Research Article

Sensitive Spectrophotometric Determination of Atenolol in Pharmaceutical Formulations Using Bromate-Bromide Mixture as an Eco-Friendly Brominating Agent

Kudige N. Prashanth and Kanakapura Basavaiah

Department of Chemistry, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India

Correspondence should be addressed to Kanakapura Basavaiah, basavaiahk@yahoo.co.in

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Three simple and sensitive spectrophotometric methods are proposed for the determination of atenolol (ATN) in bulk drug and tablets. The methods are based on the bromination of ATN by the bromine generated in situ by the action of the acid on the bromate–bromide mixture followed by the determination of unreacted bromine by reacting with a fixed amount of either meta-cresol purple (MCP) and measuring the absorbance at 540 nm (method A) and 445 nm (method B) or erioglaucine (EGC) and measuring the absorbance at 630 nm (method C). Beer’s law is valid within the concentration ranges of 1.0–20.0, 2.0–40.0 and 1.0–8.0 μg/mL for method A, method B and method C, respectively. The calculated molar absorptivities were found to be 1.20 × 10^4, 4.51 × 10^3 and 3.46 × 10^4 L/mol · cm for method A, method B and method C, respectively. Sandell’s sensitivity values, correlation coefficients, limits of detection and quantification are also reported. Recovery results were statistically compared with those of a reference method by applying Student’s t- and F-test. The novelty of the present study is the measurement of two different colors using MCP, that is, red-pink color of MCP in acid medium at 540 nm and yellowish-orange color of brominated MCP at 445 nm.

1. Introduction

Atenolol (ATN), chemically known as 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy) benzeneacetamide [1], is a β1-selective (cardio selective) adrenoreceptor antagonist drug used for antiangina treatment to relieve symptoms, improve tolerance, and as an antiarrhythmic 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], nonsuppressed ion chromatography [32], fluorometry [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 a method based on the reaction of ATN with hydroxylamine hydrochloride in NaOH medium followed by the reaction of the resultant hydroxamic acid derivative with FeCl₃ to give a red-violet ferric hydroxamate complex. Assays based on charge transfer complexation reaction of ATN with chloranilic acid have been reported by Agarwal et al. [52] and Yu et al. [53]. Korany et al. [54] have developed a method based on the treatment of a CHCl₃ 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. The slow reaction between ATN and ammonium vanadate in sulphuric acid medium resulted in two kinetic spectrophotometric methods (fixed-concentration method and fixed-time method) [55]. Al-Ghanam and Belal [56] used the reaction between ATN and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in borate buffer of pH 8 at the boiling temperature for the kinetic spectrophotometric assay of drug. The method developed by Hiremath et al. [57] is based on the oxidation of atenolol by a known excess of permanganate in alkaline media and determination of unreacted permanganate spectrophotometrically at 526 nm. Bashir et al. [58] have reported a method based on determination of ATN in basic medium, followed by addition of sodium nitroprusside to generate a coloured complex. 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 assay method based on 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 have been reported by Basavaiah et al. [60]. A similar method [61] employed bromate-bromide mixture, methyl orange as reagents in acid medium. An acid-base reaction employing phenol red has also been reported by the same authors [62]. However, many of the above methods suffered from one or other disadvantage like poor sensitivity, heating or extraction step, use of organic solvents, use of expensive chemical, and/or complicated experimental setup as can be seen from Table 1.

The aim of this study was to develop three new spectrophotometric methods for the assay of ATN based on bromination of ATN by a green brominating agent (i.e., bromine-generated in situ). The methods use bromated-bromide mixture, metacresol purple (MCP), and erioglaucine (EGC) as reagents. The proposed methods are economical compared to the previously reported chromatographic techniques. Moreover, these methods are sensitive, simple, does not involve heating or extraction step, and free from usage of hazardous chemicals. Since inexpensive and easily available chemicals are used, the developed methods evidence low cost per analysis.

2. Experimental

2.1. Apparatus. All absorbance measurements were made on a Systronics model 106 digital spectrophotometer (Systronics, Ahmadabad, India) provided with 1 cm matched quartz cells.

2.2. Materials and Reagents. All chemicals and reagents used were of analytical or pharmaceutical grade. Distilled water was used to prepare the solutions.

