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Review

Development and validation of an enantioselective SFC-MS/MS method for simultaneous separation and quantification of oxcarbazepine and its chiral metabolites in beagle dog plasma

Zhichao Yang[a], Xueyu Xu[a], Lingxia Sun[a], Xue Zhao[a], Hao Wang[a], John Paul Fawcett[b], Yan Yang[a,**], Jingkai Gu[c,*]

[a] School of Life Sciences, Jilin University, Changchun 130012, PR China
[b] School of Pharmacy, University of Otago, Dunedin, PO Box 56, New Zealand
[c] Clinical Pharmacology Center, Research Institute of Translational Medicine, The First Hospital of Jilin University, Changchun 130061, PR China

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ABSTRACT

A rapid and sensitive assay based on supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) has been developed and validated for the determination of oxcarbazepine (OXC) and its chiral metabolite licarbazine (Lic) in beagle dog plasma using carbamazepine as internal standard. Chiral analysis in a run time of only 3 min was performed on an ACQUITY UPC² ™ Trefoil™ CEL2 column (3.0 × 150 mm, 2.5 μm) at 50 °C by isocratic elution with a mobile phase of supercritical carbon dioxide (purity ≥ 99.99%) and methanol (60:40, v/v) at a flow rate of 2.3 mL/min. The assay was linear over the concentration ranges 5–1000 ng/mL for OXC and 0.5–100 ng/mL for the enantiomers of Lic with corresponding lower limits of quantitation of 5 ng/mL and 0.5 ng/mL. Intra- and inter-day precisions were in the range 0.78–14.14% with accuracies in the range −10.80% to 0.42%. The method was successfully applied to a pharmacokinetic study involving a single oral administration of 16 mg/kg OXC as Trileptal® tablets to beagle dogs.

Contents

* Corresponding author at: College of Life Science, Jilin University, 2699 Qianjin Street, Changchun 130012, PR China.
** Corresponding author.
E-mail addresses: yyan@jlu.edu.cn (Y. Yang), gujk@jlu.edu.cn (J. Gu).

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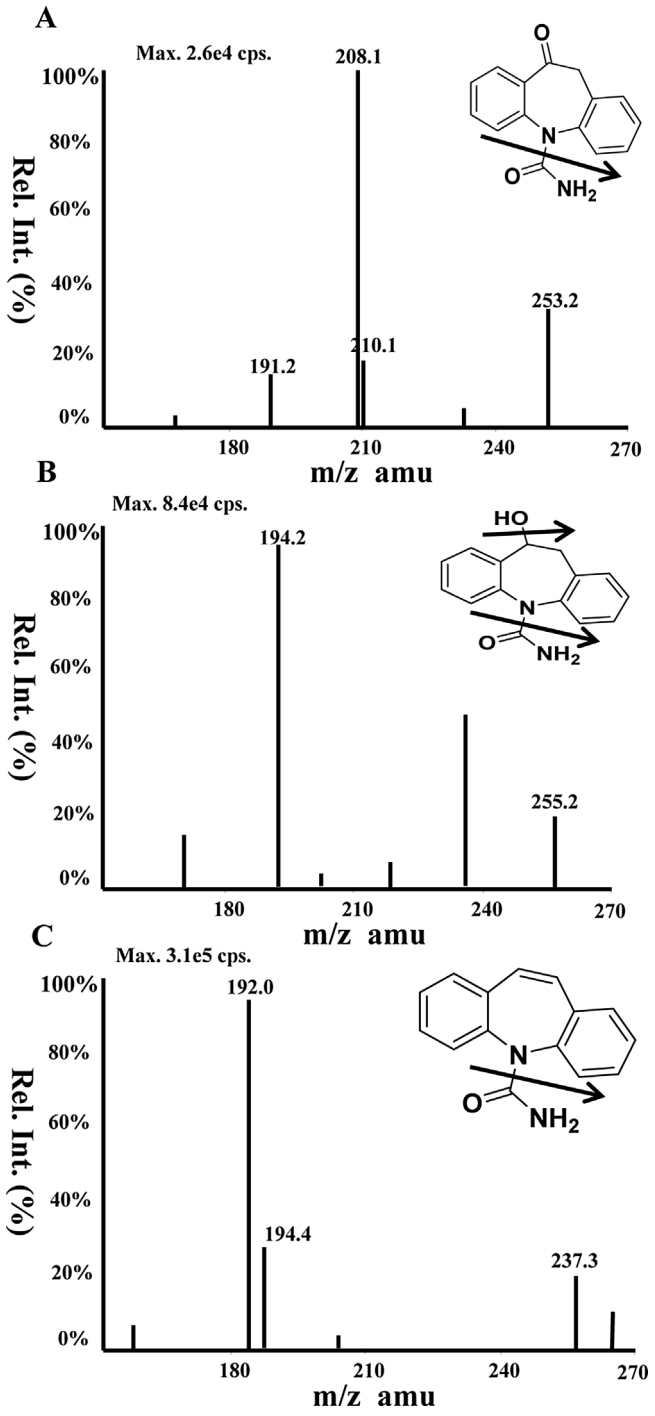

