UPLC-MS/MS analysis of the *Pimelea* toxin simplexin and its potential degradation products

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Pimelea poisoning of cattle (also known as St. George disease or Marree disease) is a uniquely Australian poisoning caused by inadvertent grazing of native herbaceous *Pimelea* plants in pastures. A novel diterpenoid orthoester, simplexin (Figure 1a) was previously isolated and identified to be the toxin responsible for the poisoning (Roberts *et al.*, 1975). It was subsequently reported that cattle fed with a diet containing increasingly low doses of simplexin showed reduced poisoning symptoms over time (Fletcher *et al.*, 2014). It has been hypothesised that some rumen microorganisms in cattle have the ability to detoxify simplexin. In this study, rumen fluid has been collected from Queensland cattle reported to graze *Pimelea* without exhibiting any poisoning symptoms. Studies are ongoing to investigate *in vitro* simplexin degradation in mixed rumen bacterial culture fermentations based on this rumen fluid (fed daily with *Pimelea trichostachya*), and in culture incubations with rumen bacteria isolated from these fermentation studies. Simplexin levels were analysed by ultra-performance liquid chromatography coupled with high resolution, accurate mass (HRAM) spectrometry (UPLC-MS/MS). The project aim is to adapt a previously developed analytical method by Chow *et al.* (2010) in the UPLC-MS/MS for analysis of simplexin levels in *in vitro* studies and investigate potential degradation products.

Samples obtained from *in vitro* studies were extracted by methanol and solid phase extraction (SPE) clean-up was performed on the complex sample matrix providing extracts capable of analysis by UPLC-MS/MS to reduce matrix effects. Fragmentation of protonated simplexin ($[M+H]^+$, *m/z* 533.31089) to two major fragment ions was utilised as transitions for quantification (*m/z* 533.31089 > 253.1223) and for verification (*m/z* 533.31089 > 267.1380) respectively (Figure 1b). Calibration curves of isolated pure simplexin over the concentration range of 10 – 2000 ng/mL produced R^2 values of 0.99 and spiked recoveries before and after extraction of 99% demonstrated that the method was both reliable and accurate for simplexin quantification. Analysis of both the fermentation and culture *in vitro* studies showed decreases in simplexin suggesting possible simplexin degradation by rumen microorganisms. However, simplexin degradation products are yet to be identified from the samples.

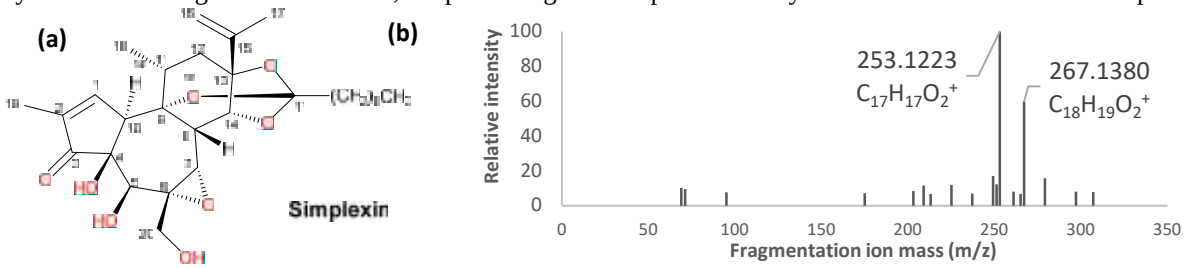

Figure 1: (a) Chemical structure of simplexin (b) Fragmentation of protonated simplexin producing two major fragment ions of m/z 253.1223 and m/z 267.1380.

Simplexin hydrolysis with acid was conducted for the identification of possible simplexin metabolites to create a simplexin metabolite database for elucidation of likely simplexin degradation pathways. Results showed the identification of possible hydrolysed products based on predicted molecular formulae and were found to share similar fragment ions to simplexin. Results from both fermentation and acid hydrolysis studies showed that the UPLC-MS/MS method can be used for simplexin quantification at parts per billion (ng/mL) concentrations and molecular formula calculations permit elucidation of unknown metabolites allowing identification of simplexin degrading rumen bacteria for probiotic development, aimed at mitigating *Pimelea* poisoning effects in cattle.

References

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