Article

Climate Effects on Ergot and Ergot Alkaloids Occurrence in Italian Wheat

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1. Supplementary Material

1.1 Tables

**Table S1.** LC conditions

|  |
| --- |
| **LC conditions** |
| **LC-MS/MS equipment** | XEVO TQ-Xs Acquity UPLC I Class Plus Waters |
| **LC Column** | UPLC BEH C18 1.7µm 100 mm x 2.1 mm Waters |
| **Mobile Phase** | A: 10 mM ammonium carbonate solution (pH 10)B: acetonitrile |
| **Flow** | 0.4 mL/min |
| **Injection volume** | 5µL |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Time** **(min)** | **Mobile phase A****(%)** | **Mobile Phase B****(%)** |
| 088.511 | 80308080 | 20702020 |

**Table S2.** Gradient Elution Program

|  |
| --- |
| **MS/MS parameters** |
| Ionization mode Capillary voltage  | ESI+ 0.50 kV  |
| Source Temperature  | 150°C  |
| Cone voltage | 35 V  |
| Desolvation Temperature   | 600°C  |

**Table S3.** MS/MS parameters

**Table S4.** Total content of 12 ergot alkaloids in sclerotia samples and corresponding wet weight.

|  |  |  |
| --- | --- | --- |
| **Samples number1** | **Wet Weight (g)** | **T-EAs2 (µg/kg)** |
| **S1** | 0.24 | 1,158,760 |
| **S2** | 0.32 | 698,640 |
| **S3** | 0.20 | 819,530 |
| **S4** | 0.20 | 110,090 |
| **S5** | 0.36 | 639,100 |
| **S6** | 0.18 | 1,421,970 |
| **S7** | 0.08 | 147,050 |
| **S8** | 0.07 | 698,960 |
| **S9** | 0.07 | 337,720 |
| **S10** | 0.08 | 702,140 |
| **S11** | 0.06 | 4,951,190 |
| **S12** | 0.02 | 1,019,080 |
| **S13****S14** | 0.030.03 | 1,208,1101,218,340 |
| **S15** | 0.01 | 2,590 |
| **WE1** | 1.00 | 2,300 |
| **WE2** | 0.52 | 70 |
| **WE3** | 0.23 | 120 |
| **WE4** | 0.47 | 50 |
| **WE5** | 0.49 | 60 |
| **WE6** | 0.14 | 30 |
| **WE7** | 0.36 | 10 |
| **WE8** | 0.76 | 20 |
| **WE9** | 0.63 | 1,450 |
| **WE10** | 0.36 | 33,110 |
| **WE11** | 0.66 | 40 |
| **WK1** | 10.00 | 1,150 |

