Toxicology of the *Amanita phalloides* (Death Cap) Mushroom

Detection of Amatoxins and Phallotoxins by Ultra-Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry

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Abstract

The increasing number of *Amanita phalloides* poisoning cases in Australia and lack of efficient treatment options has emphasised the need for detection methods which can be applied in forensic and clinical toxicology. In this study, an existing method utilised by Nomura et al. \cite{1} was adapted for the detection of *A. phalloides* toxins, \(\alpha\)-amanitin, \(\beta\)-amanitin and phalloidin, in whole blood specimens using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Various parameters were evaluated in order to develop an optimised method, which was then validated.

Optimisation of the MRM parameters was conducted using MS/MS with an electrospray interface in positive ionisation mode. This mode was found to give the greatest number of stable transitions and far greater sensitivity than negative ionisation mode. Resolution of the two amanitins was achieved using a Waters ACQUITY UPLC BEH C18 column (2.1 mm x 150 mm) and a mobile phase combination of 5 mM ammonium formate with 0.05% formic acid : 0.1% formic acid in water at a flow rate of 0.4 mL/min. The total run time of the method was 8 minutes. Two internal standards, virginiamycin B and rifampicin, were evaluated with rifampicin being chosen as the internal standard.

Samples were diluted prior to undergoing solid-phase extraction. The sample preparation method utilised a dilution step followed by SPE. Several columns were trialled, with the UCT Clean Up C18 column providing the best recovery. The SPE method was adapted from that outlined in Nomura et al. \cite{1}

The overall developed method was validated according to NATA guidelines \cite{2} and methods outlined by Shah et al. \cite{3} The parameters evaluated included selectivity, matrix effects, linearity, recovery, sensitivity, precision and limits of detection and quantification. The method produced good selectivity for each analyte, however significant matrix effects were encountered for the analytes which affected further results. Linearity studies were performed over the range of 25-500 ng/mL for the amanitins and 5-100 ng/mL for phalloidin, and gave correlation coefficients of 0.9731, 0.9825 and 0.9872 for \(\alpha\)-amanitin, \(\beta\)-amanitin and phalloidin respectively. Average recoveries ranged from 79.46%-107.99% for \(\alpha\)-amanitin, 46.96-62.19% for \(\beta\)-amanitin and 6.62-12.45% for phalloidin, suggesting the extraction method needs further improvement. The method exhibited poor sensitivity for the analytes, with slopes of 0.0031, 0.0022 and 0.0002 for \(\alpha\)-amanitin, \(\beta\)-amanitin and phalloidin.
respectively. The method was found to be precise for each of the analytes and showing no significant difference between data points, with p-values of 0.4279 for α-amanitin, 0.7265 for β-amanitin and 0.7814 for phalloidin. Limits of detection were determined to be 25 ng/mL for both amanitins and 20 ng/mL for phalloidin. Limits of quantification were determined to be 75 ng/mL for the amanitins and 60 ng/mL for phalloidin. Overall, the developed method did not pass validation, however offers a good basis for further work.
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