New Spectrophotometric Analysis of Atenolol in Pure and Pharmaceutical Formulations

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Two simple and sensitive spectrophotometric methods (A and B) for the determination of atenolol in bulk samples and pharmaceutical formulations are described. Both the methods are based on the oxidation of the drug with excess quantities of oxidants, N-bromosuccinimide (NBS) in method A and nitrous acid in method B. The excess NBS in method A is determined using a dye celestine CB blue at 1_{max} 540 nm, while in method B the excess nitrous acid is determined using a dye cresyl fast violet acetate CFVA 1_{max} 555 nm. Both these methods are applicable to pure samples as well as formulations of the drug. The results obtained by the proposed methods were in good agreement with the labelled amounts.

Atenolol (ANT), chemically known as benzene aceta-mide-4-[2-hydroxy-3-(1-methyl)aminopropoxy], is a hydrophilic b-adrenergic blocking agent, widely used in the treatment of hypertension and certain types of cardiac arrhythmias[1]. It is official in IP[2] and BP[3]. The reported techniques for its estimation include fluorometry[4-8], UV spectrophotophotometry[9-12], visible spectrophotometry[13-17], NMR spectroscopy[18-19] HPLC[20-31], TLC[32], LC[33-34] and GC[35-37]. Some of the reported spectrophotometric methods suffer from lack of sensitivity or specificity. The present communication reports two new visible spectrophotometric methods based on the susceptibility of ATN molecule to undergo oxidation. Sastry et al., developed an indirect method for the determination of some drugs using NBS/Celestine blue[38] [CB, oxazine, 51050, phenoxazin-5-ium-1-(aminocorbonyl)-7-(diethylamino)-3,4-dihydroxy chloride] or nitrous acid/cresyl fast violet acetate (CFVA, benzo (a) phenoxazin-5-ium-5-imino-9-amino acetic acid salt[39]]. In these methods the drug is treated with known excess of oxidant NBS (method A) or HNO$_2$ (Method B) and excess NBS remained after the completion of the reaction is estimated by utilizing its ability to oxidise the dye CB and there by decrease the colour intensity of the solution containing a known strength of the dye measured at 540 nm. While in method B, the excess

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nitrous acid is estimated with known excess of CFVA. The pinkish violet colour with red fluorescence (original dye) changes to yellow. The change may be due to alteration of the dye structure in which the original chromophore and auxochromes change due to nitrosation with involvement of secondary amino group of the drug.

MATERIALS AND METHODS

A Milton Roy Spectronic 1201 and Systronics 106 spectrophotometers with 1 cm matched quartz cells were used for all spectral and absorbance measurements. All solutions were prepared in double distilled water and all the chemicals used were of analytical grade. Aqueous solutions of NBS (0.01%) (Loba-Chemie, Mumbai) NaNO$_2$ (0.002%) (S.D. Fine Chemicals, Boisar), Hydrochloric Acid (5 M, E.Merck, Mumbai) CB (0.01%) and CFVA (0.005%) (CROMA-GESELLSCHAFT SCHMID & CO.) were used. All other chemicals were procured from commercial sources.

Preparation of solutions:

A stock solution containing 1 mg/ml of pure ATN was prepared by dissolving 100 mg of the drug initially in 0.1 M HCl and making up the volume to 100 ml with distilled water. Working standard solutions were prepared by further diluting the stock solution with distilled water to get 20 µg/ml (method A) and 40 µg/ml (method B).

Analytical procedure for method A:

Aliquots of standard ATN solution (0.5-5.0 ml of volume 20 µg/ml) were taken in to a series of 25 ml graduated tubes, 1.25 ml of 5 M HCl and 2.5 ml NBS (100 µg/ml) were added and the volume was made up to 15 ml with distilled water. After 10 min, 10 ml of CB was added, thoroughly mixed and the absorbance was measured after 5 min at 540 nm against distilled water. Blank solution was prepared appropriately. The decrease in absorbance corresponding to consumed NBS, which in turn to drug concentration, was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration curve was drawn by plotting the decrease in absorbance of dye CB, against amount of the drug. The amount of drug was computed from the standard calibration graph.

Analytical procedure for method B:

Aliquots of standard ATN solution (0.5-4.0 ml) of volume 40 µg/ml were placed into a series of 25 ml graduated tubes, 1.25 ml of 5 M HCl and 2 ml of sodium nitrite (20 µg/ml) were added and the volume was made up to 15 ml. After 3 min, 10 ml of CFVA was added, mixed thoroughly and the absorbance was measured after 5 min at 555 nm against distilled water. Blank experiment was carried out in similar manner omitting the compound. The decrease in the absorbance corresponding to consumed sodium nitrite, which in turn correspond to compound concentration was obtained by subtracting the absorbance of the blank solution from that of the test solution. The amount of the drug present in the sample was computed from the Beer-Lambert plot drawn between the amount of drug and decrease in the absorbance of the CFVA.