(1) Bromate-Bromide Mixture (40, 80, and 18 μg/mL). A stock standard bromate-bromide mixture solution equivalent to 500 μg/mL KBrO₃ was prepared by dissolving accurately weighed 50 mg of KBrO₃ (S. D. Fine Chem. Ltd., Mumbai, India) and 0.5 g of KBr (Merck, Mumbai, India) in water and diluted to the mark in a 100 mL calibrated flask. The stock solution was diluted appropriately with water to get the working concentrations of 40, 80, and 18 μg/mL KBrO₃ for use in method A, method B, and method C, respectively.

(2) MetaCresol Purple Solution (80 and 200 μg/mL). A 400 μg/mL stock solution was first prepared by dissolving 40 mg of dye (Loba Chemie, Mumbai, India) in 2 mL of 0.1 N NaOH and diluted to volume with water in a 100 mL calibrated flask. The solution (400 μg/mL) was diluted further with water to get the working concentrations of 80 μg/mL and 200 μg/mL MCP solutions.

(3) Erioglaucine Solution (300 μg/mL). The solution was prepared by dissolving 30 mg of dye (Loba Chemie, Mumbai, India) in water and diluting to the mark with water in a 100 mL calibrated flask.

(4) Hydrochloric Acid (5 M and 1 M). The solutions were prepared by appropriate dilution of concentrated hydrochloric acid (S. D. Fine Chem. Ltd., Mumbai, India. Sp. gr. 1.18) with water.

(5) Standard ATN Solution. Pharmaceutical grade atenolol (ATN) certified to be 99.89% pure was gifted by Cipla India Ltd., Mumbai, India, and was used as received without any further purification and analysis. A stock standard solution equivalent to 200 μg/mL ATN was prepared by dissolving accurately weighed 50 mg of pure drug with water in a 250 mL calibrated flask. This stock solution was diluted appropriately with water to get the working concentrations of 40 μg/mL for use in methods A and C, and 80 μg/mL for use in method B.

2.3. Assay Procedure

2.3.1. Method A (Measuring MCP in Acid Medium). Different aliquots (0.25–5.0 mL) of standard ATN solution (40 μg/mL) were accurately transferred into a series of 10 mL calibrated flasks using microburette and the total volume was adjusted to 5.0 mL by adding requisite volume of water. To each flask, 2 mL of 5 M HCl was added followed by 1 mL of bromate-bromide mixture (40 μg/mL in KBrO₃). The content was mixed well and the flasks were allowed to stand for 15 min with occasional shaking. Then, 1 mL of 80 μg/mL MCP was added to each flask, diluted to the mark with water, mixed well, and the absorbance of each solution was measured at 540 nm against a reagent blank after 5 min.

2.3.2. Method B (Measuring Brominated Product of MCP). Varying aliquots (0.25–5.0 mL) of a standard solution (80 μg/mL ATN) were accurately measured into a series of 10 mL calibrated flasks and the total volume was brought to
Table 1: Comparison of the proposed and the existing visible spectrophotometric methods.

