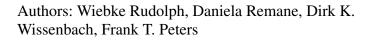
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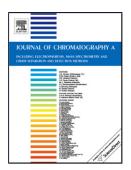
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ACCEPTED MANUSCRIPT

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Development and Validation of an UHPLC-HRMS/MS Assay for Nine Toxic Alkaloids from Endophyte-infected Pasture Grasses in Horse Serum

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Highlights (3-5 bullet points, each max 85 incl space)

- Validated determination of toxic alkaloids in horse serum
- Simultaneous extraction and separation of alkaloids from four different classes
- LODs well below 1 ng/mL for those alkaloids in horse serum

Abstract

Endophyte fungi (e.g. *Epichloë ssp.* and *Neotyphodium ssp.*) in symbiosis with pasture grasses (e.g. *Festuca arundinacaea* and *Lolium perenne*) can produce toxic alkaloids, which are suspected to be involved in equine diseases such as fescue toxicosis, ryegrass staggers, and equine fescue oedema. The aim of this study was, therefore, to develop and validate a quantification method for these and related alkaloids: ergocristine, ergocryptine, ergotamine, ergovaline, lolitrem B, lysergic acid, *N*-acetylloline, *N*-formylloline, peramine, and paxilline in horse serum.

Horse serum samples (1.5 ml) were worked up by solid-phase extraction (OASIS HLB). The extracts were analyzed by ultra high performance liquid chromatography-high resolution tandem mass spectrometry (UHPLC-HRMS/MS). Chromatographic separation was achieved by gradient elution with ammonium formate buffer and acetonitrile on a RP18 column (100 x 2.1 mm; 1.7 µm). HRMS/MS detection was performed on a QExactive Focus instrument with heated positive electrospray ionization and operated in the parallel reaction monitoring (PRM) mode. Method validation included evaluation of selectivity, matrix effect, recovery, linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, and stability.

With exception of lolitrem B solid phase extraction yielded high recoveries (73.6 - 104.6%) for all analytes. Chromatographic separation of all analytes was achieved with a run time of 25 min. HRMS/MS allowed sensitive detection with LODs ranging from 0.05 to 0.5 ng/ml and LOQs from 0.1 to 1.0 ng/ml. Selectivity experiments showed no interferences from matrix or IS, but *N*-acetylloline and *N*-formylloline were found to be ubiquitous in horse serum. Newborn calf serum was therefore used as surrogate matrix for the validation

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