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SIMPLE, SENSITIVE AND SELECTIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ATENOLOL IN PHARMACEUTICALS THROUGH CHARGE TRANSFER COMPLEX FORMATION REACTION

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Abstract: Three rapid, selective and sensitive spectrophotometric methods have been proposed for the quantitative determination of atenolol (ATN) in pure form as well as in its pharmaceutical formulation. The methods are based on charge transfer complexation reaction of ATN as n-electron donor with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 2,4-dinitrophenol (DNP) and 2,4,6-trinitrophenol (picric acid; PA) as π-acceptors to give highly colored radical anion species. The colored products were quantified spectrophotometrically at 590 nm with DDQ (method A) and at 420 nm with both DNP (method B) and PA (method C). Under the optimized experimental conditions, Beer's law is obeyed over the concentration ranges of 3–48, 2–24 and 1.5–18 μg/mL ATN for method A, method B and method C, respectively. The molar absorptivity, Sandell sensitivity, limits of detection and quantification are also reported. The effects of reaction medium, reaction time and reagent concentration on the sensitivity and stability of the complexes formed have been examined. The proposed methods were successfully applied to the determination of ATN in pure form and commercial tablets with good accuracy and precision. Statistical comparison of the results was performed using Student's *t*-test and F-ratio at 95% confidence level and the results showed no significant difference between the reference and proposed methods with regard to accuracy and precision. Further, the accuracy and reliability of the methods were confirmed by recovery studies *via* standard addition technique.

Keywords: atenolol, charge-transfer complexes, spectrophotometry, pharmaceuticals

Atenolol (ATN), chemically known as 2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide (1) is a β1-selective (cardioselective) adrenoreceptor antagonist drug commonly used for management of hypertension, prevention of heart diseases as angina pectoris and control of some forms of cardiac arrhythmia (2). It is also indicated for prophylaxis of migraine. The drug is official in Indian Pharmacopoeia (3), which describes a UV-spectrophotometric method for the assay of ATN in tablets whereas British Pharmacopoeia (4) describes high performance liquid chromatographic (HPLC) methods for its determination as official methods. Other than these official methods, several methods have been reported for the determination of ATN in pharmaceutical dosage forms, which include diffuse reflectance spectroscopy (2), liquid chromatography (LC) (5–26), HPTLC (27), ultra performance liquid chromatography (UPLC) (28), gas chromatography (GC) (29, 30), non-supressed ion-chromatography (31), fluorimetry (32, 33), differential scanning calorimetry (DSC) and thermogravimetry (TG) (34), electrophoresis (35–37), voltammetry (38), ion-selective electrode (ISE) based potentiometry (39), atomic absorption spectrometry (AAS) (40), UV-spectrophotometry (41–49), visible spectrophotometry (50–61) and titrimetry (59–61).

To the best of our knowledge, there are twelve reports on the use of visible spectrophotometry for the determination of ATN in pharmaceuticals. The reaction of ATN with hydroxylamine hydrochloride in NaOH medium followed by the reaction of the resultant hydroxamic acid derivative with $FeCl_3$ to give a red-violet ferric hydroxamate complex has served as the basis of spectrophotometric assay reported by Agrawal et al. (50). Assays based on charge transfer complexation reaction of ATN with choranilic acid have been reported by Agarwal et al. (51) and Yu et al. (52). Korang et al. (53) have developed a method based on the treatment of $CHCl_3$ 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. The

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slow reaction between ATN and ammonium vanadate in sulfuric acid medium resulted in two kinetic spectrophotometric methods (fixed-concentration method and fixed-time method) (54). Al-Ghannam and Belal (55) 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 the drug. The method developed by Hiremath et al. (56) 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. (57) have reported a method based on deamination of ATN in basic medium, followed by addition of sodium nitroprusside to generate a colored complex. Basavaiah et al. (58) 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 sulfanilic 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. (59). A similar method (60) 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 (61).

However, many of the above methods suffered from one or other disadvantages like poor sensitivity, heating or extraction step, use of expensive chemical and/or complicated experimental setup as can be seen from Table 1.