1 S= sclerotia; WE= wheat ear; WK= wheat kernels; 2 Total content of ergot alkaloids (T-EAs)

**Table S5.** The concentrations of ergot alkaloids (-ine form) in analyzed samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples number1 | Ergocornine µg/kg | Ergocristine µg/kg | Ergocryptine µg/kg | Ergosine µg/kg | Ergometrine µg/kg | Ergotamine µg/kg |
| S1 | 10,872.0 | 187,479.0 | 136,652.0 | 259,960.0 | 102,561.0 | 56,559.0 |
| S2 | < LOQ2 | 210,342.0 | 1,859.0 | 97,553.0 | 167.0 | 133,249.0 |
| S3 | < LOQ | 216,586.0 | 614.0 | 30,381.0 | 77,210.0 | 123,333.0 |
| S4 | 1,207.0 | 64.0 | 23,074.0 | 25,161.0 | 2,965.0 | 92.0 |
| S5 | 75,324.0 | 24,035.0 | 41,668.0 | 154,380.0 | 59,333.0 | 9,224.0 |
| S6 | < LOQ | 440,337.1 | 3,317.1 | 337,221.6 | 124,045.8 | 220,254.2 |
| S7 | < LOQ | 56,821.4 | 398.5 | 19,582.4 | 69.4 | 28,221.4 |
| S8 | 142,094.4 | 13,137.1 | 88,984.2 | 174,590.5 | 38,327.0 | 9,275.7 |
| S9 | 2,155.5 | 72,791.4 | 2,060.0 | 92,845.9 | 36,023.5 | 13,778.5 |
| S10 | 203,251.3 | 984.2 | 107,525.7 | 143,216.2 | 29,852.9 | 1,244.2 |
| S11 | 1,001,533.3 | 190.0 | 623,237.1 | 705,720.2 | 251,147.0 | 1,331.4 |
| S12 | 45,901.3 | 124.2 | 331,617.1 | 16,454.0 | 111,205.8 | 34.2 |
| S13 | 335,620.8 | 65.7 | 150,771.4 | 38,762.1 | 225,564.1 | 312.8 |
| S14 | 121,872.0 | 39.0 | 175,879.0 | 86,869.0 | 355,865.0 | 422.0 |
| S15 | 180.5 | 88.5 | 1,194.2 | 294.5 | 288.2 | 85.7 |
| WE1 | 118.0 | 82.0 | 56.0 | 432.0 | 674.0 | 110.0 |
| WE2 | 5.0 | < LOQ | < LOQ | 4.0 | 40.0 | < LOQ |
| WE3 | 9.0 | < LOQ | 4.0 | 10.0 | 55.0 | < LOQ |
| WE4 | 5.0 | < LOQ | < LOQ | 6.0 | 22.0 | < LOQ |
| WE5 | < LOQ | < LOQ | 5.0 | 4.0 | 33.0 | < LOQ |
| WE6 | < LOQ | < LOQ | < LOQ | < LOQ | 26.0 | < LOQ |
| WE7 | < LOQ | < LOQ | < LOQ | < LOQ | 8.0 | < LOQ |
| WE8 | < LOQ | < LOQ | < LOQ | < LOQ | 12.0 | < LOQ |
| WE9 | 3.00 | < LOQ | 18.0 | 947.0 | 30.0 | < LOQ |
| WE10 | 1,037.0 | 1,930.0 | 13,230.0 | 2,240.0 | 1,680.0 | 466.0 |
| WE11 | 3.0 | < LOQ | 10.0 | 5.0 | 14.0 | < LOQ |
| WK1 | 3.0 | 350.0 | 24.0 | 181.0 | 148.0 | 68.0 |

1 S= sclerotia; WE= wheat ear; WK= wheat kernels; 2LOQ= 2 µg/kg

**Table S6.** The concentrations of ergot alkaloids (-inine form) in analyzed samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples number1 | Ergocorninine µg/kg | Ergocristinine µg/kg | Ergocryptinine µg/kg | Ergosinine µg/kg | Ergometrinine µg/kg | Ergotaminine µg/kg |
| S1 | 10,559.0 | 95,926.0 | 83,574.0 | 122,834.0 | 70,986.0 | 20,802.0 |
| S2 | < LOQ2 | 138,875.0 | 1,364.0 | 50,141.0 | 130.0 | 64,958.0 |
| S3 | < LOQ | 216,664.0 | 641.0 | 17,975.0 | 62,254.0 | 73,870.0 |
| S4 | 3,013.0 | 67.0 | 32,031.0 | 17,584.0 | 4,791.0 | 41.0 |
| S5 | 86,187.0 | 18,347.0 | 35,447.0 | 87,265.0 | 44,131.0 | 3,758.0 |
| S6 | 844.0 | 146,938.5 | 892.9 | 68,664.9 | 36,152.0 | 43,304.2 |
| S7 | < LOQ | 24,827.7 | 192.9 | 7,414.9 | 20.0 | 9,504.2 |
| S8 | 92,710.7 | 4,330.8 | 40,957.1 | 67,154.1 | 24,540.0 | 2,861.1 |
| S9 | 2,028.0 | 40,096.9 | 1,501.4 | 43,527.0 | 25,221.3 | 5,688.9 |
| S10 | 107,953.3 | 426.2 | 41,721.4 | 52,867.6 | 12,734.7 | 361.1 |
| S11 | 114,2236.0 | 126.2 | 630,792.9 | 436,247.3 | 157,321.3 | 1,305.6 |
| S12 | 65,685.3 | 133.8 | 303,358.6 | 57,189.2 | 87,372.0 | < LOQ |
| S13 | 211,729.3 | 23.1 | 74,328.6 | 34,589.2 | 136,224.0 | 122.2 |
| S14 | 113,326.0 | 13.0 | 148,029.0 | 41,836.0 | 174,002.0 | 188.0 |
| S15 | 108.0 | 36.9 | 85.7 | 97.3 | 129.3 | < LOQ |
| WE1 | 118.0 | 75.0 | 48.0 | 238.0 | 298.0 | 53.0 |
| WE2 | 3.0 | < LOQ | < LOQ | < LOQ | 21.0 | < LOQ |
| WE3 | 8.0 | < LOQ | < LOQ | 5.0 | 33.0 | < LOQ |
| WE4 | < LOQ | < LOQ | < LOQ | 3.0 | 10.0 | < LOQ |
| WE5 | < LOQ | < LOQ | < LOQ | < LOQ | 17.0 | < LOQ |
| WE6 | < LOQ | < LOQ | < LOQ | < LOQ | 8.0 | < LOQ |
| WE7 | < LOQ | < LOQ | < LOQ | < LOQ | 4.0 | < LOQ |
| WE8 | < LOQ | < LOQ | < LOQ | < LOQ | 7.0 | < LOQ |
| WE9 | < LOQ | < LOQ | 15.0 | 421.0 | 18.0 | < LOQ |
| WE10 | 846.0 | 1,488.0 | 7,732.0 | 1,127.0 | 1,136.0 | 196.0 |
| WE11 | < LOQ | < LOQ | < LOQ | < LOQ | 8.0 | < LOQ |
| WK1 | 3.0 | 181.0 | 13.0 | 83.0 | 75.0 | 25.0 |

