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Simultaneous Estimation of Atenolol and Indapamide in Combined Tablet Dosage Form using RP-HPLC

Nitin Dubey* • **Dinesh K. Jain** • **Balvant Solanki**

College of Pharmacy, IPS Academy, Rajendra Nagar A.B. Road, Indore [Madhya Pradesh], India

Corresponding author: * **nitindubeympharm@yahoo.com**

ABSTRACT

A simple, accurate, reproducible and sensitive method for the determination of Atenolol and Indapamide was developed and validated. Atenolol and Indapamide were separated using a C-18 octadecylsilane (ODS) column (250 × 4.6 mm, id., 5 µm) with a flow rate of 1.2 ml/min. The mobile phase was methanol: acetonitrile: water (45: 25: 30 v/v/v, pH 3.5 adjusted with orthophosphoric acid), at 226 nm. The retention time of Atenolol and Indapamide was 2.34 and 4.34 min, respectively. The linearity range for Atenolol and Indapamide was 2-20 µg/ml and 5-45 µg/ml, respectively. Recovery was 100.12 ± 0.0275 for Atenolol and 99.98 ± 0.669 for Indapamide. The development method was suitable and statistically validated for all parameters.

Keywords: RP-HPLC, validation
Abbreviations: RP-HPLC, reversed phase high performance liquid chromatography

INTRODUCTION

Atenolol is chemically known as 2-[4-[2-hydroxy-3-(propen-2-ylamino)propoxy] phenyl] acetamide and belongs to the class of compounds known as anti-hypertensive. It is official in the Indian Pharmacopoeia (Anonymous 1998). It competes with sympathomimetic neurotransmitters such as catechol amines for binding at β(1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation.

Indapamide is chemically known as 4-chloro-*N*-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl benzamide and is listed in the official British Pharmacopoeia (Anonymous 2004), and belongs to the class of compounds known as anti-hypertensive and diuretics. The mechanism of action of Indapamide is not clear. It appears to act principally on the distal convoluted tubules of the nephron. The drug enhances the excretion of sodium, chloride, and water by inhibiting the transport of sodium ions across the renal tubule. The hypovolemic action of Indapamide is believed to be responsible for the drug's beneficial cardiovascular effects (Foltea *et al.* 2005).

The chemical structures of Atenolol and Indapamide are shown in **Fig. 1**.

A literature survey revealed that the assay of the Atenolol in pure and dosage forms is official in the Indian and British Pharmacopoeias (Anonymous 1998, 2004). Apart from these Pharmacopeias, several analytical methods have been reported for the determination of Atenolol in biological fluid using a spectrophotometric method (Abreu *et al.* 2003; Kasture *et al.* 2005), including column high-performance liquid chromatography (HPLC) and a degradation study.

HPLC for determination of Indapamide from tablet formulation is official in the British Pharmacopoeia (Anonymous 2004). Several analytical methods that have been reported for the determination of Indapamide in biological fluids and in bulk as well as pharmaceutical formulations, including HPLC (Foltea *et al.* 2005; Suo *et al*. 2005; Gao *et al*. 2006).

This paper describes a simple, accurate, precise, and sensitive simultaneous estimation of Atenolol and Indapamide in combined tablet dosage form using reversed-phase (RP) HPLC. The proposed methods were validated according to the International Conference on Harmonization (ICH) Guidelines (2005).

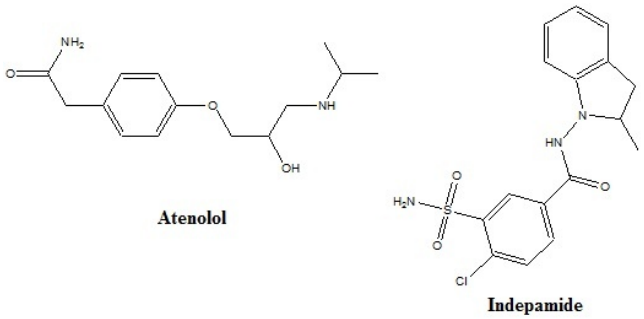

Fig. 1 Chemical structures of Atenolol and Indapamide.

