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A novel HPLC method for the determination of zonisamide in human plasma using microextraction by packed sorbent optimised by experimental design

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A novel high-performance liquid chromatography-diode array detection method based on microextraction by packed sorbent (MEPS) as a sample preparation approach is described for the determination of zonisamide (ZNS) in human plasma. MEPS parameters were optimised using a Plackett–Burman experimental design to achieve the best extraction conditions. The chromatographic separation of ZNS and chloramphenicol [internal standard (IS)] was achieved in less than 5 min on a C18-column, at 35 °C, using a mobile phase composed of acetonitrile/water (35 : 65, v/v) pumped isocratically at 1.0 mL min^{-1}. ZNS and IS were detected at 240 nm. No endogenous and exogenous interference was observed at the retention times of the analyte of interest (ZNS) and IS. A good linearity was obtained for ZNS (r^2 = 0.9960) in the range of 0.2–80 μg mL^{-1} in plasma. The method was shown to be precise (CV ≤ 13.3%) and accurate (bias ±12.3%), and the absolute recovery ranged from 63.8% to 65.2%. The stability of ZNS was demonstrated in plasma samples under all predictable handling and storage conditions. The proposed assay was applied to the analysis of human plasma samples obtained from epilepsy patients under ZNS therapy, and the results supported its usefulness for therapeutic drug monitoring in clinical practice.

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1. Introduction

Zonisamide (ZNS), chemically known as 1,2-benzisoxazole-3-methanesulfonamide,[1,2] is an antiepileptic drug licensed in the European Union for monotherapy or adjunctive therapy of partial seizures with or without generalisation.[1,3] It is characterised by unique structural, mechanistic and pharmacokinetic properties that are distinct from those of other antiepileptic drugs.[4] The mechanism of action described involves the blockage of voltage-dependent sodium and T-type calcium channels interrupting synchronised neuronal firing, thereby preventing the spread of seizure activity and reducing the recurrence of epileptic seizures.[3,5,6] In addition, this drug also has some effects on the synthesis, release and degradation of a number of different neurotransmitters, including glutamate, γ-aminobutyric acid, dopamine, serotonin and acetylcholine, which may lead to an increase in inhibitory synaptic neurotransmission.[3,6–8]

Regarding the pharmacokinetic properties, ZNS is rapidly absorbed from the gastrointestinal tract and presents a high oral bioavailability.[2,3,7] It binds moderately to human serum albumin (about 50%), but tends to accumulate in red blood cells.[3,9] In addition, ZNS is partially metabolised in the liver through cytochrome P450 3A4 (under reductive cleavage) and, subsequently, it is eliminated through the renal route.[2,9] The favourable pharmacokinetic profile of ZNS supports its increasing use. Moreover, it has the advantage of not modifying significantly the plasmatic levels of other antiepileptic drugs administered concomitantly.[3,7]

A therapeutic reference range of 10–40 μg mL^{-1} for ZNS has been proposed for appropriate seizure management.[10–12] In fact, the implementation of therapeutic drug monitoring of ZNS in routine clinical practice requires the availability of suitable bioanalytical methodologies to measure ZNS concentration levels in biological samples in order to adjust the patient's medication regimen and achieve optimal therapeutic outcomes. In this sense, several techniques have been developed and validated to quantify ZNS in human matrices (*e.g.* serum,

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