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Chiral capillary zone electrophoresis in enantioseparation and analysis of cinacalcet impurities: Use of Quality by Design principles in method development

Benedetta Pasquini[a], Serena Orlandini[a,*], Mercedes Villar-Navarro[b], Claudia Caprini[a], Massimo Del Bubba[a], Michal Douša[c], Alessandro Giuffrida[d], Roberto Gotti[e], Sandra Furlanetto[a,*]

[a] Department of Chemistry "U. Schiff", University of Florence, Via U. Schiff 6, Via della Lastruccia 3-13, 50019, Sesto Fiorentino, Florence, Italy
[b] Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, 41012, Seville, Spain
[c] Zentiva, K.S. Praha, a Sanofi Company, U Kabelovny 130, 102 37, Praha 10, Czech Republic
[d] Department of Chemical Sciences, University of Catania, Viale A. Doria 6, 95125, Catania, Italy
[e] Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy

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ABSTRACT

A capillary electrophoresis method for the simultaneous determination of the enantiomeric purity and of impurities of the chiral calcimimetic drug cinacalcet hydrochloride has been developed following Quality by Design principles. The scouting phase was aimed to select the separation operative mode and to identify a suitable chiral selector. Among the tested cyclodextrins, (2-carboxyethyl)-β-cyclodextrin and (2-hydroxypropyl)-γ-cyclodextrin (HPγCyD) showed good chiral resolving capabilities. The selected separation system was solvent-modified capillary zone electrophoresis with the addition of HPγCyD and methanol. Voltage, buffer pH, methanol concentration and HPγCyD concentration were investigated as critical method parameters by a multivariate strategy. Critical method attributes were represented by enantioresolution and analysis time. A Box-Behnken Design allowed the contour plots to be drawn and quadratic and interaction effects to be highlighted. The Method Operable Design Region (MODR) was identified by applying Monte-Carlo simulations and corresponded to the multidimensional zone where both the critical method attributes fulfilled the requirements with a desired probability $\pi \geq 90\%$. The working conditions, with the MODR limits, corresponded to the following: capillary length, 48.5 cm; temperature, 18 °C; voltage, 26 kV (26–27 kV); background electrolyte, 150 mM phosphate buffer pH 2.70 (2.60–2.80), 3.1 mM (3.0–3.5 mM) HPγCyD; 2.00% (0.00–8.40%) v/v methanol. Robustness testing was carried out by a Plackett-Burman matrix and finally a method control strategy was defined. The complete separation of the analytes was obtained in about 10 min. The method was validated following the International Council for Harmonisation guidelines and was applied for the analysis of a real sample of cinacalcet hydrochloride tablets.

1. Introduction

Cinacalcet hydrochloride (CIN) is the first agent of a new therapeutic class of calcimimetic compounds, which act by increasing the sensitivity of calcium-sensing receptors to the extracellular calcium ions, thus lowering the release of parathyroid hormone [1]. CIN is an approved medication for the treatment of secondary hyperparathyroidism in adult patients with chronic kidney disease on dialysis and hypercalcemia in patients with parathyroid carcinoma [2]. It has been commercialized as single *R* enantiomer, due to the fact that *R*-CIN is about 75 times more potent than its corresponding *S*-enantiomer [3]. According to the drug product producer (Zentiva, Praha), the main potential impurities of CIN which may be found in CIN bulk samples and dosage forms are the distomer *S*-cinacalcet (*S*-CIN), impurity 1 (I_1) and impurity 2 (I_2), whose chemical structures are shown in Fig. 1.

The effective and safe therapy with chiral drugs basically depends on the quality of the pharmaceutical dosage forms, which must be controlled both in terms of drug and potential impuri-

* Corresponding authors.
E-mail addresses: serena.orlandini@unifi.it (S. Orlandini), sandra.furlanetto@unifi.it (S. Furlanetto).

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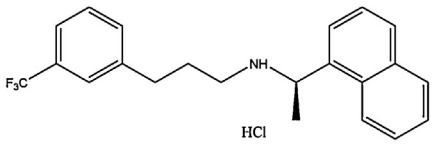
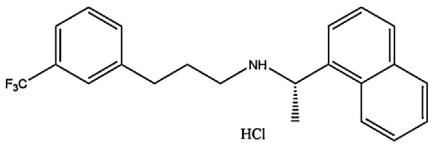
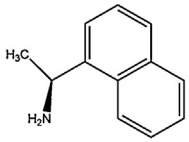


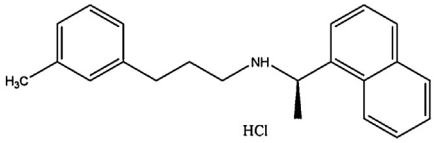
Cinacalcet hydrochloride (CIN)
(*R*)-*N*-[1-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl) phenyl]propan-1-amine hydrochloride
CAS 364782-34-3



