ISSN: 0975-766X
Available Online through
www.ijptonline.com
Research Article

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL AND INDAPAMIDE IN BULK AND TABLET DOSAGE FORM

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Received on 09-09-2010 *Accepted on 28-09-2010*

ABSTRACT

An UV spectrophotometric method using simultaneous equation was developed for the simultaneous determination of Atenolol and Indapamide in a binary mixture. In the Proposed method, the signals were measured at 225.0 nm and 240.0 nm corresponding to absorbance maxima of Atenolol and Indapamide in methanol respectively. Linearity range was observed in the concentration range of 6-30 µg/ml for Atenolol and 0-10 µg/ml for Indapamide. Concentration of each drug was obtained by using the absorptivity values calculated for both drugs at two wavelengths, 225.0 nm and 240.0 nm and solving the simultaneous equation. Developed method was applied to laboratory mixture and its Pharmaceutical formulation. The method was validated statistically and recovery study was performed to confirm the accuracy of the method. The method was found to be rapid, simple, accurate and precise.

Key words: Atenolol, Simultaneous equation, Spectrophotometric method, Indapamide.

INTRODUCTION

Indapamide (IND), Benzamide,3-(aminosulphonyl)-4 chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl is β-blocking agent, that lowers blood pressure and used for control and management of edema and widely used in treatment of hypertension[1]. Few spectroscopic methods have been reported for determination of IND as single

drug or in combination with other drugs [2]. Indapamide can determine by use of Spectrophotometrically [3,4] and also chromatographic methods[5,6]. Atenolol (ATL), chemically (*R*, *S*)-4-(2-hydroxy-3-isopropyl-aminopropoxy) phenylacetamide, is a beta-adrenoceptor antagonist. It is official in the Indian Pharmacopoeia [7]. Literature survey reveals, HPLC and HPTLC methods have also been reported for estimation of ATL in Pharmaceutical dosage forms. [8-10] and also there are various methods such as UV spectrophotometry for Atenolol [11-13].

Extensive literature survey reveals, none of the method is available that is based on estimation of Atenolol and Indapamide simultaneously by UV-spectrophotometric method. Aim of present work was to develop simple, precise, accurate and economical spectrophotometric methods for simultaneous determination of binary drug formulation. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines. [14]

MATERIALS

Spectrophotometric analysis was carried out on double beam spectrophotometer (Systronic 2201) with a fixed slit width (3 nm) using a pair of 1 cm matched quartz cells. The software system of the instrument was used for obtaining the spectra. Pure drug samples of ATL and IND were kindly gifted by Suchem Lab, Ahmadabad and Supra chemicals, Thane respectively. Methanol was procured from Merck Chemical Corporation, Mumbai. Commercial Pharmaceutical preparation (ATEN-D, Cadila Health Care, Ahmadabad) was procured from commercial source. All the reagents used were of analytical grade.

METHOD

1. Study of overlain spectra and selection of wavelength

ATL and IND, 50 mg each, were accurately weighed and dissolved separately in 50 ml methanol. From the above solutions 5 ml were diluted separately to 50 ml with methanol to produce 100 µg/ml each of ATL and IND. Suitable aliquots of these stock solutions of ATL and IND were diluted with methanol to obtain 6-30 µg/ml of ATL and 0-10µg/ml IND separately. Absorbances of the above dilutions were determined [Table 1]. Calibration curves were plotted as concentration Vs absorbance [Figure 1-2]. From the overlain spectra [Figure

3] two wave lengths, 225.0 nm and 240.0 nm were selected and absorptivity values E (1%, 1cm) of both the drugs at both wavelengths were determined for formation of simultaneous equation.

C1 = (A2ay1 – A1ay2) / (ax2ay1- ax1ay2) ---------- (1)

C2 = (A1ax2 – A2ax1) / (ax2ay1- ax1ay2) ---------- (2)