Charge-transfer complexation is an important phenomenon in biochemical and bioelectrochemial energy transfer process (62). Charge transfer phenomenon was introduced first by Mulliken. The term charge transfer gives a certain type of complex resulting from interactions of donor and acceptor with the formation of weak bonds (63, 64) and discussed widely by Foster (65). Molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge-transfer complexes which absorb radiation in the visible region.

In the present study, ATN was found to react with three π-acceptors, namely, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 2,4-dinitrophenol (DNP) and 2,4,6-trinitrophenol (picric acid; PA) to form C-T complexes, based on which three simple, rapid, selective and sensitive spectrophotometric methods were developed for the determination of ATN. The cited π-acceptors have earlier been employed for the assay of several drug substances (66–72) based on similar reaction.

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.

Materials

Pharmaceutical grade atenolol (ATN) certified to be 99.89% pure was received from Cipla India Ltd., Mumbai, India. The following pharmaceutical preparations were purchased from commercial sources in the local market and subjected to analysis: 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.

Reagents and chemicals

All reagents used were of analytical reagent grade and HPLC grade organic solvents were used throughout the investigation.

Solutions of 0.1% DDQ in 1,4-dioxane, 0.1% DNP and 0.05% PA in dichloromethane were prepared separately. A stock standard solution containing 100 µg/mL ATN was prepared by dissolving 10 mg of pure drug in acetonitrile and diluting to the mark in a 100 mL calibrated flask with the same solvent. The stock standard solution was diluted appropriately with acetonitrile to get the working concentrations of 60, 40 and 30 µg/mL ATN for use in methods A, B and C, respectively.

Construction of calibration curves

Method A (using DDQ)

Varying aliquots (0.25–4.0 mL) of a standard ATN solution (60 µg/mL) were accurately transferred into a series of 5 mL calibrated flasks using a micro burette and the total volume in each flask was brought to 4 mL by adding adequate quantity of acetonitrile. To each flask, 1 mL of 0.1% DDQ solution was added, the content was mixed well and the absorbance was measured at 590 nm against a reagent blank similarly prepared without adding ATN solution.

Method B (using DNP)

Different aliquots (0.25–3.0 mL) of standard ATN solution (40 µg/mL) were accurately trans-

Table 1. Comparison of the proposed and the existing visible spectrophotometric methods.

No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range µg/mL and ε, L/mol cm	LOD, µg/mL	Reaction time, min	Remarks	Ref.
1.	Hydroxylamine hydrochloride-iron (III)	Ferric hydroxamate complex measured	510	50–800 ($\varepsilon = 5.3\times10^2$)	NR	20–30	Less sensitive, heating required	48
2.	Chloranilic acid	Charge transfer complex measured	534	25–250	NR	-		49
3.	Chloranilic acid	Charge transfer complex measured	530	10–280	NA	NA		50
4.	Acetaldehyde -Chloranil		690	NA	NA	NA		51
5.	NH_4VO_3	Reaction rate measured	750	NA	NA	NA	heating required	52
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	53
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	54
8.	Sodium nitroprusside	Complex of ammonia and nitroprusside was measured	495	0.5–30 ($\varepsilon = 3.01\times10^5$)	0.01	5	heating required	55
9.	Chloramine-T-metol-sulfanilic acid	Unreacted chloramine-T was measured	520	2.5–25 ($\varepsilon = 3.24\times10^3$)	2.34	20	Not selective, involves multiple steps and reactions	56
10.	Chloramine-T: a) Metanil yellow b) Indigo carmine	Unreacted chloramine-T was measured	 530 610	 1–12 ($\varepsilon = 1.19\times10^4$) 2.5–20 ($\varepsilon = 6.65\times10^3$)	 0.32 0.04	 10 10	As above	57
11.	Bromate-bromide mixture- methyl orange	Unreacted bromine was measured	520	0.5–4.0 ($\varepsilon = 4.13\times10^4$)	0.07	15	As above	58
12.	Phenol red	The change in the color of phenol red was measured	430	3.0–30 ($\varepsilon = 3.47\times10^3$)	4.61	–		59
13.	a) DDQ b) 2,4-Dinitro-phenol c) Picric acid	Radical anion was measured Charge transfer complex measured	590 420 420	3.0–48 ($\varepsilon = 5.41\times10^3$) 2.0–24 ($\varepsilon = 1.13\times10^4$) 1.5–18 ($\varepsilon = 1.13\times10^4$)	0.26 0.23 0.23	– – 5	Rapid, simple, sensitive selective and no heating step. Use of one reagent and a one step reaction	This work

DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, NR: Not reported, NA: Not available.

ferred into a series of 5 mL calibration flasks as described above. One milliliter of 0.1% DNP solution was added to each flask and diluted to volume with dichloromethane. The content was mixed well and the absorbance was measured at 420 nm against a reagent blank.

Method C (using PA)

Aliquots (0.25–3.0 mL) of a standard ATN (30 µg/mL) solution were accurately transferred into a series of 5 mL calibration flasks and the total volume was brought to 3 mL by adding acetonitrile. To each flask, 1 mL of 0.05% PA solution was added and the solution made up to volume with dichloromethane. The content was mixed well and the absorbance was measured at 420 nm against a reagent blank.

Standard graph was prepared by plotting the absorbance *versus* ATN concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation.

Procedure for commercial tablets

Twenty tablets were weighed and pulverized. An amount of tablet powder equivalent to 10 mg ATN was extracted with three 30 mL portions of acetonitrile. The extracts were filtered using Whatman No. 42 filter paper; the filtrate was collected in a 100 mL calibrated flask and diluted to volume with acetonitrile. A suitable aliquot of the filtrate (100 µg/mL ATN) was diluted to get the working concentrations of 60, 40 and 30 µg/mL ATN for analysis by methods A, B and C, respectively, as described above.

Procedure for the analysis of placebo blank and synthetic mixture

A placebo blank containing starch (10 mg), acacia (15 mg), hydroxylcellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under "Procedure for commercial tablets" and then subjected to analysis.

A synthetic mixture was prepared by adding pure ATN (50 mg) to the above mentioned placebo blank and the mixture was homogenized. Synthetic mixture containing 10 mg of ATN was weighed and its solution was prepared as under "Procedure for commercial tablets". Two different aliquots were subjected to analysis by the general procedure and the concentration of ATN was found from the calibration graph or from the regression equation.

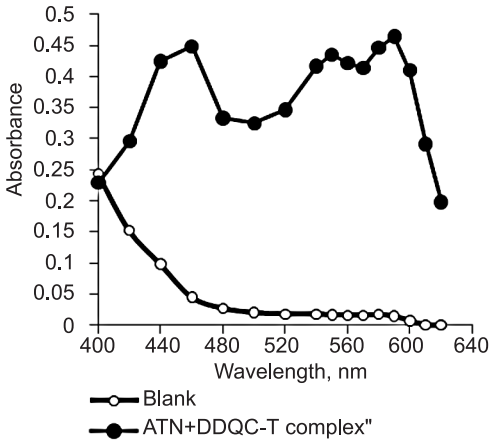

Figure 1. Absorption spectra of ATN-DDQ CT-complex

RESULTS AND DISCUSSION

Absorption spectra

The reaction of ATN as n-electron donor and the π-acceptors such as DDQ, DNP and PA results in the formation of C-T complexes. The absorption spectra of ATN-DDQ C-T complex resulted in the formation of an intense reddish violet color which