| Sl. No. | Reagent/s used                                      | Reagent used                              | \( \lambda_{\text{max}} \) (nm) | Linear range, \( \mu g/\text{mL} \) and \( \epsilon, \text{L/mol} \cdot \text{cm} \) | LOD, \( \mu g/\text{mL} \) | Reaction time, min | Remarks                        | Reference |
|--------|----------------------------------------------------|-------------------------------------------|---------------------------------|--------------------------------------------------|----------------|----------------|--------------------------------|-----------|
| (1)    | Hydroxylamine hydrochloride-iron (III)             | Ferric hydroxamate complex measured       | 510                             | 50–800 \( (\epsilon = 5.3 \times 10^2) \)         | NR            | 20–30          | Less sensitive, heating required | [51]      |
| (2)    | Chloranilic acid                                   | Charge transfer complex measured          | 534                             | 25–250                                           | NR            | —              | Less sensitive, use of organic solvents | [52]      |
| (3)    | Chloranilic acid                                   | Charge transfer complex measured          | 530                             | 10–280                                           | NA            | NA             | -do-                           | [53]      |
| (4)    | Acetaldehyde-Chloranil                              |                                           | 690                             | NA                                               | NA            | NA             | Use of organic solvents          | [54]      |
| (5)    | \( \text{NH}_4\text{VO}_3 \)                      | Reaction rate measured                    | 750                             | NA                                               | NA            | NA             | 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             | Heating required               | [56]      |
| (7)    | Potassium permanganate- in alkaline medium         | Unreacted \( \text{KMnO}_4 \) measured  | 526                             | 4 hrs                                            | 6.66–10.65    | 6.66–5.33      | 6.66–7.99                      | [57]      |
| (8)    | Sodium nitroprusside                               | Complex of ammonia and nitroprusside measured | 495                             | 0.5–30 \( (\epsilon = 3.01 \times 10^3) \)      | 0.01          | 5              | Heating required               | [58]      |
| (9)    | Chloramine-T-tolval-sulphanilic acid               | Unreacted chloramine-T measured          | 520                             | 2.5–25 \( (\epsilon = 3.24 \times 10^3) \)      | 2.34          | 20             | Less sensitive                 | [59]      |
| (10)   | Chloramine-T:                                      |                                           |                                 |                                                 |               |                |                                |           |
| (a)    | Metanil yellow                                     | Unreacted chloramine-T measured          | 530                             | 1–12 \( (\epsilon = 1.19 \times 10^4) \)        | 0.32          | 10             |                                | [60]      |
| (b)    | Indigo carmine                                     |                                           | 610                             | 2.5–20 \( (\epsilon = 6.65 \times 10^3) \)      | 0.04          | 10             |                                |           |
| (11)   | Bromate-bromide mixture- methyl orange            | Unreacted bromine measured               | 520                             | 0.5–4.0 \( (\epsilon = 4.13 \times 10^4) \)      | 0.07          | 15             |                                | [61]      |
| (12)   | Phenol red                                         | The change in the color of phenol red measured | 430                             | 3.0–30 \( (\epsilon = 3.47 \times 10^3) \)      | 4.61          | —              | Less sensitive                 | [62]      |
| (13)   | Bromate-bromide mixture:                           |                                           |                                 |                                                 |               |                |                                |           |
| (a)    | MCP                                                | Unreacted MCP in acid measured           | 540                             | 1.0–20.0 \( (\epsilon = 1.20 \times 10^4) \)    | 0.12          | 15             | Simple, sensitive and no heating step. No use of organic solvent. Use of an eco-friendly brominating reagent. |           |
| (b)    | MCP                                                | Bromo-derivative of MCP measured         | 445                             | 2.0–40.0 \( (\epsilon = 4.51 \times 10^3) \)    | 0.56          | 10             |                                |           |
| (c)    | EGC                                                | Unreacted EGC in acid measured           | 630                             | 1.0–8.0 \( (\epsilon = 3.46 \times 10^4) \)      | 0.05          | 10             |                                |           |

MCP: metacresol purple, EGC: erioglaucine, NR: not reported, NA: not available.
5 mL by adding water. To each flask were added 2 mL of 5 M HCl and 1 mL of KBrO₃-KBr solution (80 μg/mL, in KBrO₃). The content of each flask was mixed well and kept aside for 10 min with occasional swirling. At last, 1 mL of 200 μg/mL MCP solution was added to each flask and diluted up to the mark with water. The absorbance of each solution was measured after 5 min at 445 nm against water.

2.3.3. Method C (Using EGC). Aliquots (0.25–2.0 mL) of a standard ATN (40 μg/mL) solution were accurately transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 2.0 mL with water. To each flask, 5 mL of 1 M HCl was added followed by 1.0 mL of bromate-bromide mixture (18 μg/mL, in KBrO₃). The content was mixed and the flasks were let stand for 10 min with occasional shaking followed by addition of 1 mL of 300 μg/mL EGC to each flask. The solutions were diluted to the mark with water, mixed well, and the absorbance of each solution was measured at 630 nm after 5 min against a reagent blank.

2.3.4. Analysis of Commercial Tablets. Twenty tablets each containing 25, 50, or 100 mg of ATN were weighed accurately and pulverized. An amount of powdered tablet equivalent to 20 mg of ATN was transferred into a 100 mL calibrated flask and 60 mL of water was added. The content was shaken thoroughly for about 15–20 min, diluted to the mark with water, 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 of the filtrate (200 μg/mL ATN) was diluted to get the working concentrations of 40 μg/mL ATN for the assay by methods A and C, and 80 μg/mL ATN for method B.