Analysis of pharmaceutical preparations:

Tablet powder equivalent to 100 mg of ATN was weighed accurately and transferred to a 100 ml volumetric flask. The content was dissolved initially in minimum amount of 0.1 M HCl and the volume was made upto 100 ml with distilled water and filtered. The above solution was further diluted to the requisite concentrations for methods A and B were analyzed as described under the procedures for pure samples.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and intercept data from linear least squares treatment and percent relative standard deviation (from six replicate samples) were summarized in Table 1.

TABLE 1: OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS FOR ATN

Parameters	Method A	Method B
λ_{max} (nm)	540	555
Beer's law limits (µg/ml)	-4.0	0.8-6.4
Molar absorptivity (l.mol/cm)	3.57×10^4	1.46×10^4
Sandell's sensitivity (µg Cm^{-2}/0.001 Abs. Unit)	7.44×10^{-3}	1.81×10^{-2}
Regression equation (y=a+bc) Slope (b)	1.34×10^{-1}	5.47×10^{-2}
Intercept (a)	-1.0×10^{-3}	1.1×10^{-3}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation (%)*	0.40	0.66
% Range of error (confidence Limits) 0.05 level	0.43	0.70

* Calculated from 6 determinations.

The accuracy of the method was ascertained by comparing the results from proposed and reported methods statistically by the t and F tests and found not to differ significantly. In order to justify the reliability and suitability of the proposed methods, known quantities of pure drug was added to its pre analyzed dosage forms and the mixtures were analyzed by the proposed methods and the values are listed in Table 2. There is no interference from other ingredients present in formulations, in this assay method.

Method A involves two stages namely oxidation of the drug with excess NBS and the estimation of excess NBS using the dye CB. Oxidation of ATN with 2.0-3.0 ml of NBS in presence of 1 to 1.5 ml of 5 M HCl, gave maximum and reproducible absorbance values. The effect of time, temperature and acid concentration of oxidation on the absorbance of the coloured species was studied by conducting the oxidation at different temperatures for different time intervals, and different acid concentrations, oxidation time ranging from 5-20 min at room temperature gave constant and reproducible absorbance values. Prolonging the oxidation time beyond 20 min and increasing the temperature gave erratic results. Acid concentration 4-6 M is found to be necessary and 8-12 ml of CB solution afford the highest absorbance values. A waiting period ranging from 5-10 min is necessary for taking the absorbance after the addition of CB.

TABLE 2: ASSAY OF ATENOLOL IN PHARMACEUTICAL FORMULATIONS.

Pharmaceutical Formulations*	Labelled amount (mg).	Amount found by proposed method** %(mg)		Found reference method (UV)[11]	% recovery by proposed method***	
		Method A	Method B		Method A	Method B
Tablets ALOTEN 50	50	49.5±0.32 f=1.33 t=0.43	49.5±0.63 f=4.69 t=1.72	49.2±0.96	99.6±0.54	98.3±0.54
ALOTOL	50	49.7±0.52 f=2.08 t=1.35	49.9±0.582 f=2.59 t=1.92	49.3±0.56	99.7±0.42	100±0.64
ANGITOL	25	25.10±0.44 f=3.49 t=1.46	25.05±0.57 f=2.11 t=0.29	25.0±0.90	100.0±1.21	97.9±0.69
ATECARD	50	49.5±0.32 f=2.46 t=0.33	49.8±0.48 f=1.407 t=0.45	50.0±0.44	99.7±0.21	98.2±0.75

*Formulations that manufactured by four different pharmaceutical companies, Aloten (50 mg) Core Health Care limited Ahmedabad. Alotol (50 mg) Indico Remedies Mumbai. Angitol (25 mg) Ind-Swift Ltd., Chandigarh and Atecard 50 mg Dabur Pharmaceuticals Ltd., Ghazibad. **Average ± standard deviation of six determinations, the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F=5.05, t=2.57 *** Recovery of 10 mg added to the pharmaceutical formulations (average of three determinations).

Where as in method B oxidation of ATN with nitrous acid and estimation of excess HNO_2 using the dye CFVA 1-3 ml $NaNO_2$ and 1-1.5 ml of 5 M hydrochloric acid were found to be necessary. Waiting periods during successive additions were studied by means of control experiments varying one parameter at a time keeping the rest and drug fixed. The effect of acid concentration, time and temperature of oxidation were studied. Oxidation time ranging from 2-4 min at room temperature gave constant and reproducible values. The time beyond 4 min and increasing the temperature gave erratic results. CFVA solution (8-12 ml) afford the highest absorbance values. A waiting period 5-10 min is necessary before measuring absorbance after the addition of CFVA.

The proposed methods are simple and sensitive with good precision and accuracy and can be used for the routine quality control analysis of ATN in pure form as well as in pharmaceutical formulations depending upon the availability of chemicals and nature of other ingredients presents in the sample.