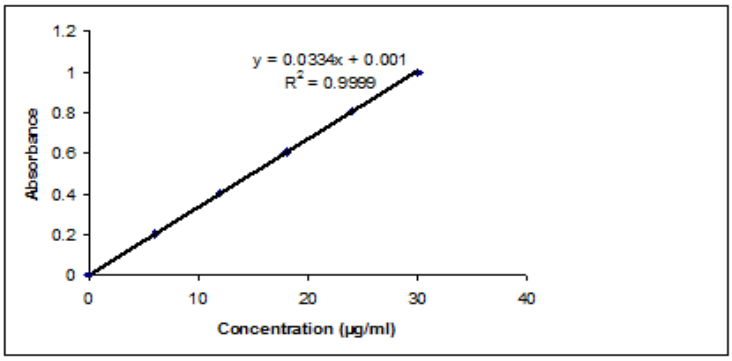

Figure-1: Showing Calibration Curve of Atenolol

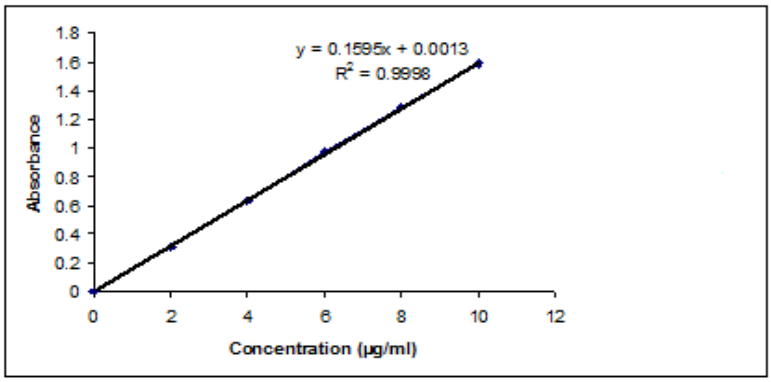

Figure-2: Showing Calibration Curve of Indapamide

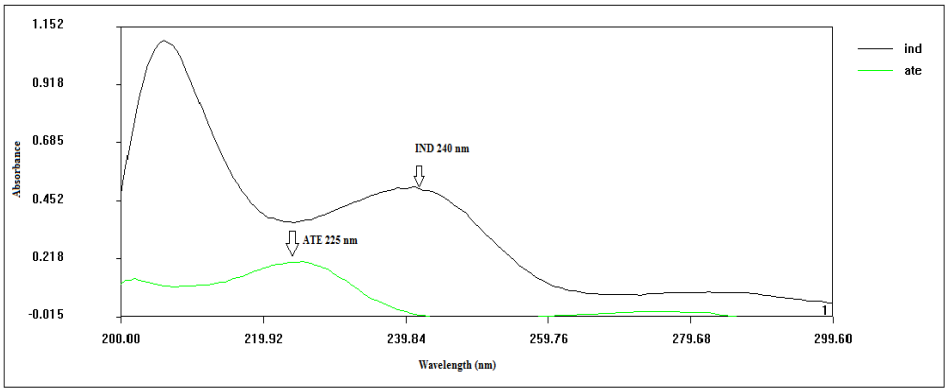

Figure 3 Showing Overlain Spectra of Atenolol and Indapamide

Table-1: Linearity Study of ATL and IND.

Sr no.	Conc of ATL [µg/ml]	Absorbance mean ± S.D. [n=3]	% R.S.D.	Conc of IND [µg/ml]	Absorbance mean± S.D. [n=3]	% R.S.D
1	06	0.2038± 0.002683	1.3164	2	0.3158±0.003271	1.0357
2	12	0.4044±0.001817	0.4493	4	0.6356±0.001673	0.2632
3	18	0.6034±0.001949	0.3230	6	0.9716±0.001342	0.1381
4	24	0.8046±0.001517	0.1885	8	1.2830±0.002828	0.2204
5	30	0.9940±0.003162	0.3181	10	1.5868±0.000837	0.0527

2. Analysis of laboratory mixture

Accurately weighed 50 mg of ATL and 2.5 mg of IND were transferred to 100 ml volumetric flask, dissolved in methanol and volume was adjusted up to the mark with same solvent. Appropriate aliquot 0.5 ml was transferred to 10 ml volumetric flask and volume was adjusted up to the mark with same solvent to obtained concentration 30 µg/ml of ATL and 1.5 µg/ml of IND. The absorbances of solutions were recorded at 225.0 nm and 240.0 nm against blank. Concentration of each drug was obtained by solving the simultaneous equation [Table 2]

