# Supplementary Materials: A Simple LC–MS Method for the Quantitation of Alkaloids in Endophyte-Infected Perennial Ryegrass

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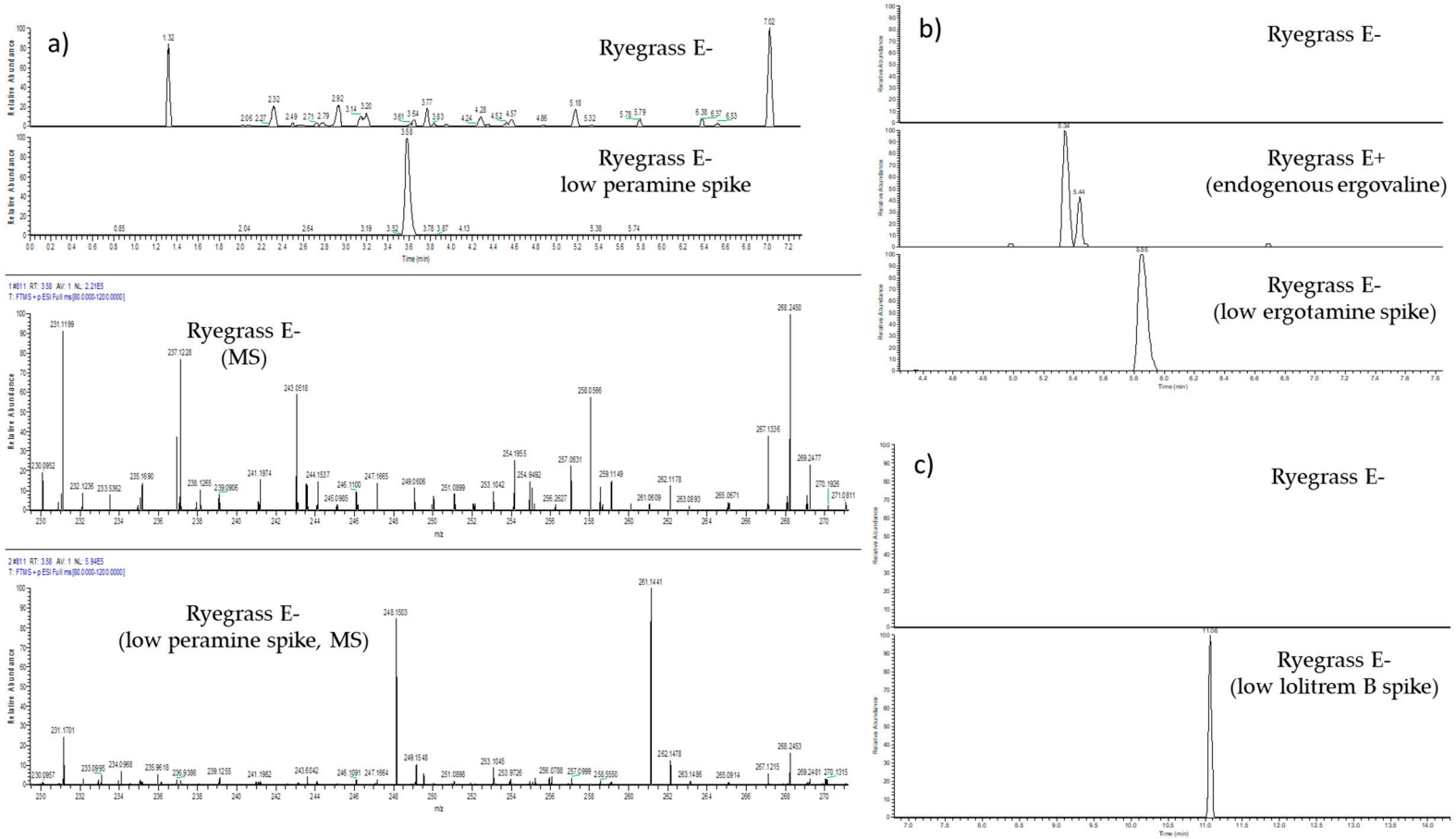