MATERIALS AND METHODS

Drugs and chemicals

Acetonitrile (HPLC grade), water (HPLC grade) and methanol (HPLC grade) were purchased from Merck (Mumbai, India). All other reagents used were of analytical grade for HPLC. Standard bulk drug samples of Atenolol (99.46% pure) and Indapamide (99% pure) were provided as a pharmaceutical dosage in the form of Aten-D tablets labeled to contain Atenolol 50 mg and Indapamide 2.50 mg/tablet (Zydus Medica, Gujrat, India).

Instrumental

An HPLC system consisting of LC 10 AT VP pump equipped with diode array detector (Shimadzu, Japan) and Luna C18 (4.6 mm id) column and class M10A software version 1.6 was used. A Rheodyne (Rohnert Park, CA) injector with 20 µL loop was used for injecting the sample.

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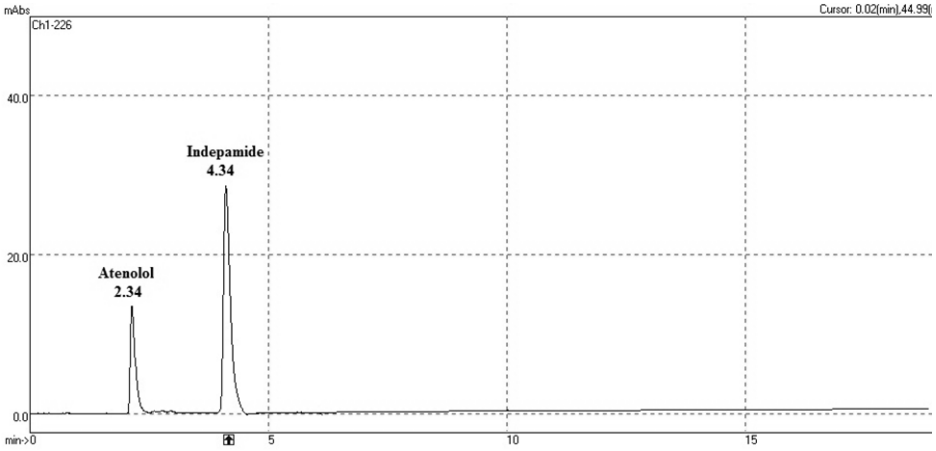

Fig. 2 Chromatogram of Atenolol and Indapamide in tablet dosage form.

Chromatographic method (RP-HPLC)

In RP-HPLC, separation and analysis of Atenolol and Indapamide were carried out on a Luna C_{18} column (4.6 mm id) with the diode array detector set at 226 nm. A mobile phase consisting of methanol: acetonitrile: water (45: 25: 30, v/v/v; and pH 3.5 adjusted with orthophosphoric acid and filtered through a 0.2 µm membrane filter, degassed) was used with a flow rate of 1.2 mL/min. The method, as described below in a-f was according to Barman *et al.* (2007) and Bharadwaj *et al.* (2007).

(a) Standard stock solutions: Standard stock solutions containing 100 µg/mL Atenolol and 100 µg/mL Indapamide were prepared by dissolving the pure drugs separately in the mobile phase.

(b) Preparation of calibration curves: Aliquots of 2, 4, 6, 8 and 10 mL of stock solution of Atenolol and 5, 10, 15, 20 and 25 mL stock solution of Indapamide were transferred into a series of 10-mL volumetric flasks and the volume was made up to the mark with the mobile phase. Each solution was injected and a chromatogram was recorded. Retention time for Atenolol and Indapamide was 2.34 and 4.34 min, respectively. The peak area of Atenolol and Indapamide were noted, and respective calibration curves were plotted as peak area against concentration of each drug.