Table-2: Analysis of Laboratory Mixture of ATL and IND

Drugs	Amount taken [µg/ml]	Amount found [µg/ml]	Amount found [%]
ATL	30	29.82	99.40
	30	29.94	99.80
	30	29.88	99.60
	30	29.91	99.70
	30	29.85	99.50
	Mean ± S.D.	29.88 ± 0.04743	99.60 ± 0.1581
	% R.S.D.	0.1587	0.1587
IND	1.5	1.49	99.77
	1.5	1.50	100.18
	1.5	1.50	100.18
	1.5	1.49	99.35
	1.5	1.49	99.77
	Mean ± S.D.	1.49 ± 0.0043	99.85 ± 0.3466
	% R.S.D.	0.2906	0.3471

3. Application of proposed method for analysis of tablet formulation

Twenty tablets **'ATEN D'** (containing 50 mg of ATL and 2.5 mg of IND**)** were weighed and ground to fine powder. A quantity of sample equivalent to 50 mg of Atenolol and 2.5 mg of Indapamide was transferred into 100 ml volumetric flask containing methanol, sonicated for 10 min; the volume was made up to the mark and filtered through Whatmann filter paper (no.41). An appropriate volume of this solution was transferred to 10 ml volumetric flask, dissolved and volume was adjusted to mark. The absorbances of the solutions were measured at 225.0 nm and 240.0 nm against blank. Concentration of each drug was obtained by solving the simultaneous equation [Table 3].

Table-3: Application of Proposed Method for Analysis of Tablet Formulation.

Drugs	Amount taken [µg/ml]	Amount found [µg/ml]	Amount found [%]
ATL	50	50.23	100.47
	50	50.29	100.59
	50	49.97	99.94
	50	50.00	100.00
	50	50.02	100.05
	Mean ± S.D.	50.10 ± 0.1468	100.21 ± 0.2977
	% R.S.D.	0.2930	0.2970
IND	2.5	2.49	99.98
	2.5	2.51	100.73
	2.5	2.49	99.73
	2.5	2.49	99.73
	2.5	2.49	99.98
	Mean ± S.D.	2.49 ± 0.0089	100.03 ± 0.4107
	% R.S.D.	0.3574	0.4105

4. Validation of proposed method

The method was validated in terms of accuracy, precision and ruggedness.

4.1 Accuracy

To assess the accuracy of proposed method, recovery experiment was performed. To the preanalyzed sample solution of ATL and IND, a known amount of standard drug solution was added that is 2 µg/ml and absorbance were recorded. The % recovery was then calculated [Table4].

Table-4: Results of Recovery Studies of ATL and IND.

Drugs	Initial amount [µg/ml]	Amount added [µg/ml]	% Drug recovered [n=3]	% R.S.D.
ATL	20	0	99.60	0.2300
	20	2	99.70	0.4363
	20	2	99.43	0.2051
	20	2	99.56	0.0753
IND	1	0	99.91	0.3640
	1	2	99.76	0.4860
	1	2	99.76	0.2429
	1	2	99.62	0.3650

4.2 Precision

Precision of the method was assessed by repeatability; determined by analyzing 30 µg/ml of ATL and1.5 µg/ml IND of drug solutions for five times; results was recorded [Table 5]. Method precision was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing ATL and IND in the concentration range of 6, 8 and 10 µg/ml for three times in the same day. Inter-day precision was determined by analyzing the same concentration of solutions daily for three days, results were recorded [Table 6].

Table-5: Results of Repeatability Studies of ATL and IND.

Drugs	Amount taken [µg/ml]	Amount found [µg/ml] [n=5]	% R.S.D.
ATL	30	29.89 ± 0.0502	0.1679
IND	1.5	1.52 ± 0.0017	0.1161

Table-6: Results of Intra-day and Inter-day Precision of ATL and IND

Drugs	Concentration [µg/ml]	Intra-day amount found [µg/ml] [n=3]	% R.S.D.	Inter-day amount found [µg/ml] [n=3]	% R.S.D.
ATL	6	5.98 ± 0.0600	1.0033	06.01 ± 0.0300	0.4991
	8	7.98 ± 0.0045	0.0563	08.00 ± 0.0624	0.7800
	10	9.98 ± 0.0416	0.4168	09.98 ± 0.0862	0.8637
IND	6	5.99 ± 0.0011	0.0183	6.00 ± 0.0173	0.2883
	8	8.01 ± 0.0015	0.0187	8.03 ± 0.0173	0.2154
	10	9.98 ± 0.0057	0.0571	9.97 ± 0.0346	0.3470

4.3 Ruggedness

Ruggedness of the method was determined by analysis of aliquots from homogeneous slot by two analyst using same operational and environmental conditions [Table 7].