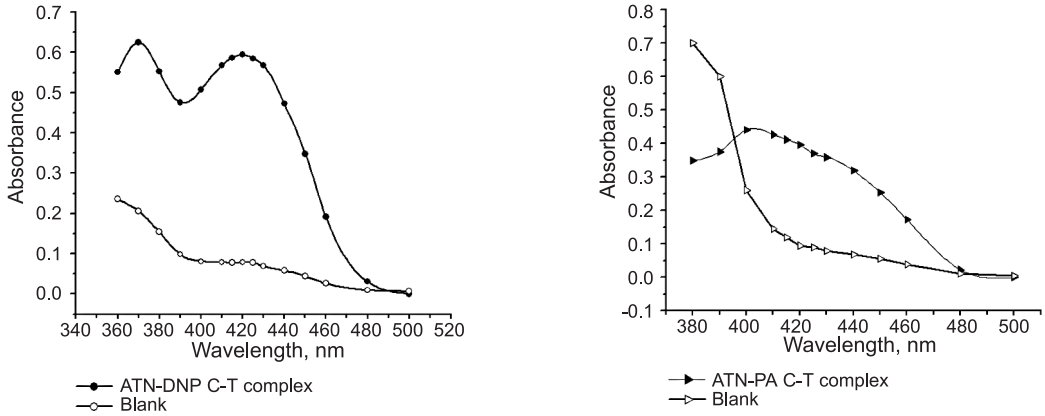

Figure 2. Absorption spectra of ATN-DNP complex (method B) and ATN-PA complex (method C)

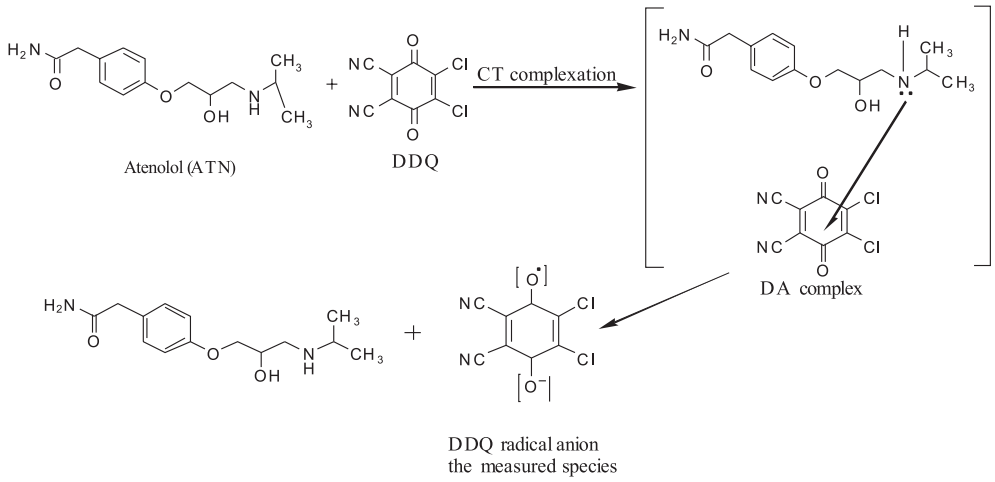

Figure 3. Probable mechanism for the formation of ATN-DDQ C-T complex

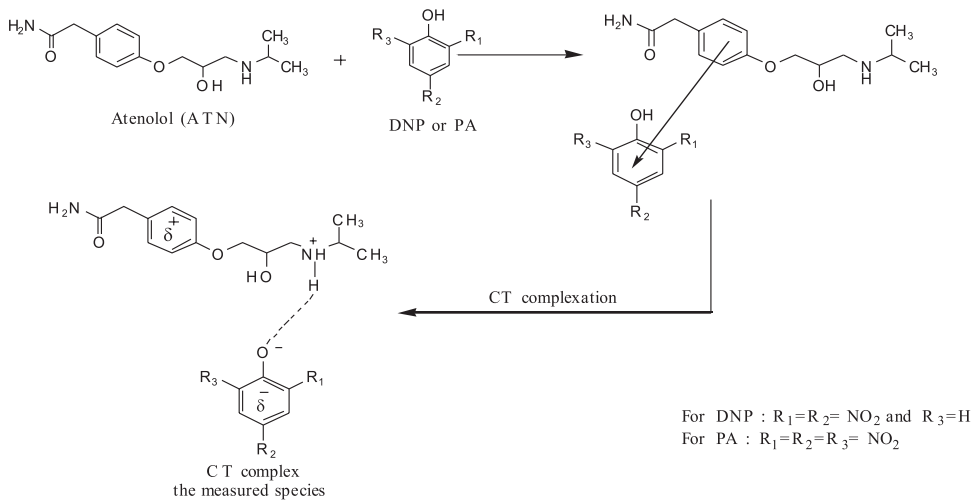

Figure 4. Probable mechanism for the formation of ATN-DNP and ATN-PA C-T complexes

