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LC–MS/MS determination of tideglusib, a novel GSK-3β inhibitor in mice plasma and its application to a pharmacokinetic study in mice

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ABSTRACT

A sensitive, specific and rapid LC-ESI–MS/MS method has been developed and validated for the quantification of tideglusib in mice plasma using warfarin as an internal standard (I.S.) as per regulatory guidelines. Sample preparation was accomplished through liquid-liquid extraction process. Chromatographic separation was performed on Atlantis dC_{18} column using mobile phase A (acetonitrile) and B (5 mM ammonium acetate in water) in a flow-gradient mode. Elution of tideglusib and the I.S. occurred at ~2.06 and 1.29 min, respectively. The total chromatographic run time was 3.2 min. A linear response function was established in the concentration range of 20.2–1008 ng/mL. The intra- and inter-day accuracy and precision were in the range of 4.61–12.6 and 6.04–11.8%, respectively. This novel method has been applied to a pharmacokinetic study in mice.

1. Introduction

Glycogen synthase kinase 3β (GSK3β) enzyme takes part in several cellular processes in physiological condition. Recently it gained importance because overexpression or over activation of this enzyme has shown an evidence in elevated production of Aβ (amyloid β), tau phosphorylation and microglia associated inflammatory process etc. leading to memory impairment [1]. Thus, GSK3β inhibition has emerged as one of the most promising therapeutic strategies in Alzheimer disease. Tideglusib (NP-12 or NP031112; Fig. 1) is a novel and potent thiadiazolidinone, which irreversibly inhibits GSK3β (IC_{50}: 60 nM), reduces tau phosphorylation and prevents apoptotic death in human neuroblastoma cells and murine primary neurons [2]. Tideglusib reduced kainic acid-induced inflammation and has a neuroprotective effect in the damaged areas of the hippocampus in animal models [3] and these findings positioned tideglusib as a potential agent for neurodegenerative disorders treatment.

Phase-II clinical trial was completed with tideglusib evaluating its efficacy, safety and tolerability and to treat mild-to-moderate Alzheimer's disease patients. In this study tideglusib was tested at 4-escalted doses ranging from 400 to 1000 mg/day [4]. Tideglusib was also tested for its efficacy and tolerability for the treatment in patients with mild-to-moderate progressive supranuclear palsy. In this study tideglusib patients received either 600 or 800 mg of tideglusib [5]. Currently two Phase-II clinical trials are on-going with tideglusib for the treatment of autism spectrum disorders [6] and congenital and juvenile-onset myotonic dystrophy [7] in adolescents. In both these studies the proposed doses ranging from 400 to 1000 mg/day. Very recent work by Neves et al. (2017) reported that tideglusib promotes natural tooth repair via mobilisation of resident stem cells into the tooth pulp [8].

To date there is no bioanalytical method reported for quantification of tideglusib in any biological matrix. In this paper, we report the development and validation of a simple, specific, sensitive and reproducible LC–MS/MS method for quantitation of tideglusib in mice plasma. The method was successfully applied to quantitate levels of tideglusib in mice pharmacokinetic studies.

2. Experimental

2.1. Chemicals and materials

Tideglusib (purity >98%) and warfarin (internal standard; I.S.; purity >98%) were purchased from Sigma-Aldrich, St. Louis, USA. HPLC grade acetonitrile and methanol were purchased from Rankem, Ranbaxy Fine Chemicals Limited, New Delhi, India. Analytical grade ammonium acetate and diethyl ether were purchased

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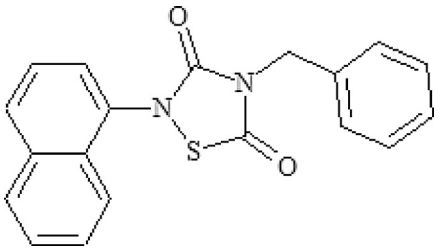

Fig. 1. Structural representation of tideglusib.

from S.D Fine Chemicals, Mumbai, India. All other chemicals and reagents were of analytical grade and used without further purification. The control mice K_2.EDTA plasma sample was procured from Animal House, Jubilant Biosys, Bangalore.

2.2. Instrumentation and chromatographic conditions

A Shimadzu HT (Shimadzu, Japan) LC system equipped with degasser (DGU-20A5), binary pump (LC-20AD) along with autosampler (SIL-HTC) was used to inject 10 μL aliquots of the processed samples on an Atlantis dC_{18} column (50 × 4.6 mm, 3 μm) which was maintained at 40 ± 1 °C. The solvents used for chromatography were filtered through a 0.45 μm membrane filter (XI5522050) (Millipore, USA or equivalent) and then degassed ultrasonically for 5 min. The isocratic mobile phase, a mixture of acetonitrile and 5 mM ammonium acetate in water at a ratio of 20:80 (v/v) was delivered at increasing flow-rate of 0.3–1.0 mL/min (0.0–1.0 min: 0.3 mL/min and 1.0–3.5 min: 1.0 mL/min) into the mass spectrometer-electro spray ionization chamber.

