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Simultaneous enantioseparation and purity determination of chiral switches of amlodipine and atenolol by liquid chromatography

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ABSTRACT

A novel, selective and robust enantiospecific HPLC method was developed for simultaneous determination of amlodipine and atenolol enantiomers. Box–Behnken design was employed to identify the effect of factors (% ethanol, % diethylamine and flow rate) and their interactions on enantioresolution and analysis time. Chromatography was performed using mobile phase comprising acetonitrile, ethanol and DEA (92:8:0.2% *v*/*v*/*v*) delivered at a flow rate of 1.2 mL min^{-1} on a Lux Cellulose-4 column. The enantiomers were monitored at a wavelength of 240 nm and separation was achieved within 8 min. The method was validated in terms of specificity, linearity, accuracy, precision, limit of detection and quantification. The method was found to be linear ($R^2 \geq 0.991$), accurate (99.8–101.4%) and precise (%RSD ≤ 3%). Additionally, fractional factorial design was used to evaluate the robustness of the method and non-significant intervals for mixture related factors were established using contour profiling. Furthermore, the pertinence of this validated method was established by analyzing three different commercially available formulations. The obtained results confirmed that the proposed method can be extended for routine enantiopurity assay of amlodipine and atenolol in pharmaceutical formulations.

1. Introduction

Chirality and its influence on molecular interactions is one of the fundamentals of life itself. It is a prominent feature of most biological processes, and the enantiomers of a bioactive molecule often exhibit different pharmacological activity. Therefore, drug regulatory agencies from USA, European Union, Canada, China and Japan have issued guidelines indicating that preferably only the active enantiomer of a chiral drug should be bought to market [1]. Henceforward, the majority of new chiral drugs will be developed as new single enantiomer chemical entities or obtained by a chiral switch. The assessment of enantiopurity of chiral switches aids in avoiding undesirable side effects and assure its therapeutic efficacy. Lately, development of direct chiral HPLC methods by employing polysaccharide chiral stationary phases (CSPs) in polar organic (PO) mode has gained considerable attention [2,3]. It offers advantages of being fast, efficient and cost-effective in chiral analysis. Therefore, in this study chiral separation was performed using polysaccharide CSPs in PO mode.

Amlodipine (AML), (Fig. 1) designated as (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate belongs to class of dihydropyridine type calcium channel blockers. AML possesses one stereogenic center at C-4 position of dihydropyridine ring and exist as enantiomeric twins. Levamlodipine (*S*-AML) is the *S*-enantiomer of (±)-AML, the eutomer, which was reported to be 1000 times more pharmacologically active than *R*-AML [4]. Levamlodipine products may contain traces of *R*-AML, as a chiral impurity resulting from levamlodipine synthesis. In recent years, there are several studies about the chiral separation of (±)-AML in various matrices by capillary electrophoresis [5], indirect chiral HPLC [6,7] and direct chiral HPLC methods [8–11]. However, in literature, there were only two HPLC methods reported for assessment of enantiomeric purity of *S*-AML. Dossou et al. [11]. proposed a direct chiral HPLC method for determination of enantiomeric purity of *S*-AML. The method mainly focuses on describing reversal order of enantiomer elution and enantioresolution using formic acid as additive. Nevertheless, the method was not applied for the real samples. Ansari et al. [12]. reported a reversed phase liquid chromatographic method for quantitation of enantiopurity of *S*-AML. The (±)-AML was separated on an ovomucoid protein column using a mobile consisting buffer and acetonitrile. However, this method suffers from poor enantioresolution (Rs = 1.43) and lack of sensitivity (LOD = 0.5 μg mL^{-1}).

Atenolol (ATN), (Fig. 1) designated as (*RS*)-2-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy]phenyl]acetamide belongs to class of β-blockers, used mainly in treatment of cardiovascular dis-

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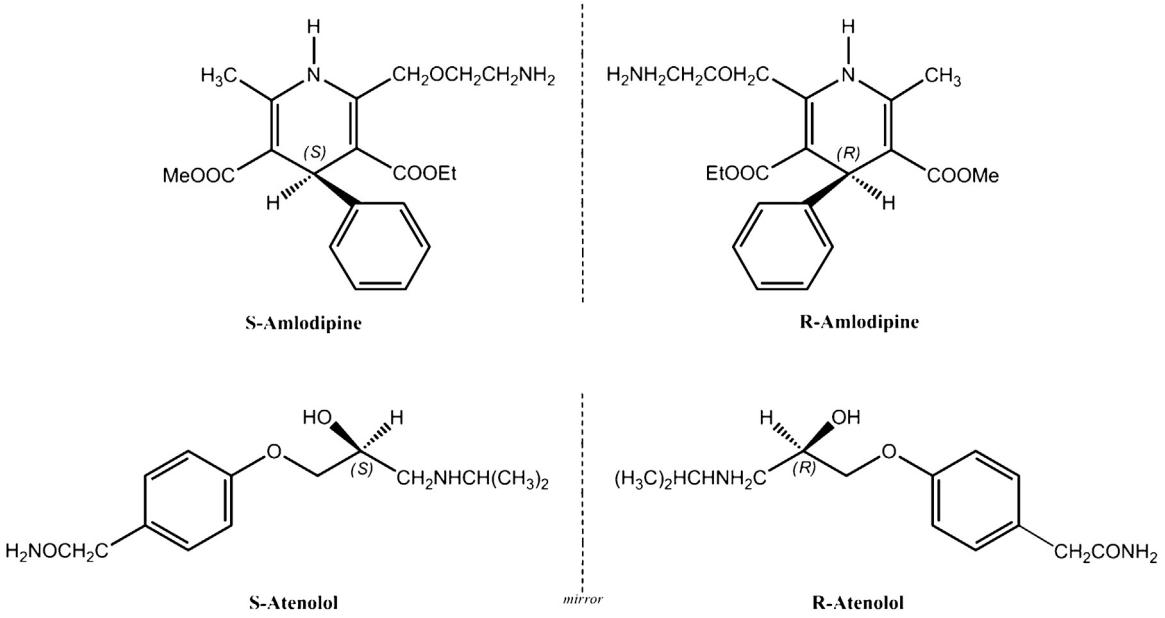

