A Rapid Detection Method for Desmethylbromethalin Using MALDI-TOF Mass Spectrometry

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Bromethalin is a neurotoxic rodenticide that is increasingly being utilized due to recent cancellations of second-generation anticoagulant rodenticides. It is marketed under the trade name Tomcat in bait block packs and bait worm products at concentrations of 0.01% and 0.025%, respectively. The toxic mechanism of bromethalin involves its metabolism to desmethylbromethalin, which uncouples oxidative phosphorylation causing cerebral edema. Because the use of bromethalin is increasing, a rapid, sensitive method to detect it for diagnostic toxicology is needed. This study highlights the use of MALDI-TOF MS for rapid detection of desmethylbromethalin in brain tissue.

MALDI-TOF MS analysis for desmethylbromethalin briefly involves the following: (1) 1 g of brain tissue is homogenized in 5% ethanol in ethyl acetate; (2) extract is centrifuged and supernatant evaporated and reconstituted in 250 µl methanol; (3) 3 µl of extract or neat desmethylbromethalin standard is mixed with 3 µl of α-cyano-4-hydroxycinnamic acid matrix; (4) 1 µl of each standard/sample is pipetted onto a stainless steel MALDI target plate; (5) standards/samples are analyzed in reflector negative mode with a voltage set at 19.99 kV, detector scan range of 0 to 1,200 Da; and (6) 1,000 laser shots are scanned and accumulated for all \([\text{M-H}]^{-}\) ions. The unique, diagnostic ions detected for desmethylbromethalin were 562.93/564.93 m/z \([\text{M-H}]^{-}\), 467.06 m/z \([\text{M-Br-NO}]^{-}\), and 450.87 m/z \([\text{M-Br-NO}_2]^ {-}\). The method detection limit for these ions was 0.5 ppm. This study is the first to highlight the utility of MALDI-TOF MS for the rapid detection of the small molecule neurotoxicant desmethylbromethalin.

Research advisor Christina Wilson writes: “Detection of the toxic metabolite desmethylbromethalin in animals previously involved laborious extraction procedures and long analysis times, resulting in delayed diagnosis and treatment in exposed animals. Kendal’s research using MALDI-TOF MS to detect desmethylbromethalin provides a more rapid diagnosis and can expedite treatment in animals exposed to bromethalin rodenticide.”

The MALDI-TOF MS ion chromatograms of brain tissue. Negative control brain (---) and brain containing 100 ppm desmethylbromethalin (---). Diagnostic ions for desmethylbromethalin detected in the brain tissue are 562.90/564.93 m/z \([\text{M-H}]^{-}\), 467.06 m/z \([\text{M-Br-NO}]^{-}\), and 450.87 m/z \([\text{M-Br-NO}_2]^ {-}\).