**Figure S1.** Extracted ion chromatograms (EIC) of non-spiked perennial ryegrass without endophyte (E−), or spiked samples at low concentrations (peramine, 8.0 ng/mL; ergotamine, 8.4 ng/mL and lolitrem B, 12 ng/mL). (**a**) The complexity of the plant matrix is demonstrated using the EIC and mass spectrum (MS) of peramine

at 3.58 min *m*/*z* 248.1501. (**b**) No co-eluting peaks were observed for ergovaline/ergotamine *m*/*z* 534.2709/582.2701 or (**c**) lolitrem B *m*/*z* 686.4037. Perennial ryegrass with standard toxic endophyte (E+, endogenous, no spike) was used to determine the elution of ergovaline.

**Table S1.** Matrix effect (ME) and recovery (RE) data for alkaloids spiked at low, medium and high concentrations in perennial ryegrass with (E+) and without (E-) endophyte.

| QC sample | Concentration | | Peramine | | | | Ergotamine | | | | Lolitrem B | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Neat std. | Post-spike | Pre-spike | Endogenous | Neat std. | Post-spike | Pre-spike | Endogenous | Neat std. | Post-spike | Pre-spike | Endogenous |
| E- | Low | Mean. | 302 | 199 | 152 | na | 41 | 31 | 26 | na | 13 | 11 | 10 | na |
| | | SD. | 3.06 | 3.63 | 2.57 | na | 1.24 | 0.82 | 1.32 | na | 0.45 | 1.00 | 0.32 | na |
| | | % RSD | 1.20 | 1.83 | 1.69 | na | 3.04 | 2.63 | 4.99 | na | 3.45 | 9.14 | 3.42 | na |
| | Med | Mean. | 2630 | 1845 | 1461 | na | 416 | 319 | 279 | na | 148 | 118 | 101 | na |
| | | SD. | 24.19 | 4.46 | 17.29 | na | 12.28 | 11.25 | 7.17 | na | 5.45 | 4.29 | 3.62 | na |
| | | % RSD | 0.92 | 0.24 | 1.18 | na | 2.95 | 3.53 | 2.57 | na | 3.69 | 3.64 | 3.59 | na |
| | High | Mean. | 18402 | 14821 | 11957 | na | 4533 | 3450 | 2916 | na | 1480 | 1044 | 835 | na |
| | | SD. | 105.53 | 67.32 | 41.42 | na | 57.74 | 75.45 | 46.21 | na | 54.25 | 35.35 | 21.89 | na |
| | | % RSD | 0.57 | 0.45 | 0.35 | na | 1.27 | 2.19 | 1.58 | na | 3.67 | 3.39 | 2.62 | na |
| E+ | NS | Mean. | na | na | na | 9373 | na | na | na | na | na | na | na | 201 |
| | | SD. | na | na | na | 132.28 | na | na | na | na | na | na | na | 4.48 |
| | | % RSD | na | na | na | 1.41 | na | na | na | na | na | na | na | 2.23 |
| | Low | Mean. | 302 | 9556 | 9534 | na | 41 | 30 | 26 | na | 13 | 217 | 215 | na |
| | | SD. | 3.06 | 117.08 | 187.48 | na | 1.24 | 0.98 | 1.13 | na | 0.45 | 6.30 | 9.45 | na |
| | | % RSD | 1.20 | 1.23 | 1.97 | na | 3.04 | 3.24 | 4.42 | na | 3.45 | 2.91 | 4.39 | na |
| | Med | Mean. | 2630 | 11145 | 10536 | na | 416 | 309 | 268 | na | 148 | 321 | 310 | na |
| | | SD. | 24.19 | 469.73 | 290.25 | na | 12.28 | 3.96 | 3.11 | na | 5.45 | 10.23 | 5.25 | na |
| | | % RSD | 0.92 | 4.21 | 2.75 | na | 2.95 | 1.28 | 1.16 | na | 3.69 | 3.19 | 1.69 | na |
| | High | Mean. | 18402 | 21748 | 19688 | na | 4533 | 3309 | 2836 | na | 1480 | 1198 | 1057 | na |
| | | SD. | 105.53 | 410.17 | 186.57 | na | 57.74 | 37.67 | 88.03 | na | 54.25 | 52.17 | 70.57 | na |
| | | % RSD | 0.57 | 1.89 | 0.95 | na | 1.27 | 1.14 | 3.10 | na | 3.67 | 4.35 | 6.68 | na |

