

## LC-MS/MS ANALYSIS OF PERAMINE AND ERGOT AND LOLINE ALKALOIDS OF GRASS ENDOPHYTES

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Fungi of *Neotyphodium* species growing as endophytes within grasses are known to produce a range of alkaloids including indoleterpenoids, ergopeptides, clavines and simple lysergyl compounds, lolines and the pyrrolopyrazine peramine. Strains of *Neotyphodium* species have been selected for the absence of lolitrem and ergovaline production *in planta* and for their persistence in pasture cultivars. These new associations have been demonstrated to have significant advantages for animal health. Peramine and pyrrolizidine alkaloids that are produced by some strains of *Neotyphodium* have anti-insect activity, but do not show any toxicity towards mammals, while the role of the clavines and lysergyl compounds is not yet clear. While the major alkaloids can be monitored by HPLC-fluorescence and UV absorbance, or in the case of lolines, GC-FID, a more comprehensive analytical approach was required to provide a broader view of alkaloid accumulation in herbage of grasses infected with selected fungal strains.

A method for the simultaneous analysis of peramine, lolines and the range of ergot alkaloid metabolites could provide a significant improvement in both time and data quality for investigations of endophyte metabolism, and for quality assurance in the development and commercial release of selected endophyte strains. We have found peramine and a wide range of ergot alkaloids, including ergopeptides, lysergyl derivatives and clavine alkaloids to be amenable to analysis by reverse-phase HPLC-ESI-MS/MS. The sensitivity and selectivity of selective reaction monitoring methods is high, and the collision-induced fragmentation patterns provide clear characterising information. Loline alkaloids also ionise very well in ESI and undergo distinctive collision-induced fragmentation but they are less amenable to HPLC analysis. Results of HPLC-MS/MS analyses of a range of endophyte-infected grasses for fungal-produced ergot alkaloids and peramine and progress towards the development of an LC-MS/MS method for the simultaneous analysis of these three classes of endophyte metabolites will be presented and discussed.