Journal of Chromatography A, 1467 (2016) 363–371

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Enantioseparation and impurity determination of ambrisentan using cyclodextrin-modified micellar electrokinetic chromatography: Visualizing the design space within quality by design framework

S. Orlandini [a], B. Pasquini [a], C. Caprini [a], M. Del Bubba [b], M. Douša [c], S. Pinzauti [a], S. Furlanetto [a,*]

[a] Department of Chemistry "U. Schiff", University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino, Florence, Italy
[b] Department of Chemistry "U. Schiff", University of Florence, Via della Lastruccia 3-13, 50019 Sesto Fiorentino, Florence, Italy
[c] Zentiva, k.s. Praha, a Sanofi Company, U Kabelovny 130, 102 37 Praha 10, Czech Republic

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 8 June 2016
Accepted 30 June 2016
Available online 1 July 2016

Keywords:
Ambrisentan
Capillary electrophoresis
Chiral separation
Experimental design
Impurities
Quality by design

ABSTRACT

A capillary electrophoresis method for the simultaneous determination of the enantiomeric purity and of impurities of the chiral drug ambrisentan has been developed following the Quality by Design principles. The selected separation system consisted of a micellar pseudostationary phase made by sodium dodecyl sulphate with the addition of γ-cyclodextrin. The effects of critical process parameters (capillary length, temperature, voltage, borate concentration, pH, sodium dodecyl sulphate concentration, γ-cyclodextrin concentration) on enantioresolution of ambrisentan and analysis time were extensively investigated by multivariate strategies involving a screening phase and Response Surface Methodology. The Design Space was defined with a desired probability level $\pi \geq 90\%$, and the working conditions, with the limits of the Design Space, corresponded to the following: capillary length, 64.5 cm; temperature, 22 °C; voltage, 30 kV (26–30 kV); background electrolyte, 100 mM borate buffer pH 9.20 (8.80–9.60), 100 mM sodium dodecyl sulphate, 50 mM (43–50 mM) γ-cyclodextrin. A Plackett-Burman design was applied for robustness testing, and a method control strategy was established. The method was fully validated according to the International Conference on Harmonisation guidelines and was applied to ambrisentan coated tablets.

1. Introduction

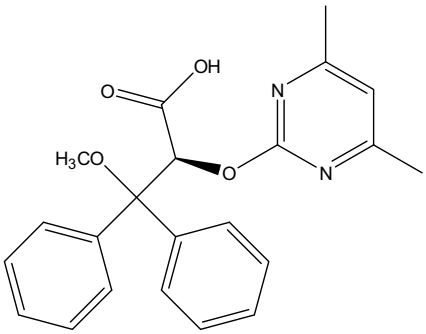
Pulmonary Arterial Hypertension (PAH) is a rare and progressive disease of the small pulmonary arteries, characterized by vascular proliferation and remodeling, which leads to an increase in pulmonary artery pressure and pulmonary vascular resistance, and, ultimately, right ventricular failure and death [1]. In PAH endothelial dysfunction leads to disturbances in the normal balance between endogenous vasoconstrictors/mitogens, such as endothelin (ET-1) and thromboxane A_2, and vasodilators/antimitotics, such as prostaglandin I_2 and nitric oxide [2]. Among these factors, ET-1, an endogenous peptide produced by vascular endothelial cells, is one of the most potent vasoconstrictors and smooth-muscle cells mitogens, and exerts its effects on ET_A and ET_B receptors. ET-1 receptor antagonists (ERAs) have emerged as a promising class of therapeutic agents in the treatment of PAH [1].

Ambrisentan (AMB) is an orally active and potent ET_A-selective ERA of the propanoic acid class, designated as an orphan drug for the treatment of PAH and chronic thromboembolic pulmonary hypertension. It is used in the treatment of patients with PAH classified as functional class II and III and in particular its efficacy has been shown in idiopathic PAH and in PAH associated with connective tissue disease. AMB is marketed as single (*S*)-enantiomer, representing the most active enantiomer, whose *in vivo* inversion to (*R*)-ambrisentan (*R*-AMB) is negligible [3]. *R*-AMB and other AMB impurities may be found in AMB bulk samples and dosage forms [4–7].

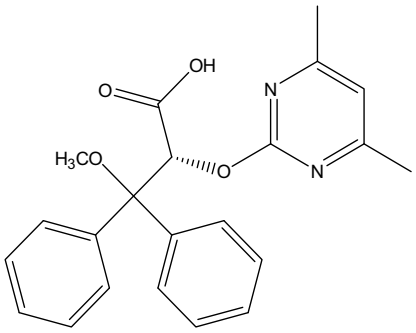
For the first time in the literature, the aim of the present study was to develop a CE method for the determination of AMB, its chiral impurity *R*-AMB and its main related substances in pharmaceutical formulations, following the new concepts of Quality by Design (QbD) [8]. CE has been already recognized as a suitable technique for the simultaneous determination of enantiomeric purity and of related substances of single-enantiomer drugs [9–14]. The four main impurities considered in this paper were AMB impurity 1 (I_1), AMB impurity 2 (I_2) and AMB impurity 3 (I_3), which were named

* Corresponding author.
E-mail address: sandra.furlanetto@unifi.it (S. Furlanetto).

