

# **Ion-Pairing Reverse-Phase High Performance Liquid Chromatography Method for Simultaneous Estimation of Atenolol and Indapamide in Bulk and Combined Dosage Form**

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*Baheti, et al.: Ion Pairing RP-HPLC of Antihypertensive Combination*

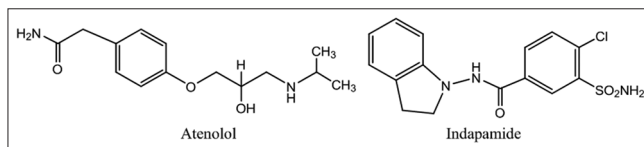
A sensitive, accurate, precise and validated ion-pairing reverse-phase liquid chromatographic method for the quantitative determination of atenolol and indapamide in bulk and tablet dosage form was developed. The proposed ion-pairing

reverse-phase high performance liquid chromatography method utilises  $C_{18}$  column with  $5\ \mu\text{m}$ ,  $150\times 4.6\ \text{mm}$  i.d. column and mobile phase consisting of 0.1% w/v solution of octane sulphonic acid, sodium salt and methanol (55:45 v/v), (pH 2.8) and ultraviolet detection at 235 nm. A linearity range of 1-250  $\mu\text{g}/\text{ml}$  and 1-25  $\mu\text{g}/\text{ml}$  for atenolol and indapamide, respectively, was obtained. The mean recoveries are 100.48 and 99.82% for atenolol and indapamide, respectively. The method was validated as per International Conference on Harmonization guidelines.

**Key words:** Atenolol, indapamide, ion-pairing, reverse-phase high performance liquid chromatography

Atenolol (ATN), chemically (R,S)-4-(2-hydroxy-3-isopropyl-aminopropoxy) phenyl acetamide, is a beta-adrenoceptor antagonist widely used in the treatment of high blood pressure, arrhythmias and angina pectoris. It is official in the Indian Pharmacopoeia<sup>[1]</sup> and the British Pharmacopoeia<sup>[2]</sup>. Indapamide (IND), chemically (4-chloro-N-(2-methyl-1-indoliny)-3-sulphamoyl benzamide) is a nonthiazide indole derivative of chlorosulphonamide has an antihypertensive action causing a drop in systolic, diastolic and mean blood pressure<sup>[3]</sup>. The chemical structures of ATN and IND are given in fig. 1. Analysis by various methods like ultraviolet (UV), high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) has been reported for analysis of these drugs individually or in combination<sup>[4-7]</sup>. The literature survey reveals three reverse-phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of ATN and IND<sup>[8-10]</sup>.

Sane *et al.*<sup>[8]</sup> reported the HPLC determination of ATN and IND by quaternary mobile phase consisting of methanol:water:glacial acetic acid:diethylamine in the ratio of 63:37:01:0.15 v/v. The retention time observed was 1.6 and 3.0 min for ATN and IND, respectively, at 241nm using a UV detector. They have used  $C_{18}$  ( $10\ \mu\text{m}$ ) column  $300\times 4\ \text{mm}$  column. Rani *et al.*<sup>[9]</sup> reported RP-HPLC method for the simultaneous determination of ATN and IND from bulk and formulations. Chromatographic separation was achieved isocratically on a Waters  $C_{18}$  column ( $250\times 4.6\ \text{mm}$ ,  $5\ \mu\text{m}$  particle size) using a mobile phase, methanol and water (adjusted to pH 2.7 with 1% orthophosphoric acid) in the ratio of 80:20. The flow rate was 1 ml/min and eluent was detected at 230 nm. The retention time of ATN and IND were 1.76 min and 3.40 min, respectively. The HPLC method reported by Kher *et al.*<sup>[10]</sup> utilises



**Fig. 1: Structure of atenolol and indapamide**

$C_{18}$ ,  $250\times 4.6\ \text{mm}$  i.d.,  $5\ \mu\text{m}$  particle size column. The flow rate of the mobile phase was adjusted to 0.8 ml/min and the injection volume was 20  $\mu\text{l}$  and detection was carried out at 241 nm. None of the reported methods have used the ion-pairing reagent for the analysis and resolution of between ATN and IND. Hence, the proposed work aims at developing simple, precise and rapid RP-HPLC method for better resolution between the analytes. The method uses the ion-pairing agent for the better resolution between analytes of interest.

