

# Analysis of the Pimelea toxin simplexin for the development of a cattle microbial probiotic

Loh, ZH<sup>1</sup>, Hungerford, NL<sup>1</sup>, Ouwkerk, D<sup>2,1</sup>, Klieve, AV<sup>1</sup>, Fletcher, MT<sup>1</sup>

<sup>1</sup>Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia

<sup>2</sup>Agri-Science Queensland, Department of Agriculture and Fisheries (QDAF), Ecosciences Precinct, Dutton Park, QLD 4102, Australia

## Introduction

- Pimelea poisoning of cattle (Fig. 1) is a uniquely Australia poisoning caused by the toxin simplexin (Fig. 2), found in native Pimelea plants [1].
- To date, there is no effective treatment for Pimelea poisoning.
- Cattle fed a diet containing increasing low doses of simplexin displayed reduced poisoning signs over time [2], suggesting adaption of rumen microorganisms to detoxify simplexin.
- This project aims to develop a protective microbial probiotic derived from the rumen fluid of field-exposed animals that is capable of detoxifying simplexin.



Figure 1: Steer affected by Pimelea poisoning

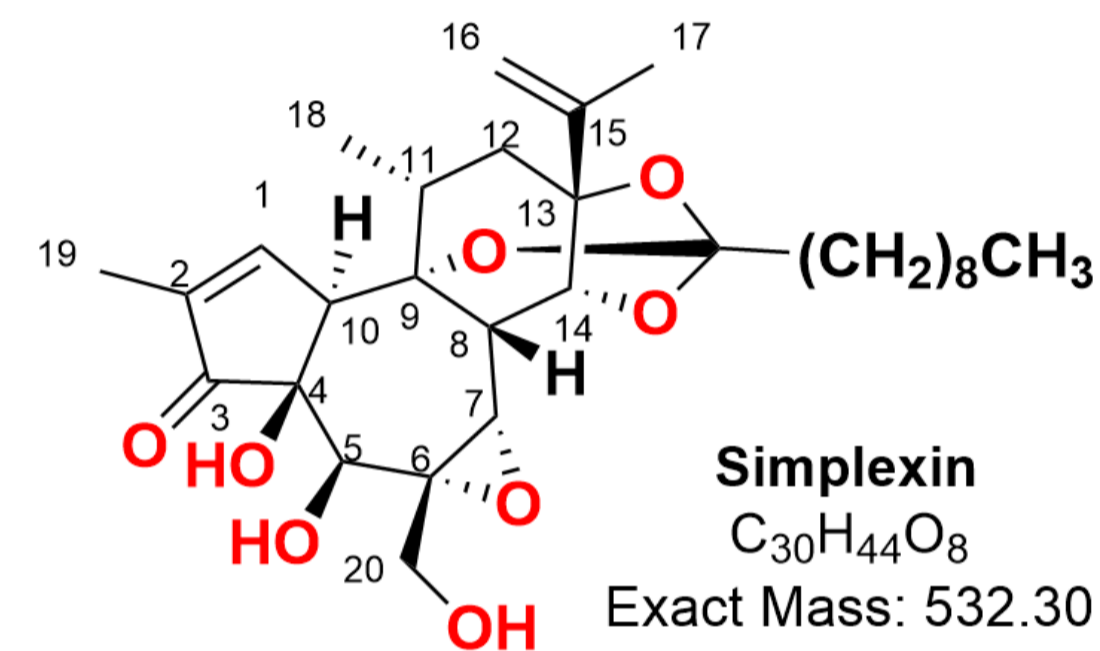


Figure 2: Simplexin chemical structure

## Results & Discussion

- Quantification of simplexin in both *in-vitro* studies showed decreased levels of simplexin.
- Acid hydrolysis of simplexin resulted in the identification of six possible hydrolysed simplexin products based on predicted molecular formulae (Fig. 4).
- Possible hydrolysed simplexin products also shared several fragmentation ions with simplexin.