The mean values (peak area) and standard deviations (SD.) are in arbitrary units, $\times 10^4$. % RSD, percent relative standard deviation. Low, medium and high spikes are: peramine at 8.0, 79.7 and 796.9 ng/mL; ergotamine at 8.4, 84.2 and 842.0 ng/mL; and lolitrem B at 12, 120 and 1200 ng/mL. NS, no spike (E+ samples used to measure endogenous peramine and lolitrem B). Data was acquired from the QE MS instrument.

**Table S2.** Comparison of mean alkaloid concentrations for peramine, ergovaline and lolitrem B (parts per million, ppm) in different glasshouse-grown perennial ryegrass-endophyte associations using three different analytical instruments. Values of significance (compared to the QE) are highlighted (*t*-test, $p < 0.05$).

| | | **Peramine** | | **Ergovaline** | | | **Lolitrem B** | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar-Endophyte** | **Statistic** | **QE** | **QQQ** | **QE** | **QQQ** | **FLD** | **QE** | **QQQ** |
| Alto-SE | mean | 50.77 | 52.63 | 0.20 | 0.20 | 0.29 | 20.28 | 18.40 |
| | SEM | 0.69 | 3.16 | 0.01 | 0.00 | 0.04 | 0.55 | 0.49 |
| | RSD (%) | 2.35 | 10.40 | 7.99 | 1.56 | 21.22 | 4.68 | 4.65 |
| | difference (%) | - | 3.65 | - | -0.91 | 48.01 | - | -9.28 |
| | *P* value | - | 0.60 | - | 0.86 | 0.07 | - | 0.06 |
| Alto-NEA3 | mean | 7.84 | 7.93 | 0.29 | 0.29 | 0.29 | n.d | n.d |
| | SEM | 0.08 | 0.43 | 0.02 | 0.01 | 0.02 | - | - |
| | RSD (%) | 1.86 | 9.31 | 9.04 | 3.25 | 11.74 | - | - |
| | difference (%) | - | 1.06 | - | -0.40 | 0.85 | - | - |
| | *P* value | - | 0.86 | - | 0.95 | 0.96 | - | - |
| Trojan-WE | mean | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| | SEM | - | - | - | - | - | - | - |
| | RSD (%) | - | - | - | - | - | - | - |
| | difference (%) | - | - | - | - | - | - | - |
| | *P* value | - | - | - | - | - | - | - |
| Trojan-NEA6 | mean | 4.59 | 4.65 | 1.05 | 0.99 | 1.11 | n.d | n.d |
| | SEM | 0.01 | 0.15 | 0.06 | 0.04 | 0.10 | - | - |
| | RSD (%) | 0.44 | 5.55 | 10.02 | 6.77 | 15.18 | - | - |
| | difference (%) | - | 1.24 | - | -6.05 | 12.15 | - | - |
| | *P* value | - | 0.72 | - | 0.43 | 0.65 | - | - |
| Trojan-NEA10 | mean | 9.05 | 9.76 | 4.74 | 4.86 | 4.38 | n.d | n.d |
| | SEM | 0.04 | 0.67 | 0.16 | 0.28 | 0.19 | - | - |
| | RSD (%) | 0.74 | 11.93 | 5.92 | 9.79 | 7.46 | - | - |
| | difference (%) | - | 7.80 | - | 2.55 | -10.05 | - | - |
| | *P* value | - | 0.35 | - | 0.72 | 0.21 | - | - |
| Trojan-NEA11 | mean | 10.12 | 9.53 | 0.06 | 0.08 | na | n.d | n.d |
| | SEM | 0.16 | 0.81 | 0.01 | 0.00 | na | - | - |
| | RSD (%) | 2.80 | 14.66 | 16.95 | 6.66 | na | - | - |
| | difference (%) | - | -5.84 | - | 41.03 | n.a | - | - |
| | *P* value | - | 0.51 | - | 0.02 | n.a | - | - |
| Trojan-NEA47 | mean | 11.55 | 12.29 | 0.39 | 0.39 | 0.18 | n.d | n.d |
| | SEM | 0.32 | 0.19 | 0.01 | 0.00 | 0.01 | - | - |
| | RSD (%) | 4.86 | 2.63 | 6.49 | 1.40 | 10.87 | - | - |
| | difference (%) | - | 6.47 | - | -1.16 | -52.68 | - | - |
| | *P* value | - | 0.12 | - | 0.78 | 0.0004 | - | - |