Working standards of ATN (purity 99.98%) and IND (purity 99.80%) were gift samples from SRS Pharmaceuticals Limited, Mumbai, India and were used without further purification. Combination drug product of ATN and IND (Label claim: ATN 50 mg and IND 2.5 mg), Aten D tablets of Zydus Cadila Ltd., India, were purchased from a local Pharmacy. Methanol (HPLC Grade, Merck Ltd., India), orthophosphoric acid (AR Grade, Loba Chemicals Ltd., Mumbai, India), octane sulphonic acid (HPLC Grade, Rankem, Faridabad, India), sodium hydroxide and hydrochloric acid (AR Grade, Qualigens Fine Chemicals, Mumbai, India) were used in analysis.

The HPLC system JASCO make consisted of binary pump PU2080 Plus, Rheodyne universal injector and Jasco UV2075 Plus UV/Vis detector with LC-Net BORWIN 1.2 software. The chromatographic separations were performed using Xterra<sup>®</sup>  $C_{18}$  column with  $5\ \mu\text{m}$ ,  $150\times 4.6\ \text{mm}$  i.d. column. Mobile phase consisted of 0.1% w/v solution of octane sulphonic acid sodium salt in water: methanol (55:45 v/v). The apparent pH of mobile phase was adjusted to  $2.8\pm 0.1$  with

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orthophosphoric acid, filtered through 0.45  $\mu\text{m}$  nylon filter and degassed in ultrasonic bath for 15 min prior to use. Wavelength was selected by scanning standard solutions of both drugs over 200-400 nm using Jasco model V-630 double beam UV/Vis spectrophotometer with a pair of 10 mm matched quartz cells. Measurements were made with injection volume 20  $\mu\text{l}$  and UV detection at 235 nm, as both the components showed reasonably good absorbance at this wavelength.

The standard stock solutions 1.0 mg/ml of ATN and IND were prepared separately by dissolving working standards in mobile phase, which was sonicated for 15 min and further diluted with the same solvent. Standard calibration solutions of ATN and IND having concentrations in the range of 1-250  $\mu\text{g/ml}$  and 1-25  $\mu\text{g/ml}$ , respectively, were prepared by diluting stock solutions with mobile phase.

Twenty tablets were weighed; powdered and mass equivalent to 50 mg of ATN and 2.5 mg of IND was taken in a 50 ml volumetric flask and dissolved in mobile phase. The solution was sonicated for 15 min and further made up to volume with mobile phase. The solution was filtered through Millipore Millex HN Nylon 0.45- $\mu\text{m}$  syringe filter and 1 ml aliquot of filtrate was diluted to 10 ml with mobile phase to obtain concentration of 100  $\mu\text{g/ml}$  of ATN and 5  $\mu\text{g/ml}$  IND. The method was validated for linearity, accuracy, precision, specificity and robustness as per ICH guidelines<sup>[11]</sup>.

The proposed method utilises mobile phase consisting of 0.1% w/v solution of octane sulphonic acid sodium salt in water:methanol (55:45 v/v) of pH 2.8 $\pm$ 0.1. The mobile phase was found to be ideal as it resolved the peaks of ATN ( $t_R$  2.0 min) and IND ( $t_R$  6.1 min), with clear line separation. Octane sulphonic acid was used as the ion-pairing agent. UV detection wavelength at 235 nm, injection volume 20  $\mu\text{l}$  and ambient temperature for column was found to be best for analysis. The typical chromatogram is given in fig. 2.

The assay results of ATN and IND in tablet were comparable with the value claimed by the label. The results presented in Table 1 indicate the suitability of the method for routine analysis of ATN and IND from their combination drug products. The proposed method has been validated for linearity,

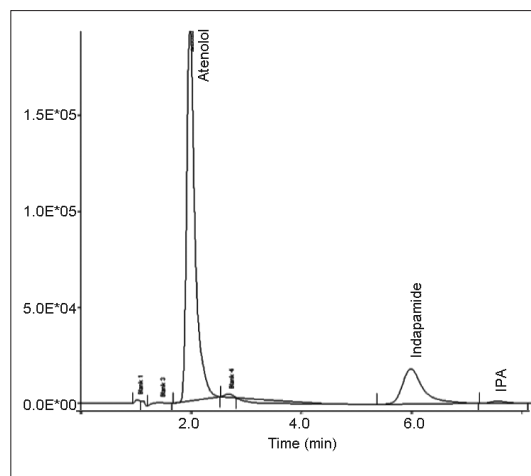


Fig. 2: Typical chromatogram of atenolol and indapamide tablet solution

**TABLE 1: ANALYSIS OF ATENOLOL AND INDAPAMIDE BY ION-PAIRING REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD**

Component	Label claim	Amount found	% Label claim* $\pm$ RSD
ATN	50 mg	50.1 mg	100.2 $\pm$ 0.25
IND	2.50 mg	2.48 mg	99.2 $\pm$ 0.60

