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Liquid chromatography separation of the chiral prodrug eslicarbazepine acetate and its main metabolites in polar organic mode. Application to their analysis after *in vitro* metabolism

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ABSTRACT

A LC method using a chiral stationary phase (CSP) with cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral selector in polar organic mode (POM) was developed for the separation of the biopharmaceutic classification system (BCS) class II chiral prodrug eslicarbazepine acetate (ESL) and its main metabolites, namely eslicarbazepine, its optical antipode, (*R*)-licarbazepine, and the achiral oxcarbazepine (OXC). The percentage of methanol (MeOH) in the mobile phase containing acetonitrile (ACN) as the main solvent was found to significantly influence analyte retention and resolution. A reversal of elution order of OXC and (*R*)-licarbazepine was observed, depending on the MeOH percentage in the mobile phase. The optimized mobile phase consisted of ACN/MeOH/acetic acid/diethylamine (95/5/0.2/0.07; v/v/v/v). The potential of this chemo- and enantioselective LC method combined with solid-phase extraction (SPE) was then evaluated for *in vitro* metabolism studies using ESL as a model case. Only eslicarbazepine could be detected after incubation of ESL in human liver microsome systems.

1. Introduction

Large advances in the development of enantioselective analysis methods have been made since the US Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products have published guidelines on the development of chiral drugs [1,2]. Indeed, the production of pure enantiomers and the quality control of chiral drug substances have become key issues for pharmaceutical industry and regulatory agencies. Nowadays, LC is an essential tool to perform the enantioseparation of chiral compounds, which can be largely attributed to the existence of many different kinds of chiral stationary phases (CSPs). Among these, polysaccharide-based CSPs are used in more than 80% of the analytical enantioseparations [3]. Different kinds of mobile phases can be used together with polysaccharide-based CSPs, corresponding to normal-phase, reversed-phase, polar organic and sub/supercritical fluid modes. The application of the polar organic mode (POM) in combination with polysaccharide-based CSPs for enantioseparations is well-established, owing to short analysis times, alternative chiral recognition mechanisms compared to other LC modes and high solubility for many analytes in the mobile phases [4].

Eslicarbazepine acetate (ESL) is an antiepileptic drug approved in the EU and USA for use as add-on therapy for adults with partial-onset seizures. Recently, the US license was extended to include its use as monotherapy [5]. After oral administration, ESL is extensively metabolized into the main active metabolite eslicarbazepine, also called (*S*)-licarbazepine [6]. Subsequently, minor chiral inversion of eslicarbazepine to R-licarbazepine occurs, presumably via oxidation to oxcarbazepine (OXC), both the latter compounds being minor metabolites (cf. Fig. 1) [7]. According to the biopharmaceutic classification system (BCS), ESL belongs to BCS class II (highly permeable-poorly soluble drug) while eslicarbazepine is categorized as BCS class I (highly permeable-highly soluble) [6].

Enantioselective LC methods coupled to solid-phase extraction (SPE) with UV or MS detection have been proposed for the separation and quantification of ESL and its metabolites eslicarbazepine, (*R*)-licarbazepine and OXC in biological samples [8–10]. One of these methods was developed in reversed-phase mode using a ChiraDex column with a run time of about 27 min [8,9]. Another method was performed in normal-phase mode using a polysaccharide-based CSP (Chiralcel® OD-H column) with a run