Table S3. Continued.

| | | **Peramine** | | **Ergovaline** | | | **Lolitrem B** | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar-Endophyte** | **Statistic** | **QE** | **QQQ** | **QE** | **QQQ** | **FLD** | **QE** | **QQQ** |
| Lp534-NEA21 | mean | 7.07 | 6.65 | n.d | n.d | n.d | n.d | n.d |
| | SEM | 0.05 | 0.43 | - | - | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | RSD (%) | 1.23 | 11.18 | - | - | - | - | - |
| | difference (%) | - | -5.87 | - | - | - | - | - |
| | *P* value | - | 0.39 | - | - | - | - | - |
| Shogun-NEA2 | mean | 1.10 | 0.59 | n.d | n.d | n.d | 1.45 | 1.37 |
| | SEM | 0.02 | 0.04 | - | - | - | 0.04 | 0.03 |
| | RSD (%) | 3.36 | 13.00 | - | - | - | 5.33 | 3.45 |
| | difference (%) | - | -46.72 | - | - | - | - | -5.57 |
| | *P* value | - | 0.0005 | - | - | - | - | 0.20 |

SEM, standard error of the mean; RSD, relative standard deviation of triplicates; n.d, not detected. The difference (%) compares the triple quadruple (QQQ) or the fluorescent detection (FLD) data with that of the Q Exactive (QE) data. No alkaloids were detected in endophyte-free plants (Trojan-WE).

**Table S3.** Retention time (RT), ion information, limit of detection (LOD), limit of quantitation (LOQ) and linearity range of alkaloids detected by the triple quadrupole MS (QQQ MS) instrument and fluoresce detection (FLD).

| Instrument | Compound | RT (min) | Ion Mass (*m*/*z*) | Quantifier Ion (*m*/*z*) | Qualifier Ions (*m*/*z*) | LOD (ng/mL) | LOQ (ng/mL) | Linearity Range (ng/mL) | Equation | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| QQQ | peramine [1] | 3.53 | 248.2 | 175.0 | 189.1 | 0.2 | 0.8 | 0.8–1593.9 | $y=44482.5x^{0.7}$ | 0.9987 |
| | ergotamine [2] | 5.75 | 582.3 | 223.1 | 208.1 | 0.2 | 0.8 | 0.8–1684.0 | y=3903.4x | 0.9998 |
| | ergovaline | 5.23 | 534.3 | 223.1 | 208.1 | - | - | - | - | - |
| | lolitrem B | 11.15 | 686.4 | 628.4 | 238.3 | 1.8 | 6.0 | 6.0–2400.0 | y=92.7x | 0.9942 |
| FLD | ergovaline/ergotamine | 5.27/6.15 | - | - | - | 2.4 | 8.0 | 8.0–210.0 | y=0.1629x | 0.9995 |

[1] The standards used for peramine were not linear on the QQQ MS, therefore, a power curve was used to calculate the $R^2$ value. [2] Ergovaline was quantified using ergotamine. The indicated RT and mass window for ergovaline was established using perennial ryegrass with standard toxic endophyte QC samples (E+).