\*n=6 experiments. ATN-Atenolol, IND-Indapamide

**TABLE 2: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD VALIDATION OF ATENOLOL AND INDAPAMIDE**

Parameters	Atenolol	Indapamide
Linearity	1-250 $\mu\text{g/ml}$	1-25 $\mu\text{g/ml}$
Equation of line Y	28644.1X	63437.98X
$r^2$	0.999	0.998
Limit of detection	0.4 $\mu\text{g/ml}$	0.33 $\mu\text{g/ml}$
Limit of quantitation	1.0 $\mu\text{g/ml}$	1.0 $\mu\text{g/ml}$

**TABLE 3: SYSTEM SUITABILITY PARAMETERS**

Parameter	Atenolol	Indapamide
Retention time (min)	2.0	6.1
Tailing factor	0.9	1.1
Theoretical plates	3298	2740
Resolution	-	7.8
% RSD	0.08	0.55

RSD-Relative standard deviation

accuracy, precision, specificity and robustness. The results of validation study are presented in Table 2. Characteristic parameters for system suitability are given in Table 3. Repeatability of measurements of peak area was carried out using three replicates of concentration (100 and 5  $\mu\text{g/ml}$  of ATN and IND, respectively). The intra- and inter-day variation of the method was carried out for three concentration levels. The low % relative standard deviation (RSD) values of within a day, day-to-day variations and analyst-to-analyst variation for ATN and IND revealed

**TABLE 4: INTRA-AND INTER-DAY REPEATABILITY OF DEVELOPED METHOD FOR ATENOLOL AND INDAPAMIDE**

	% ATN	% RSD*	% IND	% RSD*
Intra-day precision	102.4	0.59	101.3	0.81
Inter-day precision*	102.6	0.81	103.8	0.76

\*n=3 experiments. ATN=Atenolol, IND=Indapamide, RSD-Relative standard deviation

**TABLE 5: ROBUSTNESS RESULTS**

Factor	Level	% ATN	% RSD*	% IND	%RSD*
Flow rate (ml/min) ( $\pm 0.1$ ml)	0.9	99.1	0.87	99.9	0.06
	1.1	98.8	0.20	100.1	0.22
Methanol ( $\pm 2\%$ )	44.1 ml	100.7	0.44	100.2	0.25
	45.9 ml	98.7	0.58	99.8	0.87
Wavelength ( $\pm 2$ nm)	233 nm	99.4	0.53	100.0	0.77
	237 nm	99.8	0.47	99.3	0.28
pH ( $\pm 0.2$ )	2.6	99.9	0.78	101.1	0.96
	3.0	100.3	0.18	100.0	0.22

\*n=3 experiments. ATN=Atenolol, IND=Indapamide, RSD-Relative standard deviation

**TABLE 6: RECOVERY DATA**

Component	% Spiked	% Recovered $\pm$ RSD*
ATN	80	99.32 $\pm$ 0.28
ATN	100	99.50 $\pm$ 0.19
ATN	120	102.62 $\pm$ 0.62
IND	80	99.07 $\pm$ 0.15
IND	100	100.39 $\pm$ 0.23
IND	120	100.01 $\pm$ 0.85

\*n=3 experiments. ATN=Atenolol, IND=Indapamide, RSD-Relative standard deviation

that the proposed method is precise. The results of the intra- and inter-day repeatability and robustness study of the methods are presented in Tables 4 and 5, respectively.

Accuracy of the method was checked by recovery study using standard addition method, at three different concentration levels. The preanalysed samples were spiked with standard ATN and standard IND and the mixtures were analysed by the proposed method. Recovery of standard drugs added was found to be 99.32-102.62% for ATN and 99.07-100.39% for IND with the value of % RSD less than 2 indicating that the proposed method is precise for the simultaneous estimation of ATN and IND in combined drug products. Results of recovery study are shown in Table 6.

The proposed method was found to be advantageous over the reported methods because of better resolution of 7.78 of peaks of ATN and IND. The

precision and accuracy of method was within the expected pharmacopoeial limits with % RSD less than 2. From the above study, it can be concluded that the proposed method is novel, accurate and precise. The better resolution between ATN and IND and validation of the method indicates that it can be adopted for routine dissolution testing and purity determination of tablets containing these Active Pharmaceutical Ingredients.

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