2.3.5. Analysis of Placebo Blank. A placebo blank of the composition: talc (45 mg), starch (35 mg), acacia (25 mg), methyl cellulose (40 mg), sodium citrate (25 mg), magnesium stearate (35 mg), and sodium alginate (30 mg) was made and its solution was prepared in 25 mL calibration flask as described under Section 2.3.4, and then subjected to analysis using the procedures described above.

2.3.6. Analysis of Synthetic Mixture. To the placebo blank of the composition described above, 20 mg of ATN was added and homogenized, transferred to a 100 mL calibrated flask, and the solution was prepared as described under Section 2.3.4, and then subjected to analysis by the procedures described above. The analysis was used to study the interferences of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate, and sodium alginate.

3. Results and Discussion

3.1. Absorption Spectra. The proposed methods are based on the determination of residual bromine generated in situ after the reaction between the drug and bromine is judged to be complete. The red-pink color of unreacted MCP in acid medium was absorbed maximally at 540 nm (method A). The residual bromine was then used to brominate MCP yielding yellow-colored bromo-derivative product with λ_max at 445 nm (method B). Similar to method A, the green color of unreacted EGC in acid medium peaked at 630 nm (method C). The absorption spectra of all methods are presented in Figure 1.
3.2. Chemistry. Atenolol is reported to undergo bromination by bromine generated in situ by the action of the acid on the bromate-bromide mixture [61]. The solution of bromate-bromide mixture in acid medium behaves as an equivalent solution of bromine and has been used for the assay of several pharmaceutical compounds [63–66]. The present investigation deals with three spectrophotometric methods for the assay of ATN using bromine generated in situ as eco-friendly brominating agent and avoiding the use of highly toxic and hazardous liquid bromine. The proposed methods are indirect and based on the bromination of ATN by the bromine followed by the determination of unreacted bromine by reacting with a fixed amount of either MCP or EGC and measuring the absorbance at the respective wavelengths. The reaction between ATN and bromine generated in situ uses electrophilic substitution reaction at one orthoposition to the alkoxy group on the benzene ring. The unreacted bromine was determined by its reaction with either MCP or EGC. The reaction of bromine with MCP involved two simultaneous processes, that is, decrease in the pink color of MCP in acid medium at 540 nm (method A) and increase in the yellowish-orange color at 445 nm (method B) due to the bromination of the dye. Similar to method A, unreacted bromine would react with EGC and the decrease in the absorbance of the green color of EGC in acid medium was measured at 630 nm (method C). The tentative reaction scheme is given and illustrated in Figure 2.

3.3. Basis of the Methods. ATN, when added in increasing concentrations to a fixed concentration of in situ bromine, consumed the latter and there occurred a concomitant fall in bromine concentration. When a fixed concentration of MCP was added to decreasing concentrations of bromine, a concomitant increase in the absorbance of MCP resulted at 540 nm and at the same time decrease in the absorbance resulted at 445 nm. Similarly, when a fixed concentration of EGC was added to decreasing concentrations of bromine, a corresponding increase in the absorbance of EGC was observed at 630 nm. These were observed as a proportional increase in the absorbance at 540 nm (method A) or 630 nm (method C) and decrease at 445 nm (method B) with increasing the concentration of ATN.

3.4. Optimization of Reaction Variables
3.4.1. Effect of Reagent Concentration. Preliminary experiments were performed to fix the upper limits of the MCP and EGC that could produce a reasonably high absorbance, and these were found to be 80, 200 μg/mL for MCP in methods A and B, and 300 μg/mL for EGC in method C. Bromate concentrations of 4.0 and 1.8 μg/mL in the presence of excess bromide were found optimum to bleach the dye color in method A and method C, respectively, whereas 8.0 μg/mL KBrO₃ produced a reasonable maximum absorbance at
445 nm in method B. Hence, different concentrations of ATN were reacted with 1.0 mL each of 40, 80, and 18 μg/mL bromate in methods A, B, and C, respectively.