S-cinacalcet (*S*-CIN)
(*S*)-*N*-[1-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl)phenyl] propan-1-amine hydrochloride
CAS 694495-47-1



Impurity 1 (I_1)
(*R*)-(+)-1-(1-naphthyl)ethylamine
CAS 3886-70-2



Impurity 2 (I_2)
N-[3-(3-methylphenyl)propyl]-*N*-[(1*R*)1-(1-naphthyl) ethyl]amine hydrochloride
CAS 253337-60-9

Fig. 1. Molecular structures of the compounds.

ties content and enantiomeric purity. In particular, the assessment of enantiomeric purity is required by regulatory agencies prior to the marketing of optically active drugs, and in this context the efficient separation of enantiomers represents a fundamental analytical task. Enantioseparation can be effectively obtained with several chromatographic techniques including HPLC, ultra-high performance liquid chromatography, nano-liquid chromatography and TLC [4–7]. Anyway, for routine analysis it is important to have at disposal fast and low cost analytical methods with simple sample preparation and eco-friendly characteristics as small reagent consumption. CE easily fulfills these requirements and has been effectively applied during all stages of drug discovery as well as for the quality control of the finished pharmaceutical products. Among the well-known advantages of CE there are its inherent flexibility as well as the various available operative modes, high separation efficiency, low sample and reagent consumption [8]. In pharmaceutical analysis, CE has been widely applied to the determination of the main component as well as for the purity of drugs with regard to related substances and stereoisomeric impurities [9]. It represents one of the major techniques not only for the achievement of analytical scale enantioseparations but also for a better understanding of the fine intermolecular recognition mechanisms which take place [10–13]. In addition, CE utilizes a wide spectrum of chiral selectors as buffer additives for chiral separation, without the need for expensive columns as for chromatography. The most commonly used chiral selectors are cyclodextrins (CyDs), due to their wide variety of cavity size, side chain, degree of substitution and charge [14]. When added to plain buffers, CyDs are able to resolve enantiomeric guests due to formation of diastereomeric inclusion complexes, which present different electrophoretic mobilities. When the background electrolyte (BGE) contains pseudostationary phases as micelles or microemulsions, it has been recently suggested that more complex phenomena may take place [15,16]. In the latter case also the monomers of surfactant can interact with the CyD, possibly involving the formation of ternary complexes 1:1:1 of CyD:enantiomer:surfactant, or giving rise to the displacement of CyD bound enantiomeric guests with surfactant tails due to competitive binding. In any case the affinity of the enantiomers for the CyD can be effectively modulated.

The analysis of CIN and two process-related impurities has been performed by HPLC [17], and stability indicating chromatographic methods involving forced degradation studies have been recently presented [18–21]. Enantioseparation of CIN and its *S*-enantiomer was obtained by chiral normal phase HPLC [22], by indirect reversed-phase HPLC with chiral auxiliaries as derivatizing agents and by direct thin-layer chromatography [23], and by polysaccharide chiral reversed-phase HPLC [24]. Recently, a LC/MS-MS method for separation and determination of CIN enantiomers in rat plasma on chirobiotic V column packed with vancomycin has been presented [25]. To the best of our knowledge, CE methods for the determination of I_1 and I_2 have not been reported in previous literature. There is only one reported CE method for the chiral separation of CIN [26], which was developed by univariate experiments to test the enantiopurity of CIN in tablets without considering any of the other potential impurities which can be found in the pharmaceutical dosage form.

For the first time in the literature, the purpose of this study was to set up a CE method for the simultaneous determination of CIN, its chiral impurity *S*-CIN and its main impurities I_1 and I_2 in pharmaceutical formulations, for fulfilling the requirements of an effective Quality Control strategy. Method development has been carried out by Quality by Design (QbD), a risk-management approach following the recent guidelines in the pharmaceutical field [27]. QbD has been recently focused in the field of separation methods [28,29], and its application in this study made it possible to identify not only a single optimum point but a multidimensional zone where the desired quality of analytical data for determining all the four analytes was achieved.

QbD has been defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" and has the aim of improving product quality and of increasing regulatory flexibility [27]. In terms of application to analytical methods, Analytical Quality by Design (AQbD) emphasizes the need to thoroughly understand the analytical system by an in-depth study of critical method parameters (CMPs) based on risk assessment and multivariate tools [28,29]. Such strategy enables the robust optimization of the analytical method in compliance with the recommendations of US Food and Drug Administration (US FDA) [30] and with the guidelines of International Council for Harmonisation (ICH) [27], and represents a great step forward with respect to the traditional quality-by-testing approach. As a matter of facts, the latter furnishes data and information limited to the experiments run by the operator, without studying the possible interactions between CMPs and without building a model relating the CMPs to critical method attributes

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