Fig 1. Chemical structures of amlodipine and atenolol enantiomers.

eases. In ATN, the stereogenic center resides at the *N*-*N*-dimethyl propoxy side chain, resulting in existence of enantiomeric pair. *S*-Atenolol (*S*-ATN) is the *S*-enantiomer of (±)-ATN, the eutomer, which alone is responsible for the β-adrenoceptor blocking activity [13]. In literature, many studies were described for quantitation of ATN enantiomers in various matrices by capillary electrophoresis [14], indirect chiral HPLC [15,16] and direct chiral HPLC methods [17–19]. Eaga et al. [20]. reported a method for determination of atenolol enantiomers in pharmaceutical formulations. The atenolol enantiomers were separated on a chiral AGP column by employing a mobile phase comprising sodium phosphate buffer and methanol in 95:5 *v*/*v*. The method suffers from drawbacks like poor enantioresolution (Rs < 1.5) and more tailing. Moreover, protein based chiral columns are comparatively expensive, have low column life and offer low separation efficiencies [21]. Furthermore, none of the reported methods were utilized for chiral quality control of *S*-ATN in enantiopure formulation.

Recently, formulations based on chiral switches of (±)-AML and (±)-ATN were launched in Indian market [22]. The fact that there are no reported methods available that can simultaneous quantify (±)-AML and (±)-ATN enantiomers, motivated us to develop an enantiospecific HPLC method for enantiopurity control of *S*-AML and *S*-ATN in marketed formulations. Hence, in this study, we propose a simple, sensitive and robust method for simultaneous determination of AML and ATN enantiomers and applied for enantiopurity assay of Asomex [*S*-AML], Atpure [*S*-ATN] and Asomex AT [*S*-AML & (±)-ATN].

Additionally, in this study, chromatographic optimization was performed using Design of Experiments (DoE) methodology. DoE approach offers advantage of generating large amount of data from minimum number of experiments and helps in understanding the factors interaction with the responses [23].

2. Experimental

2.1. Apparatus

The chromatographic separation was achieved on an HPLC system equipped with two LC 20 AD solvent delivery pumps and a SPD-M20A PDA detector (Shimadzu Corporation, Kyoto, Japan). Chromatograms were recorded and processed using LC Solutions® software (Version 1.11SP1). Chromatographic separation was performed on a cellulose tris(4-chloro-3-methylphenylcarbamate) (Lux Cellulose-4, 250 × 4.6 mm, 5 μm) column Phenomenex® (Torrance, CA, USA). Other chiral selectors tested in this study were, cellulose tris(3,5-dimethyl phenylcarbamate) (Lux Cellulose-1, 250 × 4.6 mm, 5 μm) and cellulose tris(3-chloro-4-methylphenyl carbamate) (Lux Cellulose-2, 250 × 4.6 mm, 5 μm).

2.2. Chemicals and reagents

Working standards of AML (99.5%) and ATN (99.2%) were procured from Cipla Limited., (Mumbai, India). Pure enantiomers of *S*-AML (99.8%) and *S*-ATN (99.7%) were obtained from Emcure Pharmaceuticals, (Pune, India). Ethanol (EtOH) and acetonitrile (MeCN) of HPLC grade and diethylamine (DEA) of analytical reagent grade were purchased from M/S SD Fine Chemicals (Mumbai, India). The tablets Asomex (*S*-AML, 5 mg), Atpure (*S*-ATN, 25 mg) and Asomex AT (*S*-AML, 2.5 mg and (±)-ATN, 50 mg) from Emcure Pharmaceuticals (Pune, India) were procured from local pharmacy.

2.3. Chromatographic conditions

Chromatographic analysis was performed by using mobile phase comprising a mixture of MeCN, EtOH and minor quantities of DEA. UV detection of the analytes was performed at 240 nm. The mobile phase was prepared by mixing the appropriate quantities of MeCN with EtOH, followed by addition of minor amounts of DEA to the mixture as per design. All mobile phases were passed through 0.45 μm membrane filter Gelman Science (Mumbai, India) and degassed in an ultrasonic bath before use.

2.4. Design of experiments

Experimental runs were performed according to the matrix of experiments generated using Box–Behnken design (BBD). BBD's belongs to a class of rotatable or nearly rotatable second order designs. In this design, the treatment combinations are at the midpoints of edges of the design space and at the center and require three levels of each factor [23]. BBD's are extremely beneficial when