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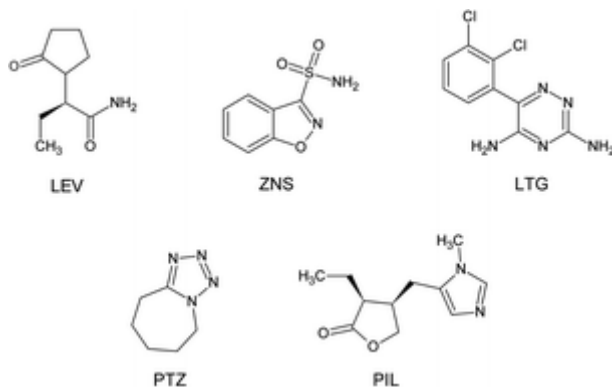
**Analytical Methods**

## First HPLC method for the simultaneous quantification of levetiracetam, zonisamide, lamotrigine, pentylenetetrazole and pilocarpine in rat plasma and brain

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### Abstract

A new high-performance liquid chromatography-diode-array detection (HPLC-DAD) method is herein reported for the first time for the simultaneous determination of three antiepileptic drugs (AEDs) [levetiracetam (LEV), zonisamide (ZNS) and lamotrigine (LTG)] and two convulsant agents [pentylenetetrazole (PTZ) and pilocarpine (PIL)] in rat plasma and brain matrices. Chromatographic separation of all analytes and 7-hydroxycoumarin, used as internal standard (IS), was achieved in less than 19 min on a C<sub>18</sub> column maintained at 30 °C using a mobile phase composed of acetonitrile (7.5%) and a mixture (92.5%) of water–triethylamine (99.5 : 0.05, v/v, pH 6.4) pumped at a flow rate of 1 mL min<sup>-1</sup>. The analytes and the IS were detected at 205, 215 or 240 nm. This method showed to be selective and the calibration curves were linear ( $r^2 \geq 0.991$ ) over the concentration ranges of 0.4–100 µg mL<sup>-1</sup> for LEV, 2–100 µg mL<sup>-1</sup> for PTZ, 0.75–100 µg mL<sup>-1</sup> for PIL, 0.15–100 µg mL<sup>-1</sup> for ZNS and 0.3–50 µg mL<sup>-1</sup> for LTG in both rat matrices. The overall data of precision and accuracy were in accordance with international guidelines on bioanalytical method validation. The analytes were extracted from the rat matrices by a liquid–liquid extraction procedure and the absolute recovery of the analytes was precise and reproducible. Additionally, this novel HPLC-DAD method was applied in a preliminary pharmacokinetic study in rats, which involved the target AEDs and convulsant agents, demonstrating to be a useful bioanalytical tool to support future non-clinical studies.



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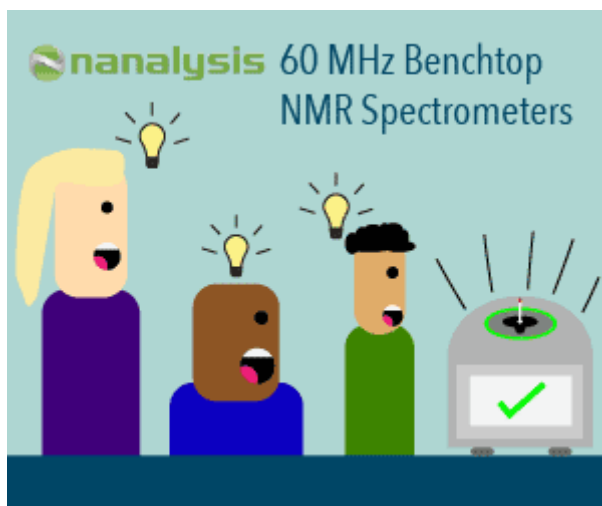
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