REFERENCES:

1. Reynolds, J.E.F., Eds., In; Martindale; The Extra Pharmacopoeia; 28th Edn., the Pharmaceutical Press, London, 1982, 1337.
2. Pharmacopoeia of India, 4th Edn., Ministry of Health and Family Welfare, Government of India, New Delhi, 1996, 72.
3. British Pharmacopoeia, Vol. I HMSO, London, 1988, 49.
4. Wang, J., Quin, Z., Dong, J. and Zhang, J., Shanghai Yike Daxue Xuebao, 1988, 15, 417.
5. Johansson, M. and Forsomo-Bruce, H., J. Chromatogr., 1988, 432, 265.
6. Flouvat B., Bazin, M., Lucsko, M., Roux, A. and Guedon, J., Ann. Biol., 1978, 36, 339.
7. Schaefer, M. and Mutschler, E., J. Chromatogr., 1979, 169, 477.
8. Kaye, C.M., Brit. J. Clin. Pharmacol., 1974, 1, 84.
9. Veronico, M., Ragno, G. and Vetuschi, C., Spectrosc. Lett., 1955, 28, 407.
10. Vetuschi, C. and Ragno, G., Int. J. Pharm., 1990, 65, 177.
11. Haung, J. and Jin, J., Zhongguo-Yiyao Congye Zazhi, 1989, 20, 19.
12. Odonez, R.G., Consuegra, M.S. and Ibanez, C.S., Rev. Cubana Farm., 1987, 21, 243.
13. Sultan, S.M., Acta Pharm. Hung., 1992, 62, 311.
14. Sungur, S. and Yurdakul G., Marmara Univ. Eczacilik Derg., 1991, 7, 63.

15. Zakhari, N.A., Hassan, S.M. and El-shabrowy, Y., **J. Pharm. Biomed. Anal.**, 1991, 9, 421.

16. Abdel-Hay, M.H., Korang, M.A., Galal, S.M. and El-Sayed, M.A., **Alexandria J. Pharm. Sci.**, 1987, 1, 11.

17. Korany, M.A., Abdel-Hay, M.H. Galal, S.M. and El-Sayed M.A., **J. Pharm, Belg.**, 1985, 40, 178.

18. Kartasho V.S., Shorshnev, S.V. and Arzamastsev, A.P., **Farmatsiya, (Mascow)** 1993, 42, 24.

19. Iorio, M.A., Mazzeo F.A. and Doldo, A., **J. Pharm. Biomed. Anal.**, 1987, 5, 1.

20. Gonzalez, A.G., Herrador, M.A. and Asuero, A.G., **Int. J. Pharm.**, 1995, 123, 149.

21. Shafiee, A. and Shojaie, F., **J. Sch. Pharm. Med. Sci; Univ. Tehran.**, 1992, 2, 261.

22. Pawlak, Z. and Clark, B.J., **J. Pharm. Biomed. Anal**, 1992, 10, 329.

23. Erram, S.V. and Tipnis, H.P., **Indian Drugs**, 1992, 29, 436.

24. Sankar, S.R., Nanjan, M.J., Vasudevan, M., Sharat N. and Suresh, B., **Indian J. Pharm, Sci.**, 1997, 59, 171.

25. Cong, L. and Liu, Y., **Yaown Feuxl Zazhl**, 1989, 9, 175.

26. Sa Sa, S.I., **J. Llq. Chromatogr.**, 1988, 11, 929.

27. Sa Sa, S.I., Jalal, I.M., and Khalil, H.S., **J. Liq. Chromatogr.**, 1988, 11, 1673.

28. El-Yazigi, A., **J. Pharm. Sci.**, 1984, 73, 751.

29. Patel, B. R., Kirschbaum, J.J. and Poet, R.B., **J. Pharm. Sci.**, 1981, 70, 336.

30. El-Dawy, M.A., Habeeb, A.A., Mabrouk, M.M., and El-Bastawissy I.E., **Mansoura J. Pharm. Sci.**, 1993, 9, 116.

31. Ficarra, R. Ficarra, P., Tommasini, A., Calabro, M.L. and Guarniera F.C., **Farmaco, Ed. Part.**, 1985, 40, 307.

32. Ojanpera, I. and Vuori, E., **J. Llq. Chomatogr.**, 1987, 10, 3595.

33. Ghanem, R., Bello, M.A., Callejon, M. and Guiraum, A., **J. Pharm, Biomned. Anal.**, 1996, 25, 383.

34. Daldrup, T., Michalke, P. and Boehme, W., **Chomatogr. Newsl.**, 1982, 10, 1.

35. You, Ch and Fang, H., **Yaowu. Fenxl Zazhl**, 1992, 12, 195.

36. Rao G.R., Avadhanulu, A.B., Giridhar, R., Panthulu,A.R.R. and Kokate, C.K., **Eastern Pharmacist**, 1990, 33, 113.

37. Yamaji, A., Kataoka, K., Kanamori, N., Olshi, M. and Hiraoka, E., **Yakugaku Zasshi**, 1985, 12, 105.

38. Sastry, C.S.P., Srinivas, K.R. and Krishna Prasad, K.M.M., **Microchemica Acta**, 1996, 122, 77.

39. Sastry, C.S.P., Rama Srinivas, K. and Krishna Prasad K.M.M., **Anal. Lett.**, 1996, 29, 1639.