Quantitation was achieved by MS/MS detection in positive ion mode for analyte and I.S. using a MDS Sciex (Foster City, CA, USA) API-5500 mass spectrometer, equipped with a Turboionspray™ interface at 600 °C temperature and 5000 V ion spray voltage. The source parameters viz., curtain gas, GS1, GS2 and CAD were set at 35, 50, 55 and 11 psi. The compound parameters viz., declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) were 40, 10, 43, and 10 V for tideglusib and 100, 10, 19, and 12 V for the I.S. Detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of the *m*/*z* 335 precursor ion to the *m*/*z* 91 product ion for tideglusib and *m*/*z* 309–251 for the I.S. Quadrupole Q1 and Q3 were set on unit resolution. The dwell time was 100 msec. The analytical data were processed by Sciex Analyst software (version 1.6.2).

2.3. Preparation of standard solutions of analyte and the I.S.

Tideglusib and the I.S. were weighed accurately into volumetric flasks using an analytical micro balance. The primary stock solutions of tideglusib and the I.S. were prepared at 1000 μg/mL in methanol. The primary stock solutions of tideglusib and the I.S. were stored at −20 °C, which were found to be stable for thirty days (data not shown). The primary stock of analyte was successively diluted in methanol:water (8:2, v/v) to prepare secondary stocks and working solutions to prepare calibration curve (CC) for tideglusib. Working stock solutions were stored approximately at 4 °C for a week (data not shown). Working stocks were used to prepare plasma calibration standards. A working I.S. solution (50 ng/mL) was prepared in methanol. Blank mice plasma was screened prior to spiking to ensure that it was free from endogenous interference at retention times of tideglusib and the I.S. Eight point calibration standards samples (20.2–1008 ng/mL) were prepared by spiking the blank mice plasma with appropriate concentration of tideglusib. Samples for the determination of precision and accuracy were prepared by spiking control mice plasma in bulk with tideglusib at appropriate concentrations 20.2 ng/mL (LLOQ, lower limit of quantitation), 60.5 ng/mL (LQC, low quality control), 420 ng/mL (MQC, medium quality control) and 756 ng/mL (HQC, high quality control) and 50 μL plasma aliquots were distributed into different tubes. All the samples were stored at −80 ± 10 °C.

2.4. Recovery

The efficiency of tideglusib and the I.S. extraction from mice plasma was determined by comparing the responses of the analytes extracted from replicate QC samples (n = 6) with the response of tideglusib from post extracted plasma standard sample at equivalent concentrations by liquid-liquid extraction. Recovery of tideglusib was determined at QC low and QC high concentrations and the recovery of the I.S. was determined at 50 ng/mL. The recovery of tideglusib and the I.S. was determined by comparing the peak areas of extracted plasma standards to the peak areas of post extraction plasma samples spiked at corresponding concentration.

2.5. Sample preparation

To an aliquot (50 μL) of mice plasma 10 μL of I.S. working stock solution and 1.0 mL of diethyl ether were added and vortex mixed on a vortex mixer for 10 s. The mixture was centrifuged for 5 min at 2850*g* in a refrigerated centrifuge (Eppendorf 5424R) maintained at 5 °C. Clear supernatant (800 μL) was evaporated under a gentle stream of nitrogen and the residue was dissolved in 200 μL of mobile phase and 10 μL was injected onto LC–MS/MS system for analysis.

2.6. Method validation

A full validation according to the FDA guidelines [9] was performed for the assay in mice plasma.

2.6.1. Specificity and selectivity

The specificity of the method was evaluated by analyzing mice plasma samples from at least six different lots to investigate the potential interferences at the LC peak region for tideglusib and the I.S.

2.6.2. Matrix effect

The effect of mice plasma constituents over the ionization of tideglusib and I.S. was determined by comparing the responses of the post extracted plasma QC samples (*n* = 6) with the response of tideglusib from neat standard samples (5 μL of each tideglusib spiked into 45 μL of methanol instead of blank plasma) at equivalent concentration. Matrix effect for tideglusib was determined at LQC and HQC, whereas the matrix effect over the I.S. was determined at a single concentration of 50 ng/mL. Post-column infusion method defined by Bonfiglio et al. was used to evaluate the matrix effect [10]. Matrix factor was determined as ratio of peak response in presence of matrix (post-extracted) to mean peak response in neat solution (*n* = 6).

2.6.3. Calibration curve

Linearity was assessed by weighted linear regression ($1/X^2$) of each analyte:I.S. peak area ratio based on four independent calibration curves prepared on each of four separate days using eight-point calibration curve. The calibration curve had to have a correlation coefficient (*r*) of >0.99 or better. The acceptance criteria for each back-calculated standard concentration were ± 15% deviation from the nominal value except at LLOQ, which was set at ±20% [9]. The