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Short communication

Rapid quantification of donepezil in whole blood and serum by LC-MS/MS

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Introduction

Donepezil (DPZ) is a pharmaceutical agent utilized in treating Alzheimer's disease, exerting its therapeutic effect via acetylcholinesterase inhibition (Shigeta and Homma, 2001). Its therapeutic concentration range is established between 30 and 70 ng/mL, whereas concentrations exceeding 100 ng/mL are considered toxic (Schulz et al., 2020). DPZ undergoes hepatic metabolism mediated by CYP2D6 and CYP3A4 isoenzymes and is eliminated via the kidneys. It exhibits a prolonged elimination half-life, varying from approximately 60 h in younger individuals to around 100 h in the geriatric population. Although its clinical use is generally safe, more severe adverse reactions, like hypotension and bradycardia, have been reported during concomitant administration of DPZ with beta-blockers (Gust et al., 2020). Given its narrow therapeutic index and a target patient group comprised predominantly of the elderly, there is an elevated risk of intoxication associated with DPZ therapy. Thus, suspected DPZ intoxication demands rapid analytical methods to ensure timely clinical intervention. This study aims to develop an efficient method for DPZ quantification and evaluate its applicability for clinical and forensic settings, in two referent matrices.

Materials and methods

Methanol, water, acetonitrile (ACN) and formic acid (FA), all with LC-MS grade, were purchased from Carlo Erba (France). Analytical standards of DPZ and Diazepam d5 were supplied by Chiron (Norway) and Lipomed (Switzerland), respectively. Drug free blood and serum were obtained from the Institute for Transfusion Medicine, R.N. Macedonia.

Sample preparation was performed according to procedure proposed by Chen et al. (2019), with slight modifications. Working solutions for DPZ and internal standard (Diazepam d5) were prepared in a 50% methanol and stored at -20 °C. Calibrators and quality controls (QC) were prepared by blood and serum spiking with appropriate amount of DPZ working solutions. Internal standard (IS) was added to all samples achieving 100 ng/mL final concentration. Samples underwent protein precipitation of 50 µL blood or serum using ACN up to 1 mL. Samples were than centrifuged at 11 000 rpm for 10 min. Equal amounts of supernatant and water (500 µL) were vortexed and filtered through 0.2 µm syringe filter. Twenty µL of this solution were injected for LC-MS/MS analysis.

Analyses were carried out on Nexera UPLC system and LCMS 8030 (Shimadzu, Japan) triple

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quadrupole mass spectrometer equipped with ESI operated in positive ion mode. Chromatographic separation was achieved using Kinetex C18 LC column (50 x 2.1 mm; Phenomenex, USA). Mobile phases consisted 0.1% FA in water and 0.1% FA in ACN in gradient mode, delivered at a flow rate of 0.4 mL/min, resulting in 6 min run time. Quantification was performed using MRM mode with *m/z* 379.90→90.95 for DPZ and *m/z* 289.80→153.90 for IS. Analytical data was acquired and integrated by Lab Solution software (Shimadzu, Japan).

Results and discussion

The developed method provides a selective determination of DPZ, exhibiting favorable chromatographic peak characteristics with a retention time (RT) of 0.37 min. Matrix-match calibration was established in whole blood, achieving a linear dynamic range of 10 –1000 ng/mL. Whole blood was preferentially selected as the calibration matrix due to the limited availability of serum specimens typically encountered in forensic toxicology casework. To evaluate matrix-dependent effects, a comparative analysis of DPZ in whole blood and serum was conducted across three QC levels: Low (QC1): 30 ng/mL; medium (QC2): 450 ng/mL; high (QC3): 900 ng/mL. The extraction recovery was 52.41% for whole blood and 61.19% for serum. Under experimental conditions, this variance proved negligible. The calculated serum-to-blood concentration ratio across all evaluated QC levels was 1.04. This consistent ratio validates the extraction efficiency and confirms the cross-applicability of the method for both biological matrices. However, quantitative interpretation must account for the high plasma protein binding (96%) of DPZ, which theoretically results in lower total concentrations in whole blood relative to serum samples (Grossberg, 2003). The primary analytical advantages of this method include a low sample volume, which is paramount in forensic investigation, and a short analysis time of approximately 1 h, fulfilling a critical demand for emergency clinical toxicology. Moreover, this method features an exceptionally wide linear dynamic range that is rarely reported in current literature (Huang, 2022; Jeong, 2018). This allows for the immediate testing of high-concentration samples without needing dilution.

Conclusion

The described method enables rapid and accurate DPZ quantification in both tested samples, demonstrating its utility in clinical and forensic toxicology. Comparative analysis following ACN protein precipitation revealed no significant difference in extraction recovery between blood and serum. However, further validation using authentic patient samples is required to establish exact correction factor for interpreting quantitative data derived from different matrices.

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