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Analytica Chimica Acta 596 (2007) 132–140
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Simultaneous and enantioselective liquid chromatographic determination of eslicarbazepine acetate, *S*-licarbazepine, *R*-licarbazepine and oxcarbazepine in mouse tissue samples using ultraviolet detection

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Received 5 March 2007; received in revised form 30 May 2007; accepted 31 May 2007
Available online 3 June 2007

Abstract

Herein is reported, for the first time, a simple and reliable chiral reversed-phase liquid chromatographic method coupled to ultraviolet (UV) detection for simultaneous determination of eslicarbazepine acetate (ESL) and its metabolites, *S*-licarbazepine (*S*-LC), *R*-licarbazepine (*R*-LC) and oxcarbazepine (OXC), in mouse plasma and brain, liver and kidney tissue homogenates. All analytes and the internal standard were extracted from plasma and tissue homogenates by a solid-phase extraction procedure using Waters Oasis® hydrophilic–lipophilic balance cartridges. The chromatographic separation was performed by isocratic elution with water/methanol (88:12, v/v), pumped at a flow rate of 0.7 mL min^{-1}, on a Lichro-CART 250-4 ChiraDex (β-cyclodextrin, 5 μm) column at 30 °C. The UV detector was set at 225 nm. Calibration curves were linear ($r^2 \geq 0.996$) in the ranges 0.4–8 μg mL^{-1}, 0.1–1.5 μg mL^{-1} and 0.1–2 μg mL^{-1} for ESL and OXC and in the ranges 0.4–80 μg mL^{-1}, 0.1–15 μg mL^{-1} and 0.1–20 μg mL^{-1} for *R*-LC and *S*-LC in plasma, brain and liver/kidney homogenates, respectively. The overall precision not exceeded 11.6% (%CV) and the accuracy ranged from −3.79 to 3.84% (%bias), considering all analytes in all matrices. Hence, this method will be a useful tool to characterize the pharmacokinetic disposition of ESL in mice.

Keywords: Eslicarbazepine acetate; Oxcarbazepine; Mouse tissue samples; Enantioselective liquid chromatography; Bioanalytical method validation

1. Introduction

Eslicarbazepine acetate (ESL) [S-(−)-10-acetoxy-10,11-dihydro-5H-dibenz/b,f/azepine-5-carboxamide], previously known as BIA 2-093, is a novel central nervous system (CNS)-active drug presently completing phase III clinical trials, as add-on therapy in refractory partial epilepsy, and undergoing phase II clinical trials, as monotherapy in partial epilepsy and in bipolar disorder [1]. Chemically, it shares with oxcarbazepine (OXC) the dibenzazepine nucleus bearing the 5-carboxamide substituent, but is structurally different at the 10,11-position (Fig. 1) [2]. These molecular variations result in differences in their metabolism and, consequently, in their pharmacological properties. Briefly, OXC is an achiral prodrug which, in humans, is stereoselectivelly reduced in liver to the pharmacologically active licarbazepine metabolite, appearing in plasma as *S*-licarbazepine (*S*-LC) and *R*-licarbazepine (*R*-LC) in approximately a 4:1 enantiomeric ratio [3,4]. On the other hand, the chiral prodrug ESL is quickly and extensively metabolized to *S*-LC (95–98%) and, in a minor extent, to *R*-LC and OXC [5,6]. Unlike OXC, ESL appears to present a more favourable metabolic pathway, with a higher S/R licarbazepine enantiomeric ratio, without losing anticonvulsant potency [6,7].

To our knowledge, up to date, in spite of OXC to be used in the clinical practice for several years and ESL to be in final phase of clinical trials, few studies have investigated the systemic

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0003-2670/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2007.05.056

disposition of their licarbazepine enantiomers [6,8–11]. Furthermore, the differential pharmacokinetic disposition of *R*-LC and *S*-LC in brain, liver and kidney tissues remains unknown, contributing for that perhaps the lack of an economical chiral assay. In fact, only three chiral liquid chromatographic methods with ultraviolet detection (LC-UV) are reported in the literature for the quantification of licarbazepine enantiomers [8,12,13], and they were all developed under normal phase chromatographic columns with expensive mobile phases essentially composed of *n*-hexane. In addition, another chiral liquid chromatographic method with mass spectrometry detection (LC–MS) has also been referred to determine ESL and its metabolites *S*-LC, *R*-LC and OXC, but it was not yet completely described [6,14].