exhibited three maxima at 590, 550 and 460 nm (Fig. 1). These bands can be attributed to the formation of DDQ radical anions arising from the complete transfer of n-electrons from donor to acceptor moieties in acetonitrile. The absorption band at 590 nm was selected as analytical wavelength keeping in view the sensitivity of the reaction product and blank absorbance. Similarly, the reaction of ATN with DNP or PA results in the formation of an intense yellow product which exhibits absorption maxima at 420 nm (Fig. 2).

Reaction mechanism

The chemistry used in method A is based on the reaction of the basic nitrogen of ATN as n-donor with DDQ as π-acceptor to form charge transfer complex, which subsequently dissociates into radical anions depending on the polarity of the solvent used (72). In polar solvents, such as acetonitrile, complete electron transfer from the donor to the acceptor moiety takes place with the formation of intensely colored radical anions (Fig. 3) (73), according to the following equation:

$$\ddot{D} + A \longrightarrow \left[\ddot{D} \rightarrow A\right] \xrightarrow{\text{Polar solvent}} \ddot{D}^{+} + A^{\bullet -}$$

charge transfer complex — radical anion

When an aromatic amine is combined with a polynitrophenol, one type of force field produces an acid-base interaction, and the other, an electron donor-acceptor interaction. The former interaction leads to

the formation of true phenolate by proton-transfer, and the latter, to a true molecular compound by charge-transfer (74). Based on this, the mechanism for method B and method C can be discussed in terms of transfer of electronic charge from the benzene ring of ATN, an electron-rich molecule (a Lewis-base donor), to the ring of DNP or PA, an electron-deficient molecule (a Lewis-acid acceptor), and at the same time the proton of the hydroxyl group of DNP or PA will transfer to the secondary amine of ATN (Fig. 4). The explanation for the produced color in method B and method C lies in the formation of complexes between the pairs of molecules ATN-DNP and ATN-PA, and this complex formation leads to the production of two new molecular orbitals and, consequently, to a new electronic transition (75).

Optimization of experimental variables

Many experimental variables, which found to affect the color intensity and stability of the resulting complexes, were optimized to achieve maximum sensitivity and adherence to Beer's law.

Effect of reagent concentration

The optimum concentration of the reagent required to achieve maximum sensitivity for the color developed in each method was ascertained by adding different amounts of the reagent DDQ, DNP or PA to a fixed concentration of ATN. The results showed that 1.0 mL each of 0.1% DDQ, 0.1% DNP and 0.05% PA solution was optimum for the production of maximum and reproducible color intensity (Fig. 5).

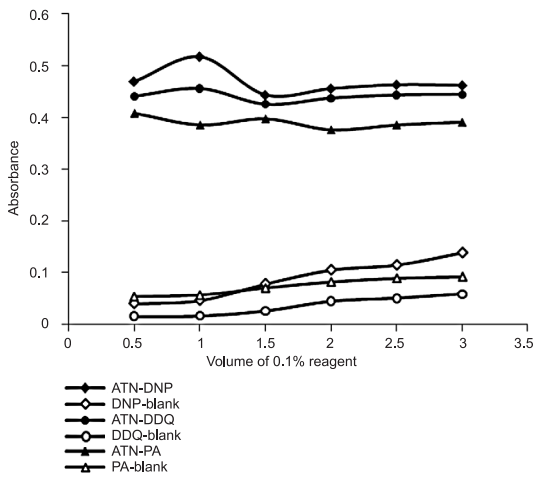

Figure 5. Effect of reagent concentration on the formation of ATN-DDQ complex, (24 µg/mL ATN), ATN-DNP complex, (12 µg/mL ATN) and ATN-PA complex, (9 µg/mL ATN)

