) 783-788

[27] A.P. Argekar, S.G. Powar, J. Pharm. Biomed. Anal. **21** (2000) 1137-1142

[28] A.P. Argekar, J.G. Sawant, Liq. Chromatogr. Related Technol. **22** (1999) 1571-1578

[29] D.D. Rao, N.V. Satyanarayana, S.S. Sait, Y.R. Reddy, Mukkanti, Chromatographia **70** (2009) 647-651

[30] G.S. Sadana, A.B. Ghogare, Indian Drugs **28** (1990) 142--145

[31] G.R. Rao, A.B. Avadhanulu, R. Giridhar, A.R.R. Pantulu, C.K. Kokate, East. Pharm. **33** (1990) 113-115

[32] R. Ghanem, M.A. Bello, M. Callejon, A. Guiraum, J. Pharm. Biomed. Anal. **15** (1996) 383-388

[33] H.C. Zhao, N.H. Yan, J.X. Guo, Fenxi Shiyanshi **13** (1994) 36-38

[34] M. Gajewska, G. Glass, J. Kostelecki, Acta Pol. Pharm. **49** (1992) 1-4

[35] G. Pyramides, J.W. Robinson, S.W. Zito, J. Pharm. Biomed. Anal. **13** (1995) 103-110

[36] K.A. Azzam, A.A. Elbashir, M.A. Elbashir, B. Saad, S.A. Hamid, Anal. Lett. **42** (2009) 1458-1470

[37] A. Shafaati, B.J. Clark, J. Pharm. Biomed. Anal. **14** (1996) 1547-1554

[38] X.W. Zhou, J.Q. Lv, Z.R. Zeng, Fenxi Kexue Xuebao **23** (2007) 30-32

[39] R.N. Goyal, S.P. Singh, Talanta **69** (2006) 932-937

[40] M. Shamsipur, F. Jalali, S. Haghgoo, Anal. Lett. **38** (2005) 401-410

[41] M.A. El Ries, Anal. Lett. **28** (1995) 1629-1639

[42] C.V.N. Prasad, C. Parihar, K. Sunil, P. Parimoo, J. Pharm. Biomed. Anal. **17** (1998) 877-884

[43] C.V.N. Prasad, C. Parihar, T.R. Chowdhary, S. Purohit, Parimoo, Pharm. Pharmacol. Comm. **4** (1998) 325-330

[44] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, Farmaco. **51** (1996) 733-738

[45] S. Singh, R. Jain, Indian Drugs **34** (1997) 678-679

[46] S. Wang, Yaowu Fenxi Zazhi **10** (1990) 110-111

[47] J. Huang, J. Jin, Zhongguo Yiyao Gongye Zazhi **20** (1989) 19-20

[48] D. Satyanarayana, K. Kannan, R. Manavalan, Chem. Anal. (Warsaw) **51** (2006) 771-784

[49] W. Wehner, Die Pharmazie **55** (2000) 543-544

[50] M.C.F. Ferraro, P.M. Castellano, T.S. Kaufman, Anal. Bioanal. Chem. **377** (2003) 1159-1164

[51] Y.K. Agrawal, K. Raman, S. Rajpu, S.K. Menon, Anal. Lett. **25** (1992) 1503-1510

[52] S.P. Agarwal, V. Singhal, A. Prakash, Indian J. Pharm. Sci. **60** (1998) 53-55

[53] L.L. Yu, J.C. Liu, H.K. Li, Yaowu Fenxi Zazhi **30** (2010) 538-540

[54] M.A. Korang, M.H. Abdel-Hay, S.M. Galal, M.A. Elsayed, J. Pharm. Belg. **40** (1984) 178-184

[55] S.M. Sultan, Acta Pharm. Hung. **62** (1992) 311-317

[56] S.M. Al-Ghannam, F. Belal, J. AOAC Int. **85** (2002) 817--823

[57] G.C. Hiremath, R.M. Mulla, S.T. Nandibewoor, Chem. Anal. (Warsaw) **50** (2005), 449-455

[58] N. Bashir, S.W.H. Shah, M. Bangesh, Riazullah, J. Sci. Ind. Res. **70** (2011) 51-54

[59] K. Basavaiah, U. Chandrashekar, P. Nagegowda, Acta Cienc. Indica Chem. **XXXC** (2004) 075-079

[60] K. Basavaiah, U. Chandrashekar, P. Nagegowda Indian J. Chem. Technol. **11** (2004), 769-776

[61] K. Basavaiah, U. Chandrashekar, P. Nagegowda, J. Serb. Chem. Soc. **71** (2006) 553-556

[62] K. Basavaiah, U. Chandrashekar, B.C. Somashekar, Proc. Nat. Acad. Sci. India **75** (2005) 233-238

[63] K. Basavaiah, Indian J. Chem. Technol. **13** (2006) 360-366

[64] A.M. El-Didamony, Drug Test. Analysis **2** (2010) 122-129

[65] A.M. El-Didamony, E.A.H. Erfan, Spectrochim. Acta, A **75** (2010) 1138-1145

[66] K. Basavaiah, K. Tharp, J. Mex. Chem. Soc. **52** (2008) 193-200

[67] International Conference on Harmonization, 1996, Validation of analytical procedures: test and methodology. ICH, London.

KUDIGE NAGARAJ PRASHANTH
KANAKAPURA BASAVAIAH
SAMEER A. M. ABDULRAHMAN
NAGARAJU RAJENDRAPRASAD
KANAKAPURA BASAVAIAH
VINAY

Department of Chemistry, University of Mysore, Manasagangotri, Mysore, Karnataka, India

NAUČNI RAD

PRIMENA BROMATE-BROMIDNE SMEŠE KAO ZELENOG BROMIRAJUĆEG AGENSA ZA SPEKTROFOTOMETRIJSKO ODREĐIVANJE ATENOLOLA U FARMACEUTSKIM PREPARATIMA

Razvijene su dve osetljive spektrofotometrijske metode za kvantitativnu analizu atenalola (ATN) u čistoj supstanci i u farmaceutskoj formulaciji. Metode se zasnivaju na reakciji bromiranja ATN sa poznatom količinom bromat-bromidne smeše u kiseloj sredini i određivanju viška neizreagovanog broma. Rezidualni brom je određen spektrofometrijski reakcijom sa viškom jodida i merenjem koncentracije oslobođenog I_3^- jona na 360 nm (metoda A) ili reakcijom sa skrobom i merenjem intenziteta boje jod-skroba na 570 nm (metoda B). Pod optimalnim uslovima ATN se može određivati po metodi A u koncentracijskom opsegu 0,5-9,0 μg ml^{-1} i po metodi B u koncentracijskom opsegu 0,3-6,0 μg ml^{-1}. Predložene metode su uspešno primenjene za analizu komercijanih farmaceutski formulacija i dobijeni rezultati su u dobroj saglasnosti sa referentnom metodom. Pouzdanost metode je utvrđena određivanjem recovery vrednosti metodom standardnog dodatka.

Ključne reči: atenolol, određivanje, bromat-bromidna smeša, spektrofotometrija, farmacija.