Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1127 (2006) 286–294
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Enantiomeric purity methods for three pharmaceutical compounds by electrokinetic capillary chromatography utilizing highly sulfated-γ-cyclodextrin as the chiral selector

Tore Ramstad *

Pharmaceutical Sciences, Pfizer Corp., Kalamazoo, MI 49001, USA

Received 27 February 2006; received in revised form 29 May 2006; accepted 6 June 2006
Available online 27 June 2006

Abstract

Methods for enantiomeric purity by electrokinetic chromatography were developed and validated for three pharmaceutical compounds, each utilizing highly sulfated-γ-cyclodextrin (HS-γ-CD) as the chiral recognition agent. Two of the compounds are weak bases, hence charged at low pH, and the third is a quaternary nitrogen compound, charged at all pH. In each instance quantification was via an authentic reference standard with addition of an internal standard. Separation was on a 61 cm × 50 μm untreated capillary under reverse polarity with a background electrolyte of 5% HS-γ-CD in pH 2.50 lithium phosphate buffer. Each method was validated with respect to the usual validation parameters, notably recovery and precision, yielding results, including limits of detection and quantitation, that allow reporting the minor enantiomer to 0.1% and less. In applying the methods, all batches of bulk drug tested were shown to be of enantiomeric purity ≥99.9%.

Keywords: Chiral analysis of pharmaceutical compounds; CD-mediated CE; Electrokinetic chromatography; Validation of CE methods; Highly sulfated-γ-cyclodextrin (HS-γ-CD)

1. Introduction

Development of drug candidates as single enantiomers has become the norm within the pharmaceutical industry. As the usual expectation is an enantiomeric purity (excess) of 99.9% or greater, sensitive analytical methods for testing the purity are needed. The field of chiral analysis is dominated by HPLC, with vendors offering a plethora of columns and supporting applications literature. As most enantioseparations are accomplished using normal-phase HPLC, the emergence in recent years of SFC as a bona fide challenger to HPLC [1,2] is not surprising. As the third member of the triumvirate, CE plays a lesser role. However, for compounds of sufficient polarity and water solubility, CE is often the technique of first choice [3], although lack of widespread implementation of CE in control laboratories can be a deterrence to method development.

The many reviews on chiral CE attest to its state of development [4–12]. CE has been applied to virtually every class of drug. The usual means of effecting separation is to introduce a carrier,[1] most commonly a cyclodextrin (CD), originally derivatized, neutral CDs [11,15], now mostly charged, principally sulfated CDs [16–18]. Although CE's greatest strength is for water-soluble compounds, the use of micellar electrokinetic chromatography (MEKC) [19–23], microemulsion EKC (MEEKC) [24–27], and nonaqueous CE (NACE) [28,29] extend its range to virtually any compound. While in the early years most reported applications were only semi-quantitative, in recent years an increasing number of reports are for CE methods validated to the same standards as HPLC procedures [30–40].

We report here on chiral CE methods developed for three drugs. Two compounds are structural analogs, two are weak bases, and all are positively charged at low pH, and consequently are amenable to similar electrophoretic treatment. For each compound, highly sulfated-γ-cyclodextrin (HS-γ-CD), a so-called randomly substituted CD [18], proved most effective

* Tel.: +1 269 833 6687; fax: +1 269 833 5349.
E-mail addresses: donna.s.socha@pfizer.com, toreram@earthlink.net.

[1] With the introduction of a carrier, the technique is more appropriately referred to as eletrokinetic chromatography (EKC) [13,14].