Effect of solvent

In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as 1,4-dioxane, chloroform, acetonitrile, acetone, *t*-butanol, 2-propanol and dichloromethane, and the reaction of ATN with DDQ, DNP or PA was followed. In method A, as shown in Fig. 6, acetonitrile was best suited for preparation of DDQ solution. The dichloromethane solvent was found to be the ideal solvent for preparation of both DNP and PA for method B and method C, respectively (Fig. 6). Similarly, the effect of the diluting solvent was studied for all methods and the results showed that the ideal diluting solvent to achieve maximum sensitivity was acetonitrile in method A and dichloromethane in method B and method C.

Effect of reaction time and stability of the C-T complexes

The optimum reaction times were determined by measuring the absorbance of the complex formed upon the addition of reagent solution to ATN solution at room temperature. The reaction of ATN with DDQ in method A and DNP in method B was instantaneous while complete color development was attained after 5 min with PA. The absorbance of the resulting C-T complexes remained stable for at least 45 min for method A and for more than 24 h for methods B and C.

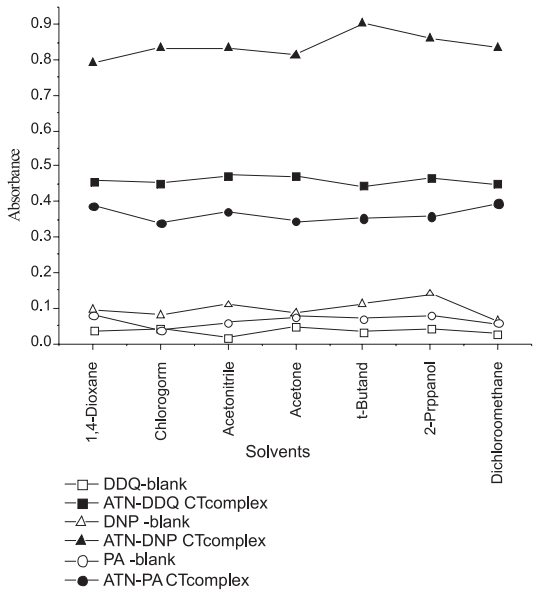

Figure 6. Effect of solvents on the formation of ATN-DDQ complex, (24 µg/mL ATN), ATN-DNP complex, (20 µg/mL ATN) and ATN-PA complex, (9 µg/mL ATN)

Method validation

The proposed methods were validated for linearity, sensitivity, selectivity, accuracy, precision, robustness, ruggedness and recovery according to the current ICH guidelines (76).

Linearity and sensitivity

Under the optimum conditions, a linear relation was obtained between absorbance and concentration of ATN in the ranges given in Table 2. The calibration graph in each instance is described by the equation: Y = a + b X, (where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL). The correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection (LOD) and quantification (LOQ) are calculated as per the current ICH guidelines (76) and compiled in Table 2. LOD and LOQ were calculated according to the same guidelines using the following formulae:

$$LOD = \frac{3.3\sigma}{s} \quad \text{and} \quad LOQ = \frac{10\sigma}{s}$$

where σ is the standard deviation of six reagent blank determinations and s is the slope of the calibration curve.

Table 2. Regression and analytical parameters.

Parameter	Method A	Method B	Method C
λ_{max} (nm)	590	420	420
Beer's law limits (µg/mL)	3–48	2–24	1.5–18
Molar absorptivity (L/mol cm)	5.41×10^3	1.13×10^4	1.13×10^4
Sandell sensitivity* (µg/cm^2)	0.0493	0.0236	0.0236
Limit of detection (µg/mL)	0.26	0.23	0.23
Limit of quantification (µg/mL)	0.80	0.70	0.69
Regression equation, Y**			
Intercept (a)	0.0091	0.0387	–0.0120
Slope (b)	0.0197	0.0387	0.0446
Correlation coefficient (r)	0.9992	0.9997	0.9999
Standard deviation of intercept (S_a)	0.01078	0.00650	0.00287
Standard deviation of slope (S_b)	0.00040	0.00045	0.00026

*Limit of determination as the weight in µg/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.
**$Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in µg/mL.