3.4.2. Effect of Reaction Medium. Hydrochloric acid was found to be an ideal medium for the two steps involved in all the three methods (Figure 3). In method A, the effect of (1.0–3.0 mL of 5 M HCl) was studied and the results showed that 2.0 mL of 5 M HCl was optimum for the bromination reaction of the drug as well as the dye. Taking into account the maximum absorbance of the measured species and the minimum absorbance of the blank, 2.0 mL of 5 M HCl was fixed. In method B, 2.0 mL of 5 M HCl was found optimum and any excess of the acid up to 3.0 mL would not affect the absorbance of the measured species. In method C, 5.0 mL of 1 M HCl was found optimum to achieve maximum absorbance for the sample and minimum absorbance for the blank.

3.4.3. Reaction Time and Color Stability. The reaction time between ATN and the bromine generated in situ was found to be 15 min in method A and 10 min in both method B and method C. After completion the reaction between the drug and the bromine, the residual bromine would brominate the dyes and this bromination process was found to be complete in 5 min for all three methods. The absorbance of the measured species was constant up to 24 hours.

3.5. Validation of the Proposed Methods

3.5.1. Linearity. A linear relation is found between absorbance and concentration of ATN within Beer’s law range given in Table 2. The calibration graphs are described by the equation:

\[
Y = a + bX, \tag{1}
\]
Table 3: Evaluation of intraday and interday precision and accuracy.

| Method  | ATN taken (µg/mL) | Intraday (n = 7) | Interday (n = 5) |
|---------|-------------------|------------------|------------------|
|         | ATN found (µg/mL) | ATN found (µg/mL) | %RSD b | %RE c | ATN found (µg/mL) | %RSD b | %RE c |
| Method A |                   |                   |                   |       |       |                   |       |       |
| 4.00    | 4.14              | 1.49             | 1.71             | 4.09  | 1.86  | 2.25             |       |       |
| 8.00    | 8.12              | 0.75             | 1.56             | 8.16  | 1.34  | 2.00             |       |       |
| 12.00   | 4.0               | 0.67             | 1.04             | 12.31 | 1.28  | 2.58             |       |       |
| Method B |                   |                   |                   |       |       |                   |       |       |
| 8.00    | 8.22              | 1.74             | 2.69             | 8.19  | 2.14  | 2.38             |       |       |
| 16.00   | 16.25             | 1.06             | 1.58             | 16.44 | 2.08  | 2.75             |       |       |
| 24.00   | 24.63             | 0.56             | 2.62             | 24.85 | 1.72  | 3.54             |       |       |
| 2.00    | 1.99              | 1.64             | 0.46             | 2.05  | 2.14  | 2.50             |       |       |
| Method C |                   |                   |                   |       |       |                   |       |       |
| 4.00    | 4.09              | 2.09             | 2.44             | 4.14  | 2.56  | 3.50             |       |       |
| 6.00    | 6.07              | 1.47             | 1.09             | 6.16  | 2.63  | 2.67             |       |       |

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

Table 4: Robustness and ruggedness.

| Method | ATN taken, µg/mL | Parameters altered | Method robustness | Method ruggedness |
|--------|------------------|-------------------|------------------|------------------|
|        |                  | Volume of acid, ml a | RSD, % (n = 3) | RSD, % (n = 3) | RSD, % (n = 3) | RSD, % (n = 3) |
| A      | 4.00             | 1.26               | 1.46             | 1.34             | 2.64             |                   |
|        | 8.00             | 0.72               | 1.72             | 0.85             | 3.18             |                   |
|        | 12.00            | 0.64               | 1.28             | 1.03             | 3.03             |                   |
|        | 8.00             | 0.85               | 1.39             | 1.42             | 2.86             |                   |
| B      | 16.00            | 0.52               | 0.92             | 1.17             | 2.47             |                   |
|        | 24.00            | 1.18               | 1.15             | 1.33             | 3.26             |                   |
|        | 2.00             | 1.26               | 1.26             | 1.06             | 3.42             |                   |
|        | 6.00             | 0.96               | 1.39             | 0.88             | 2.78             |                   |
| C      | 4.00             | 1.08               | 0.76             | 1.24             | 2.37             |                   |
|        | 6.00             |                   |                  |                  |                  |                   |

a In methods A and B, the volume of 5 M HCl was 1.8, 2.0, and 2.2 mL whereas in method C, the volume of 1 M HCl was 4.8, 5.0, and 5.2 mL. b The reaction time in methods A was 14, 15, and 16 min whereas in methods B and C, the same was 9, 10, and 11 min.

