

**IDENTIFICATION OF GAMMA-HYDROXYBUTYRATE IN BIOLOGICAL FLUIDS BY LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY**

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Gamma-hydroxybutyrate (GHB) is a substance that belongs to depressors of the central nervous system, and its mechanism of action involves GABA receptors binding. In recent years, there has been an increase in GHB abuse. The maximum GHB blood concentration is reached 20-40 min after oral ingestion, elimination half-life being 30-50 min. About 1-5% of GHB is excreted unchanged in urine, detected only 3-10 h after ingestion. GHB abuse risk is a small difference between dose that causes euphoria and reduced inhibition and the dose that can lead to loss of consciousness cardiorespiratory depression. GHB blood and urine identification is important in poisoning with unknown etiology diagnosis. This work aimed to present method of liquid chromatography with tandem mass spectrometry (UPLC-MS-MS) GHB detection in biological fluids. There were 21 cases of GHB abuse recorded at the Poison Control Centre, in 2018-2020 period. Immunoassays for the detection of GHB in urine still stayed unavailable for routine laboratory practice, so we developed UPLC-MS-MS method for its detection in urine and serum. Samples were prepared by solid-phase extraction on Oasis HLB cartridges. GHB separation from matrix compounds was performed on a C18 column by mixture of acetonitrile and 5 mmol/L formate buffer pH 3.5 as mobile phase. GHB identification was performed on the basis of the characteristic mass ion m/z 105, 87 and 45. Since GHB overdose can often lead to coma and even death, analytical GHB confirmation in biological fluids has great importance because facilitates poisoning diagnosis and speed up patients treatment.

## **IDENTIFIKACIJA GAMA-HIDROKSIBUTIRATA U BIOLOŠKIM TEČNOSTIMA PRIMENOM METODE TEČNE HROMATOGRAFIJE SA TANDEM MASENOM SPEKTROMETRIJOM**

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Gama-hidroksibutirat (GHB) je supstanca koja pripada depresorima centralnog nervnog sistema, a svoje dejstvo ostvaruje vezivanjem za GABA receptore. Poslednjih godina zabeležen je porast zloupotrebe GHB-a. Maksimalna koncentracija GHB-a u krvi postiže se posle 20-40 min, dok je poluvreme eliminacije 30-50 min. Oko 1-5% GHB-a se eliminiše nepromenjeno urinom i može se detektovati svega 3-10 h od ingestije. Opasnost prilikom zloupotrebe GHB-a predstavlja mala razlika između doze koja izaziva euforiju i smanjenje inhibicije i doze koja može dovesti do gubitka svesti i kardiorespiratorne depresije. Identifikacija GHB-a u krvi i urinu je važna u dijagnostici trovanja nepoznate etiologije. Cilj ovog rada je da prikaže metodu tečne hromatografije sa tandem masenom spektrometrijom (UPLC-MS-MS) za dokazivanje GHB-a u biološkim tečnostima. U periodu od 2018-2020. godine u Centru za kontrolu trovanja zabeležen je 21 slučaj zloupotrebe GHB. Imunotestovi za dokazivanje GHB u urinu su još uvek nedostupni u rutinskom laboratorijskom radu, te je razvijena UPLC-MS-MS metoda za njegovu detekciju u urinu i serumu. Uzorci su pripremani čvrsto-faznom ekstrakcijom na Oasis HLB kertridžima. Razdvajanje GHB-a od komponenata matriksa izvršeno je na C18 koloni uz korišćenje smeše acetonitrila i 5 mmol/L formijatnog pufera pH 3,5 kao mobilne faze. Identifikacija GHB-a vršena je na osnovu karakterističnih masenih jona m/z 105, 87 i 45. S obzirom da predoziranje GHB-om često može da dovede do kome, pa čak i smrti, analitička potvrda GHB-a u biološkim tečnostima ima veliki značaj, jer može da olakša dijagnozu trovanja i ubrza lečenje bolesnika.