(c) Procedure for analysis of tablet formulation: 20 tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg Atenolol and 2.5 mg Indapamide was weighed and transferred to a 50-mL volumetric flask containing about 35 mL mobile phase, ultrasonicated for 5 min, and the volume was made up to the mark with the mobile phase. The solution was filtered through Whattman (Florham Park, NJ) No. 41 paper, 0.2 mL filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The tablet sample solution was injected, the chromatogram was obtained and the peak areas were recorded. A representative chromatogram is given in **Fig. 2**. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves.

(d) Recovery studies: Accuracy of the method were analyzed by recovery studies carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and 120%.

(f) Precision: Precision of the method was checked by 3 replicate readings at 3 concentration levels of within range expressed as RSD values phase.

Statistical analysis

Means, standard deviation (SD), relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003.

RESULTS AND DISCUSSION

For RP-HPLC, chromatographic conditions were optimized to achieve the best resolution and peak shape for Atenolol and Indapamide. Different mobile phases containing methanol, acetonitrile and water were examined (data not shown), and the mobile phase methanol: acetonitrile: water (45: 25: 30, v/v/v, pH 3.5 adjusted with orthophosphoric acid) was selected as optimal for obtaining well-defined and resolved peaks. The instrument was precise indicated by a %RSD < 1.

Straight line calibration curves were obtained for Atenolol and Indapamide. **Table 1** summarizes linear regression equation, correlation coefficient, SD, and limit of detection (LOD) and limit of quantitation (LOQ) values for method all the statistical validation parameters were satisfactory as per the ICH Guidelines (2005). System suitability parameters for RP-HPLC are listed in **Table 2**.

The proposed methods were also evaluated in the assay of commercially available tablets containing Atenolol and Indapamide. Six replicates were performed on an accurately weighed amount of tablets. For Atenolol, recovery (mean%, ± SD, n = 6) was 100.12 ± 0.29. For Indapamide it was 99.98 ± 0.50 (**Table 3**).

For Atenolol, the recovery ranged from 99.75 to

Table 1 Regression analysis of calibration curve using RP-HPLC.

Parameters	Atenolol	Indapamide
λ_{max}	226	242
Linearity range µg/mL	2-20	5-45
Correlation coefficient	0.9938	0.9961
Linear regression equation [b]		
Intercept	0.0041	2056.7
Slope	30531	62490
SD [c]	4594.43	19741.11
Detection limit, µg/mL	0.49	1.042
Quantitation limit, µg/mL	1.504	3.15

[a] Detection wavelength for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL).
[c] SD = standard deviation.

Table 2 System suitability parameters for RP-HPLC method.

Parameters	Atenolol	Indapamide
Calibration curve range, µg/mL	2-10	5-25
Theoretical plate number	2258	4901
HETP [a]	0.0110	0.0051
Tailing factor	2.08	1.98
Capacity factor (k')	-	0.85
Resolution	-	8.99

[a] HETP = Height equivalent to theoretical plate, cm

Table 3 Results of analysis of commercial formulation.

Sample	Label claim, mg/tablet		% Claim, estimated [a]	
No.	Atenolol	Indapamide	Atenolol	Indapamide
1	50	2.5	99.68± 0.52	100.20 ± 1.64

[a] Mean ± Relative standard deviation, n = 6.

Table 4 Recovery studies of Atenolol and Indapamide using RP-HPLC.

Drug	Concentration taken µg/ml for methods	Concentration added µg/ml for methods	Total concentration found µg/ml	Recovery % [a]
Atenolol	20	16	35.91	99.75 ± 0.0642
	20	20	40.08	100.02 ± 0.275
	20	24	44.26	100.60 ± 0.100
Indapamide	1	0.8	1.79	99.71 ± 0.189
	1	1	2.00	100.40 ± 0.669
	1	1.2	2.19	99.83 ± 0.354

[a] mean ± relative standard deviation (n = 3).

100.60% with RSD values ranging from 0.1 to 0.5%. For Indapamide, the recovery ranged from 99.72 to 100.42% with RSD values ranged from 0.03 to 0.7%. Results of recovery studies are reported in **Table 4**.

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