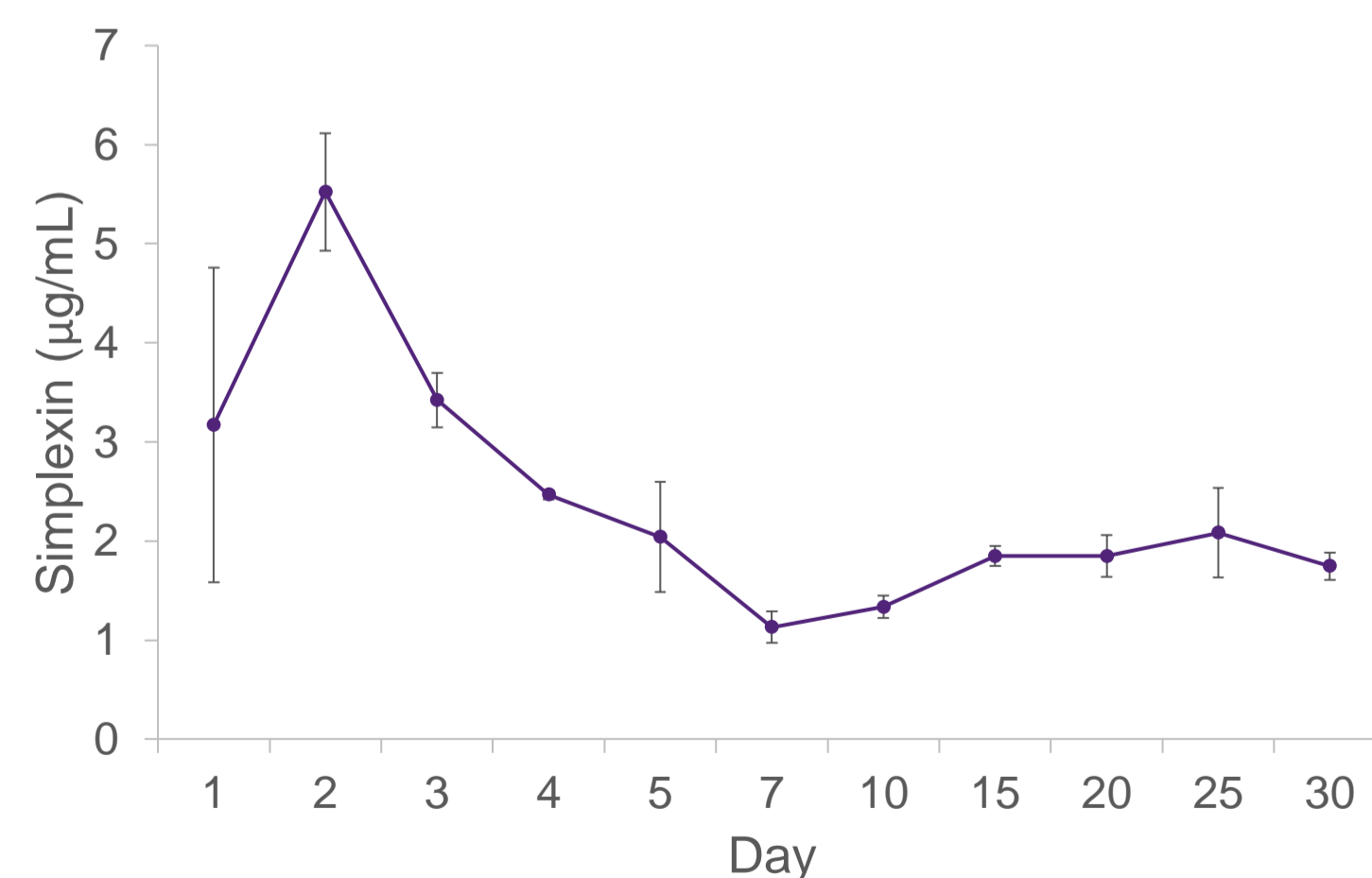


Figure 3: Simplexin levels in *in-vitro* rumen fermentation study

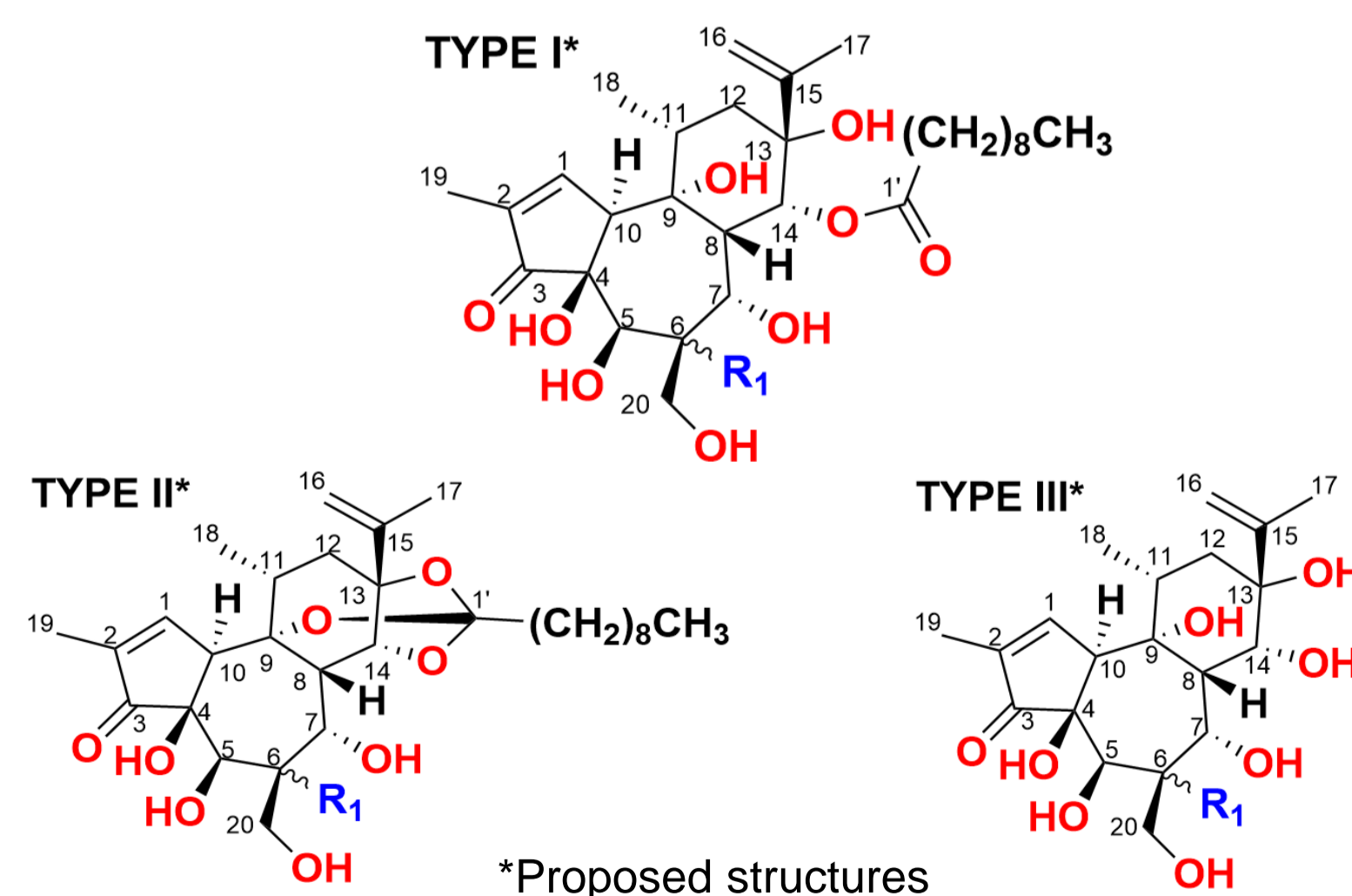
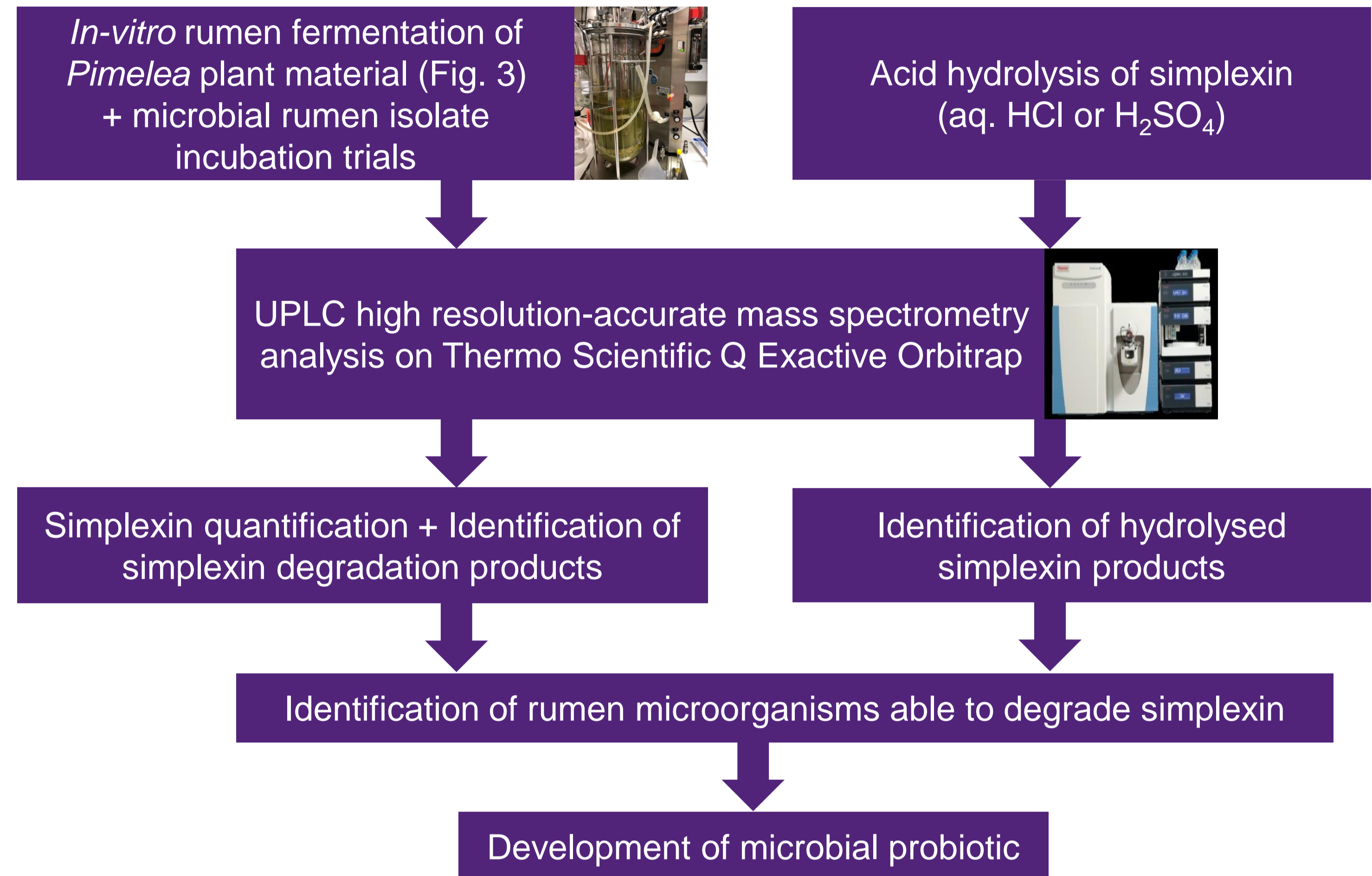


Figure 4: Proposed simplexin products identified from hydrolysis with aq. HCl (R<sub>1</sub> = Cl) and H<sub>2</sub>SO<sub>4</sub> (R<sub>1</sub> = OH)

## Methods & Materials



## Conclusions

- UPLC-MS/MS analysis enabled simplexin quantification at low concentrations (ng/mL).
- Simplexin decrease in *in-vitro* studies suggestive of potential simplexin degradation by rumen microorganisms.
- Possible simplexin hydrolysed products can be identified using the analytical method.

## Future studies

- Identification and characterisation of simplexin degradation products in both *in-vitro* fermentations and bacteria isolate incubation studies.
- Identified rumen isolates capable of degrading simplexin will be further investigated.

## References

- [1] P. Freeman, E. Ritchie, W. Taylor, Australian Journal of Chemistry, **1979**, 32, 2495-2506.
- [2] MT. Fletcher, S. Chow, SM. Ossedryver, Journal of Agriculture and Food Chemistry, **2014**, 62, 7402-7406.