Table 3. Evaluation of intra-day and inter-day precision and accuracy.

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]
A	12.00	11.83	1.31	1.41	12.24	1.56	2.04
	24.00	23.81	0.78	0.81	24.42	0.97	1.75
	36.00	35.65	1.06	0.98	36.66	1.28	1.84
B	8.00	8.19	1.23	2.37	8.23	1.66	2.85
	12.00	12.11	1.01	0.90	12.29	2.01	2.42
	16.00	16.17	0.71	1.07	16.38	1.85	2.39
C	6.00	5.91	0.57	1.47	6.13	1.74	2.18
	9.00	9.14	0.25	1.55	9.23	1.36	2.56
	12.00	11.79	0.82	1.72	12.29	1.54	2.38

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

Table 4. Robustness and ruggedness.

Method		Method robustness		Method ruggedness	
		Parameters altered			
	ATN taken µg/mL	Reagent volume, mL[a] RSD, % (n = 3)	Reaction time[b] RSD, % (n = 3)	Inter-analysts RSD, % (n = 3)	Inter-instruments RSD, % (n = 3)
A	12.00	0.56		1.24	2.48
	24.00	1.24		0.85	2.75
	36.00	1.35		0.63	2.16
B	8.0	1.08		1.45	1.67
	12.0	1.26		1.38	2.39
	16.0	1.48		1.13	1.86
C	6.0	0.79	1.45	0.75	2.75
	9.0	0.57	1.03	1.36	2.18
	12.0	1.14	0.94	1.12	1.67

[a] In all methods, the volume of reagent was 0.8, 1.0 and 1.2 mL. [b] The reaction was instantaneous in methods A and B and the reaction time was 4, 5 and 6 min for method C.

Table 5. Results of analysis of tablets by the 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	25	100.3 ± 0.58	99.06 ± 1.17 t = 2.12 F = 4.07	99.82 ± 1.37 t = 0.72 F = 5.58	98.97 ± 1.32 t = 2.00 F = 5.18
Atekind-50	50	99.67 ± 0.67	98.21 ± 1.11 t = 2.52 F = 2.74	101.1 ± 1.06 t = 2.55 F = 2.50	98.31 ± 1.18 t = 2.24 F = 3.10
Aten-100	100	100.6 ± 0.82	100.9 ± 1.91 t = 0.32 F = 5.43	101.5 ± 1.06 t = 1.5 F = 1.67	99.32 ± 1.71 t = 1.51 F = 4.35

[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.

Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, pure drug (ATN) solution at three different concentration levels (within the working range) were prepared and analyzed in seven replicates during the same day (intra-day precision) and on five consecutive days (inter-day precision) and the results are presented in Table 3. The percentage relative error (RE%) was = 2.85 which indicates that the accuracy of the methods is satisfactory. Percentage relative standard deviation (RSD %) for intra-day was = 1.23 and for inter-day it was ≤ 2.01 indicating repeatability and usefulness of the proposed methods in the routine analysis**.**

Selectivity

The selectivity of the proposed methods for the analysis of ATN was evaluated by placebo blank and synthetic mixture analyses. The recommended procedures were applied to the analysis of placebo blank and the resulting absorbance readings in all methods were the same as that of the reagent blank, confirming no interference from the placebo. The analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries of 98.3 ± 2.13, 99.1 ± 1.76 and 98.9 ± 1.91 (n = 5) for method A, method B and method C, respectively. The results of this study showed that the inactive ingredients did not interfere in the assay indicating the high selectivity of the proposed method and applicability to use for routine determination in pure and in tablets form.

Robustness and ruggedness

To evaluate the robustness of the methods, two important experimental variables: volume of reagent

Table 6. Results of recovery study by standard addition method.