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.06.009

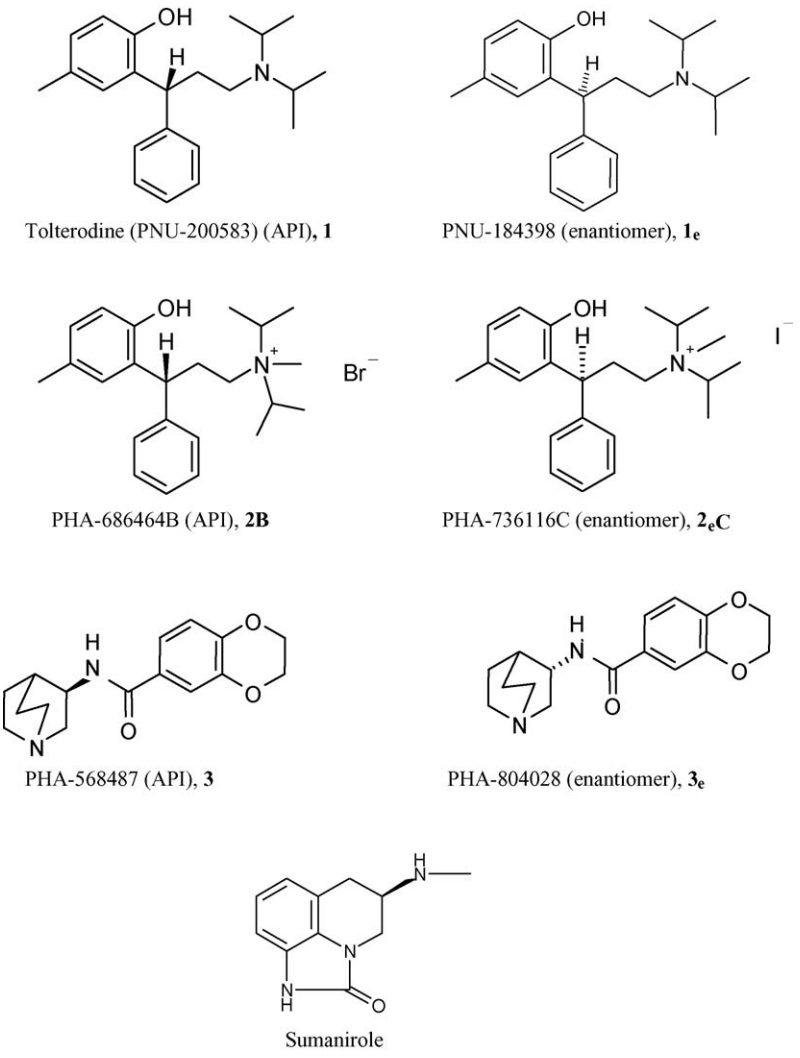

Fig. 1. Structures of the studied compounds plus the internal standard sumanirole.

for separation of the enantiomers. Tolterodine L-tartrate (PNU-200583E), **1E**, is the active ingredient in Detrol and Detrol LA, a treatment for incontinence (Fig. 1). PHA-686464B (B for bromide), **2B**, is an antimuscarinic compound that was under development for treatment of asthma and chronic obstructive pulmonary disorder (Fig. 1). PHA-568487E (E for fumarate), **3E**, a quinuclidine, was under development as a treatment for the cognitive deficits associated with schizophrenia (Fig. 1). The method for PHA-686464B was developed as a joint chiral/achiral impurities assay, but only the chiral component is discussed in this report. The CAS names of the three compounds are: tolterodine L-tartrate, 2-[(1*R*)-3-[bis(1-methylethyl) amino]-1-phenylpropyl]-4-methylphenol (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1) (salt); PHA-686464B, (γ*R*)-2-hydroxy-*N*,5-dimethyl-*N*,*N*-bis(1-methylethyl)-γ-phenylbenzenepropanaminium bromide; and PHA-568487E, *N*-(3*R*)-1-azabicyclo [2.2.2]oct-3-yl-2,3-dihydro-1,4-benzodioxin-6-carboxamide (2*E*)-2-butenedioate (1:1). Note that tolterodine and PHA-686464 are structurally very similar, differing only in a tertiary versus a quaternary nitrogen. In order to avoid the use of company-designated numbers throughout this report, the compounds are designated as shown in Fig. 1. Where a salt is intended, it is denoted by a capital letter suffix. The minor enantiomer corresponding to each compound is denoted by e as a subscript.

Each of these compounds possesses properties that make it a viable candidate for enantioseparation by capillary electrophoresis: (1) good water solubility (>5 mg mL^{-1}); (2) tolterodine and **3**, both weak bases, are fully protonated, and hence charged at low pH, ordinarily the region of first choice for chiral analysis by CE; **2**, as the quaternary analog of tolterodine, is positively charged at all pH; (3) although, in order to achieve adequate detection limits, short wavelength detection is required for all three, this poses little challenge for CE, as the background absorbance is low due to the short optical pathlength; and (4) the site of asymmetry is of a size that should fit inside the cavity of an appropriately sized CD; furthermore, the combination of a positively charged analyte and a negatively charged CD ordinarily promotes separation [39].