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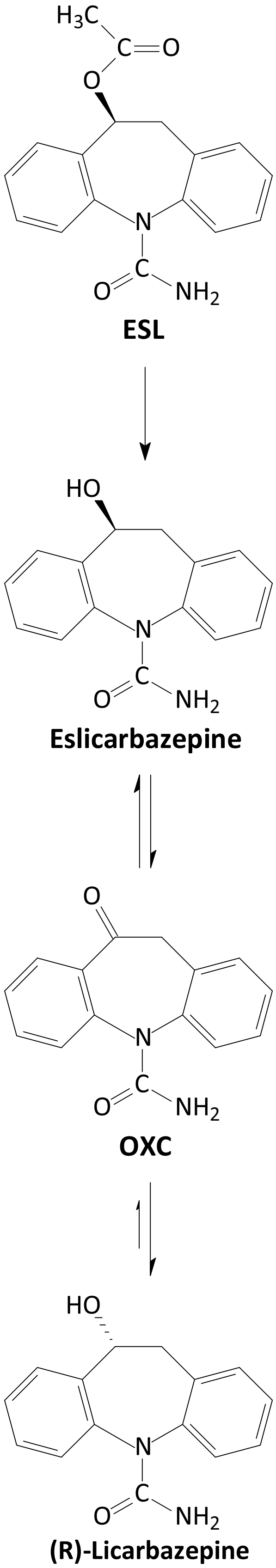

Fig. 1. Metabolic pathways of ESL [7].

time of 8 min [10]. It is worth noting that the normal-phase mode has some economical and environmental disadvantages related to the cost and toxicity of the mobile phase components. Mone et al. developed a LC method in POM using cellulose tris(3,5-dichlorophenylcarbamate) immobilized column (Chiralpak IC-3) for the enantiomeric purity determination of eslicarbazepine acetate [11]. Nevertheless, using this method, eslicarbazepine and its optical antipode, (*R*)-licarbazepine, were only partially resolved, with a run time of 23 min. To the best of our knowledge, the separation of ESL and its metabolites eslicarbazepine, (*R*)-licarbazepine and OXC in biological samples has never been investigated in LC using a polysaccharide-based CSP in POM.

Microsomes, which contain a wide variety of metabolic enzymes, are used to perform *in vitro* drug metabolism and therefore to predict drug pharmacokinetics as well as to study reactions of phase I and/or II metabolism [12,13]. Moreover, there are some advantages to carry out *in vitro* experiments rather than *in vivo*, such as reduced use of animals and the possibility to study the isoforms involved in the metabolic pathway as well as drug–drug interactions [14].

In this paper, the potential of a LC method using a polysaccharide-based CSP in POM for studying the *in vitro* metabolism of a drug using microsomal fraction from human livers was investigated as a chemo- and enantioselective bioanalytical tool. This approach was applied to the determination of ESL and its metabolites, used as a model case. To achieve this goal, a CSP with cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral selector was chosen. The LC method combined with sample preparation using SPE was optimized in terms of analysis time and analyte resolution.

2. Materials and methods

2.1. Instrumentation

The chromatographic system from Agilent Technologies (Waldbronn, Germany) consisted in a binary pump, an automatic injector, a thermostated column compartment and a diode array detector, all of 1100 series. The Chemstation software was used for system control and data acquisition. The chiral column Sepapak-2 (250 mm x 4.6 mm I.D.; 5 μm), equivalent to Lux Cellulose-2 from Phenomenex, was provided by Sepaserve (Münster, Germany). The chiral selector adsorbed on aminopropyl silica was cellulose tris(3-chloro-4-methylphenylcarbamate). The analytical column was protected with a SecurityGuard Lux Cellulose-2 cartridge (4 × 3.0 mm I.D.) purchased from Phenomenex (Utrecht, The Netherlands).

Sample evaporation was performed on a vacuum concentrator (Labconco, Kansas City, USA).

2.2. Chemicals and reagents

ESL, racemic licarbazepine and phenacetine were provided by Sigma-Aldrich (Saint-Louis, MO, USA). OXC was supplied by EDQM (Strasbourg, France).

Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were provided by J.T. Baker (Deventer, The Netherlands). Diethylamine (DEA) was from Sigma-Aldrich. Acetic acid (AcA) and orthophosphoric acid 85% were provided by VWR Chemicals (Leuven, Belgium).

Sodium dihydrogen phosphate (NaH_2PO_4), di-sodium hydrogen phosphate (Na_2HPO_4) and magnesium chloride ($MgCl_2$) were purchased from Merck (Darmstadt, Germany). NADPH was purchased from AppliChem (Darmstadt, Germany).