Table-7: Results of Ruggedness of ATL and IND

Drug	Amount taken [µg/ml]	Analyst I [n=3]	% R.S.D.	Analyst II [n=3]	% R.S.D.
ATL	30	99.83 ± 0.4457	0.4464	99.90 ± 0.6361	0.6367
IND	1.5	99.60 ± 0.200	0.4784	99.49 ± 0.4849	0.4873

RESULTS AND DISCUSSION

In this simultaneous equation method, the overlain spectra of drugs showed the λmax of 225.0 nm and 240.0 nm for ATL and IND respectively. Both the drugs obeyed linearity range 6-30 µg/ml and 0-10 µg/ml correlation coefficient (r2) were found to be <1 in both cases. The absorptivity values were calculated and along with absorbances, these values were submitted in equation (1) and (2) to obtain concentration of drugs. The percentage purity of drugs in binary mixture was found to be 99.60 ± 0.1581 % for ATL and 99.85 ± 0.3466 %

for IND. The percentage purity of drugs in combined dosage form was found to be 100.21 ± 0.2977 % for ATL and 100.03 ± 0.4107 % for IND. The accuracy of the method was determined by performing recovery study by standard addition method. The % recoveries were found near to 100 for both drugs. The experiment was repeated three times in a day for intra-day and on three different days for inter-day precision. The method was found to be precise as % RSD for intra-day and inter-day precision were found to be <2. The method was found to be rugged as the percentage purity of the drugs determined by two different analysts were 99.83 ± 0.4457 for ATL, 99.60 ± 0.2000 for IND and 99.90 ± 0.6361 for ATL, 99.49 ± 0.4849 for IND.

CONCLUSION

The proposed method for simultaneous determination of ATL and IND is a suitable technique for reliable analysis of the commercial formulation containing combination of these drugs and may be successfully applied in control laboratories for their determination in combined dosage form. The results of validation showed that the proposed method is simple, linear, accurate and precise and it can be employed in routine assay of ATL and IND in combined dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Dr. S. P. Pawar, Principal, P.S.G.V.P.M's College of Pharmacy, Shahada for providing necessary facilities and Mr. V. H. Bankar, Asst. Professor, P.S.G.V.P.M's College of Pharmacy, Shahada for their valuable guidance. The authors are also thankful to Suchem Lab, Ahmadabad and Supra chemicals, Thane for providing gift samples of drugs.

References

1. A. Kar, "Medicinal Chemistry", 4th edition, New Age International Ltd.,2007, pp 466-470.
2. N. Erk, Journal of Pharmaceutical and Biomedical Analysis, Vol 26,2001 , pp43-52.
3. Y.K. Agrawal, F.D. Majumdar, Anal. Lett. Vol 28, 1995, p1691.
4. M.Y. Ebeid, B.A. Moussa, A.A. Nasr, F.A. Ashour, A.A. Malek, Egypt J. Pharm. Sci. Vol 35, 1994, p587.

5. M.V. Padval, H.N. Bhargava, J. Pharm. Biomed. Anal. Vol 11, 1993, p1033.
6. R.B. Miller, D. Dadgar, M. Lalande, J. Chromatogr. Biomed. Appl. Vol 614, 1993, p293.
7. Indian Pharmacopoeia, Volume 2, Ministry of Health and Family Welfare, Government of India, The Indian Pharmacopoeia Commission, Ghaziabad, 2007, pp749-750.
8. S.I. sa'sa, I.M. Jalal, H.S. Khalil, J., "Liquid Chromatography", Vol 8, 1988, pp1673–1696.
9. A.C. Keech, P.M. Harrison, A.J. Mclean, J. Chrom. Biomed. Appl. Vol 70, 1988. pp 234–236.
10. A.P. Argekar, S.G. Powar, Journal of Pharmaceutical and Biomedical Analysis, Vol 21, 2000, pp1137–1142.
11. J. Huang, J. Jin, Zhongguo yiyao gongye Zazhi, Vol 1, 1989, pp19–20.
12. S. Wong, Yaowu fenxi Zazhi, Vol 2, 1990, pp110–111.
13. S.V. Erram, H.P. Tipnis, Indian Drugs, Vol 9, 1993, pp460–467.
14. ICH, Q2 (R1), Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and methodology, International Conference on Harmonization (ICH), Geneva, Nov., 2005.

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