Fig. 1. Full-scan product ion mass spectra of $[M+H]^+$ ions for (A) OXC, (B) Lic and (C) CBZ.

1. Introduction

Oxcarbazepine (10,11-dihydro-10-oxo-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide, OXC) is a second generation antiepileptic drug marketed as Trileptal® tablets for monotherapy or adjunctive therapy of partial seizures and generalized tonic-clonic seizures in adults and children [1,2]. After oral administration, OXC is rapidly metabolized by cytosolic reductase enzymes to 10-hydroxycarbazepine (licarbazepine, Lic) which is pharmacologically more active than the parent compound and essentially responsible for its antiepileptic effects [3,4]. Lic has a chiral center at position 10 and appears in human plasma as an enantiomeric mixture of *S*-(+)-licarbazepine (*S*-Lic) and *R*-(−)-licarbazepine (*R*-Lic) in a ratio of approximately 5:1 [5,6]. This implies that the reductase is stereoselective and that chiral analysis is necessary to fully understand the pharmacokinetic and pharmacodynamic effects of OXC.

OXC and the enantiomers of Lic have been previously quantitated in biological samples by chiral capillary electrophoresis [7], high performance liquid chromatography (HPLC) [8] and liquid chromatography-tandem mass spectrometry (LC–MS/MS) [9–12]. The most sensitive method has a lower limit of quantitation (LLOQ) of 50 ng/mL [10,12–15] for each analyte and a run time of 8 min. In addition, the resolution of *S*- and *R*-Lic was unsatisfactory. Thus there remains a need for a rapid and sensitive chiral assay for the determination of OXC and the enantiomers of Lic.

This paper reports the use of chiral supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) for the simultaneous determination of OXC, *S*- and *R*-Lic. SFC uses supercritical carbon dioxide ($SCCO_2$) as the main component of the mobile phase [16,17] and is characterized by high resolution at high flow rates providing rapid analysis times with no reduction in sensitivity or efficiency for some chiral or achiral compound [18,19]. SCCO2 has relatively low polarity but the polarity of the mobile phase can be altered through addition of polar solvents like methanol (with which it is completely miscible) and/or additives like trifluoroacetic acid and isopropylamine. This enables molecules with a wide range of polarities to be simultaneously analysed [20,21]. Compared with HPLC, SFC has a number of advantages. First, the use of non-toxic SCCO2 as the main mobile phase component decreases the use and need to dispose of volatile organic solvents thereby reducing environmental pollution and the health risk to laboratory personnel. Secondly SCCO2 is cheap, recyclable and widely available which greatly reduces operating costs. Moreover, SFC is an environmental friendly technique due to its use of less organic solvent. The method was fully validated according to "Guidance for Industry-Bioanalytical Method Validation" of the USA Food and Drug Administration (FDA) [22] and successfully applied to a pharmacokinetic study of oral OXC in beagle dogs.

2. Materials and methods

2.1. Chemicals and reagents

Trileptal® tablets were purchased from Novartis Pharma (Basel, Switzerland). OXC, *S*-Lic, *R*-Lic and carbamazepine (CBZ) for use as internal standard (IS) (purity >99.5% in all cases) were purchased from J&K Chemicals Ltd. (Shanghai, China). HPLC grade isopropanol, methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Perchloric acid, methanol dichloromethane, diethyl ether, hexane and ethyl acetate were purchased from Beijing Chemical Plant (Beijing, PR China). Distilled water, prepared from demineralized water, was used throughout the study. All other chemicals were HPLC grade.