Tablets studied	Method A			Method B				Method C			
	ATN in tablets, µg/mL	Pure ATN added, µg/mL	Total found, µg/mL	Pure ATN recovered*, % ± SD	ATN in tablets, µg/mL	Pure ATN added, µg/mL	Total found, µg/mL	Pure ATN recovered* % ± SD	ATN in tablets µg/mL	Pure ATN added, µg/mL	Total found, µg/mL	Pure ATN recovered*, %±SD
Atenex 25	11.88	6	18.14	104.4 ± 2.77	7.99	4	12.16	104.28 ± 2.21	5.94	3	9.03	102.97 ± 2.92
	11.88	12	24.54	105.48 ± 0.57	7.99	8	16.43	105.46 ± 2.65	5.94	6	12.21	104.49 ± 2.70
	11.88	18	30.46	103.21 ± 2.83	7.99	12	20.77	106.47 ± 1.11	5.94	9	15.23	103.19 ± 1.05
Atekind 50	11.77	6	17.98	103.54 ± 2.76	8.09	4	12.40	107.76 ± 2.51	5.90	3	9.04	104.52 ± 2.16
	11.77	12	24.10	102.83 ± 2.84	8.09	8	16.75	108.21 ± 2.76	5.90	6	12.33	107.20 ± 2.07
	11.77	18	30.68	105.04 ± 1.51	8.09	12	21.19	109.18 ± 1.40	5.90	9	15.37	105.25 ± 1.80
Aten 100	12.12	6	18.35	103.87 ± 2.88	8.12	4	12.49	109.26 ± 1.51	5.96	3	9.08	104.01 ± 2.37
	12.12	12	24.5	103.21 ± 2.15	8.12	8	16.61	106.12± 2.75	5.96	6	12.5	109.06 ± 1.76
	12.12	18	30.9	104.37 ± 0.77	8.12	12	20.61	104.05 ± 1.44	5.96	9	15.85	109.92 ± 1.91

Mean values of three determinations.

in all the methods and reaction time in method C, were altered incrementally and the effect of this change on the absorbance of the C-T complexes was studied. The results of this study are presented in Table 4 and indicated that the proposed methods are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts and on three different spectrophotometers by the same analyst. From the %RSD values presented in Table 4, one can conclude that the proposed methods are rugged.

Application to analysis of tablets containing ATN

The proposed methods were successfully applied to the determination of ATN in three brands of tablets and the results are compiled in Table 5. The results obtained were statistically compared with those obtained by the reference method (3) by applying the Student's *t*-test for accuracy and F-test for precision at 95% confidence level. As can be seen from Table 5, the calculated *t*- and F- values at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees of freedom. This indicates that there are no significant differences between the proposed methods and the reference method with respect to accuracy and precision.

Recovery study

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ATN at three concentration levels (50, 100 and 150 % of that in tablet powder) and the total was analyzed by the proposed methods. The results of this study are presented in Table 6 and indicate that the excipients present in the tablets did not interfere in the assay.

CONCLUSIONS

This paper presents three visible spectrophotometric methods for the quantitative determination of atenolol in pure drug and tablets. The proposed methods are based on charge-transfer complexation reaction, and have the advantages of simplicity, speed, accuracy and precision, and use of inexpensive equipment compared to the reported chromatographic methods. The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Whereas most of the reported methods rely on the use of multiple reagents/reactions, the proposed methods employ a

single reagent/reaction, with minimal manipulation which results in considerably increased precision. The DDQ method is less sensitive than both DNP and PA methods as can be seen from the lower molar absorptivity value. The methods are characterized by wide linear dynamic ranges and high sensitivity compared to many existing spectrophotometric methods. Moreover, the proposed methods are free from the usual analytical complications like heating or extraction steps and can be performed at room temperature. Thus, the proposed methods are useful for the quality control and routine analysis of ATN in pharmaceuticals because there is no interference from the common excipients usually found in commercial tablets.

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