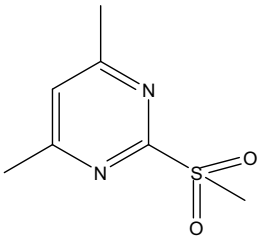
http://dx.doi.org/10.1016/j.chroma.2016.06.082
0021-9673/



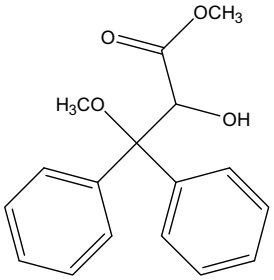
Ambrisentan (AMB)
(+)-(2*S*)-2-[(4,6-Dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid
CAS 177036-94-1



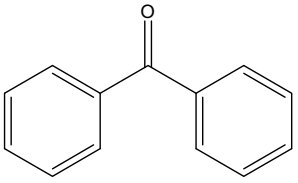
R-Ambrisentan (*R*-AMB)
(-)-(2*R*)-2-[(4,6-Dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid
CAS 1007358-76-0



Impurity 1 (I_1)
4,6-Dimethyl-2-methyl-sulfonyl-pyrimidine
CAS 35144-22-0



Impurity 2 (I_2)
2-Hydroxy-3-methoxy-3,3-phenylpropionic acid methyl ester
CAS 178306-47-3



Impurity 3 (I_3)
Diphenylmethanone
CAS 119-61-19

Fig. 1. Structures of ambrisentan and potential impurities.

according to their migration order, and the impurity enantiomer (*R*-AMB). Their structures are reported in Fig. 1.

Various analytical methods have been reported in the literature for the assay of AMB in bulk drug and pharmaceutical dosage forms, including spectrophotometry [15–17] and RP-HPLC [18]. Analysis of AMB in biological fluids has been performed by HPLC [19] and LC–MS/MS [20–26]. Stability indicating RP-HPLC methods for AMB have been set up in forced degradation studies [16,27–29]. Chromatography was used to analyze different sets of AMB impurities, including two [5] or more [4,6,7] of the four considered impurities. In particular, Douša et al. optimized an enantioselective HPLC method, which made it possible to simultaneously separate *R*-AMB, I_1, I_3, and (*S*)-2-hydroxy-3-methoxy-3,3-diphenylpropionic acid in less than 10 min [4]. The chiral separation of AMB and (*R*)-enantiomer was also carried out by LC using a cellulose based chiral stationary phase [30].

QbD is a concept of quality recently introduced in the pharmaceutical industry and its implementation has been the object of specific guidelines [8]. The application of QbD to analytical methods ensures a controlled risk-based development [31,32], representing an advantageous alternative approach to the Quality-by-Testing methodology [33]. The use of QbD in the development of a separation method for impurity assay has been recently reported with success, as it makes it possible an in-depth understanding of the parameters affecting analytical method performances [34–41]. QbD can also meet another great analytical challenge, represented by enantiomeric purity testing in quality control of single-enantiomer drugs, where large enantiomeric separation power and high sensitivity are required [42]. Up to now, the use of QbD in the development of enantiomeric separations has been reported only in polar organic solvent chromatography using a polysaccharide-based stationary phase for three drug candidates [43] and in CE for the determination of enantiomeric purity of levosulpiride [44].

In this study, the CE method development was carried out within QbD framework, with the target of establishing a Design Space (DS) where the analysis performances meet predefined quality attributes with a selected degree of probability. QbD scouting made it possible to select Micellar ElectroKinetic Chromatography (MEKC) with the addition of γ-cyclodextrin (γ-CD) as operative mode. The incorporation of QbD strategy in CE method development enabled dealing with optimization challenges in a rational and systematic way, providing the key for a better comprehension of the separation. A first screening phase was followed by Response Surface Methodology (RSM) [45], which allowed the DS to be defined in combination with Monte-Carlo simulations [46]. The method was validated according to International Conference on Harmonisation (ICH) guidelines [47] and then applied to real samples made by coated tablets.

2. Materials and methods

2.1. Chemicals and reagents

The reference standards of AMB and its impurities (*R*-AMB, I_1, I_2, I_3) were kindly furnished by Zentiva (Praha, Czech Republic).