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

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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 β1-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 Pharmacopoeias, 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 Indapa-
Chromatographic method (RP-HPLC)

In RP-HPLC, separation and analysis of Atenolol and Indapamide were carried out on a Luna C18 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, 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. 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%.

(e) 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 < 1.2%

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 method 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 100.20 ± 0.29. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves.
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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| 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            | 99.75 ± 0.189 |
|                 | 1               | 1               | 2.00            | 100.40 ± 0.669 |
|                 | 1               | 1.2             | 2.19            | 99.83 ± 0.354 |

* mean ± relative standard deviation (n = 3).