Actually, the clinical development of a new drug always runs together with non-clinical studies, since for ethical aspects many experiments could not be performed in humans. Therefore, the availability of an accurate and easy-to-use LC method to quantify ESL and its metabolites in an appropriate experimental model will be important to complete the pharmacokinetic data that are arising from clinical trials. Thus, bearing in mind the metabolism specie-dependent of ESL and being mouse the most relevant specie to humans in this case [5,6,14], the purpose of this paper is to describe the first chiral reversed-phase LC-UV method developed and validated to simultaneous determination of ESL and its metabolites *S*-LC, *R*-LC and OXC in mouse plasma and brain, liver and kidney tissue homogenates.

2. Experimental

2.1. Chemicals

Standards of ESL (BIA 2-093, lot number 0000012976, 100% pure), *S*-LC (BIA 2-194, lot number PC020131B, 99.79% pure), *R*-LC (BIA 2-195, lot number PC040414, 100% pure), OXC (lot number 97.12.17, >98% pure) and BIA 2-265 (lot number PC050704, 97.4% pure) used as internal standard (I.S.), were kindly supplied by BIAL (Porto, Portugal) (Fig. 1). Methanol (LC grade, SDS), water milli-Q (LC grade, >15 MΩ, home-made), acetonitrile, ethyl acetate, sodium dihydrogen phosphate dihydrate, di-sodium hydrogen phosphate dehydrate and hydrochloric acid fuming 37% were purchased from Merck KGaA (Darmstadt, Germany).

2.2. Animal experiments

Adult male CD-1 mice obtained from Harlan-Interfauna (Barcelona, Spain), weighing 30–35 g, were housed in local animal facilities with light (12 h light/dark cycle) and temperature (22 ± 1 °C) controlled environment. A regular chow diet (4RF21, Mucedola, Italy) and tap water were available *ad libitum* until the experimental procedures. Mice not subjected to any pharmacological treatment were used as a source of drug-free mouse plasma and brain, liver and kidney tissues, which were used as blank matrices in the validation studies. For that, blood samples were collected into heparinised tubes by decapitation preceded of cervical dislocation. The plasma was separated by centrifugation at 4000 rpm for 10 min (4 °C) and stored at −30 °C until use. After exsanguination, brain, liver and kidneys were quickly removed, weighed and then homogenised (4 mL g^{-1}) in a 0.1 M sodium phosphate buffer (pH 5). The tissue homogenates were centrifuged at 4800 rpm for 15 min (4 °C) and the supernatants were also stored at −30 °C.

All animal experimentation was conducted in accordance with the European Directive (86/609/EEC) for the accommodation and care of laboratory animals and the experimental

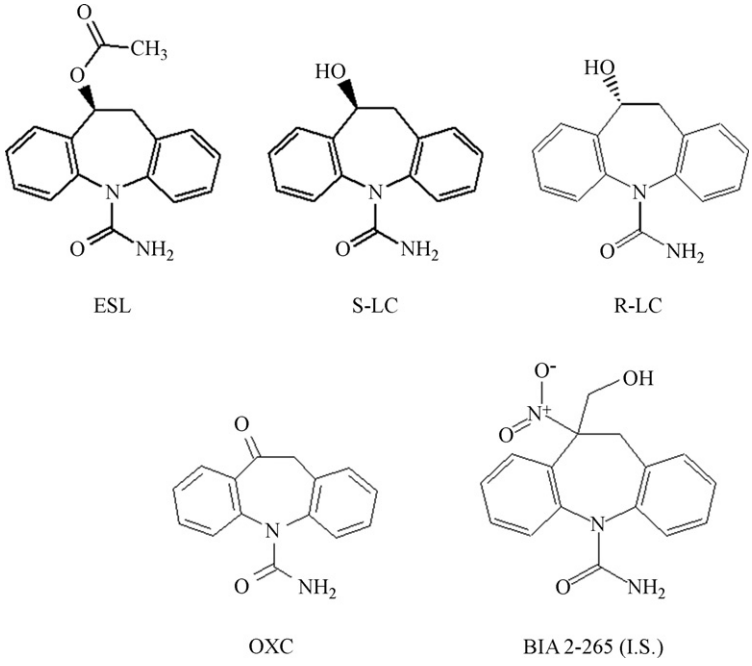

Fig. 1. Chemical structures of eslicarbazepine acetate (ESL), *S*-licarbazepine (*S*-LC), *R*-licarbazepine (*R*-LC), oxcarbazepine (OXC) and BIA 2-265 used as internal standard (I.S.).