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                              |
|                   |                       | Method C                              |
| Atenex-25 b       | 25                    | 100.3 ± 0.58                          |
|                   |                       | t = 1.11                              |
|                   |                       | F = 3.34                              |
|                   |                       | 101.0 ± 1.09                          |
|                   |                       | 99.65 ± 0.96                          |
|                   |                       | 101.0 ± 1.12                          |
| Atekind-50 c      | 50                    | 99.67 ± 0.67                          |
|                   |                       | t = 2.32                              |
|                   |                       | F = 4.18                              |
|                   |                       | 100.6 ± 1.11                          |
|                   |                       | 101.1 ± 1.37                          |
|                   |                       | 99.72 ± 1.69                          |
| Aten-100 d        | 100                   | 100.6 ± 0.82                          |
|                   |                       | t = 0.03                              |
|                   |                       | F = 1.83                              |
|                   |                       | 99.65 ± 0.96                          |
|                   |                       | 101.0 ± 1.12                          |

a Mean value of five determinations. b,d Marketed by Zydas Healthcare, East Sikkim, India, c Marketed by Mankind Pharma Ltd., New Delhi, India, 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 | Method C |
|-----------------|----------|----------|----------|
| Pure ATN added, μg/mL | ATN in tablets, μg/mL | Total found, μg/mL | Pure ATN recovered* percent ± SD | ATN in tablets, μg/mL | Total found, μg/mL | Pure ATN recovered* percent ± SD | ATN in tablets, μg/mL | Total found, μg/mL | Pure ATN recovered* percent ± SD |
| Pure ATN added, μg/mL | | | | | | | | | |
| Pure ATN recovered* | | | | | | | | | |
| Atenex 25 | 4.08 | 2.0 | 6.07 | 99.5 ± 2.29 | 7.97 | 4.0 | 12.01 | 101.00 ± 2.74 | 2.02 | 1.0 | 3.05 | 103.00 ± 1.76 |
| | 4.08 | 4.0 | 8.03 | 98.75 ± 2.79 | 7.97 | 8.0 | 15.93 | 99.50 ± 2.35 | 2.02 | 2.0 | 4.09 | 103.5 ± 1.98 |
| | 4.08 | 6.0 | 10.15 | 101.17 ± 1.22 | 7.97 | 12.0 | 20.10 | 101.08 ± 0.98 | 2.02 | 3.0 | 5.11 | 103.00 ± 1.52 |
| | 3.99 | 2.0 | 6.06 | 103.5 ± 1.91 | 8.05 | 4.0 | 12.16 | 102.75 ± 2.15 | 1.99 | 1.0 | 3.00 | 101.00 ± 0.97 |
| Atekind 50 | 3.99 | 4.0 | 8.14 | 103.75 ± 1.04 | 8.05 | 8.0 | 16.15 | 101.25 ± 2.76 | 1.99 | 2.0 | 4.02 | 101.50 ± 1.58 |
| | 3.99 | 6.0 | 10.23 | 104.00 ± 2.51 | 8.05 | 12.0 | 20.44 | 103.25 ± 2.40 | 1.99 | 3.0 | 5.03 | 103.33 ± 1.79 |
| | 4.02 | 2.0 | 6.10 | 104.00 ± 2.31 | 8.09 | 4.0 | 12.18 | 102.25 ± 1.56 | 1.99 | 1.0 | 3.02 | 103.00 ± 2.13 |
| Aten 100 | 4.02 | 4.0 | 8.16 | 103.5 ± 1.43 | 8.09 | 8.0 | 16.35 | 103.25 ± 2.45 | 1.99 | 2.0 | 4.05 | 103.00 ± 2.06 |
| | 4.02 | 6.0 | 10.29 | 104.5 ± 2.77 | 8.09 | 12.0 | 20.34 | 102.08 ± 1.47 | 1.99 | 3.0 | 5.07 | 102.67 ± 1.91 |

* Mean value of three determinations.
Three sensitive spectrophotometric methods for the determination of ATN in bulk drug as well as in tablets. The proposed methods have the advantages of utilization of bromine generated in situ as a green brominating reagent, free from critical experimental conditions, and complicated procedures such as heating or extraction step. The reagents used in the proposed methods are cheap, readily available, and the procedures do not involve any tedious sample preparation. These advantages encourage the application of the proposed methods in routine quality control analysis of ATN in pharmaceutical formulations.

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