1 S= sclerotia; WE= wheat ear; WK= wheat kernels; 2LOQ= 2 µg/kg

**Table S7.** Validation Parameters for each EA -ine form at six spiking levels

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Samples | Spiking Levelµg/kg | Mean Conc µg/kg | SD1 | RSDr2 | Mean Recovery % |
| Ergocornine | **2** | 1.5 | 0.16 | 10.79 | 79.3 |
| **10** | 8.3 | 0.66 | 7.90 |
| **50** | 43.2 | 4.32 | 9.99 |
| **150** | 112.9 | 5.80 | 5.14 |
| **250** | 213.0 | 17.42 | 8.18 |
| **600** | 422.5 | 36.00 | 8.52 |
| Ergocristine | **2** | 1.4 | 0.16 | 11.16 | 74.7 |
| **10** | 7.4 | 0.78 | 10.44 |
| **50** | 39.5 | 1.92 | 4.86 |
| **150** | 107.2 | 2.59 | 2.41 |
| **250** | 188.9 | 21.49 | 11.37 |
| **600** | 444.1 | 28.40 | 6.39 |
| Ergocryptine | **2** | 1.4 | 0.18 | 12.90 | 77.4 |
| **10** | 7.7 | 0.69 | 8.87 |
| **50** | 41.4 | 4.29 | 10.34 |
| **150** | 105.1 | 8.29 | 7.89 |
| **250** | 197.9 | 15.46 | 7.81 |
| **600** | 504.7 | 55.71 | 11.04 |
| Ergosine | **2** | 1.6 | 0.20 | 12.33 | 86.9 |
| **10** | 8.8 | 0.68 | 7.77 |
| **50** | 44.0 | 3.41 | 7.74 |
| **150** | 120.6 | 6.29 | 5.22 |
| **250** | 233.1 | 21.69 | 9.30 |
| **600** | 548.4 | 62.44 | 11.38 |
| Ergometrine | **2** | 1.6 | 0.12 | 7.41 | 90.7 |
| **10** | 8.5 | 0.48 | 5.61 |
| **50** | 38.1 | 2.56 | 6.69 |
| **150** | 129.8 | 7.47 | 5.75 |
| **250** | 231.5 | 13.82 | 5.97 |
| **600** | 717.8 | 52.49 | 7.31 |
| Ergotamine | **2** | 1.5 | 0.25 | 16.12 | 76.7 |
| **10** | 7.8 | 0.82 | 10.47 |
| **50** | 39.5 | 2.70 | 6.82 |
| **150** | 107.7 | 10.31 | 9.57 |
| **250** | 202.7 | 24.59 | 12.13 |
| **600** | 439.2 | 77.67 | 17.68 |

1 Standard Deviation; 2The Coefficient of Variation (CV%) of the repeatability was calculated by analyzing blank samples in six replicates at the six fortified levels.

**Table S8.** Validation Parameters for each EA -inine form at six spiking levels

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Samples | Spiking Levelµg/kg | Mean conc µg/kg | Sd1 | RSDr2 | Mean Recovery% |
| Ergocorninine | **2** | 1.6 | 0.19 | 11.50 | 88.9 |
| **10** | 8.9 | 0.64 | 7.11 |
| **50** | 47.6 | 6.41 | 13.46 |
| **150** | 121.3 | 8.70 | 7.17 |
| **250** | 230.5 | 30.00 | 13.01 |
| **600** | 549.7 | 71.81 | 13.06 |
| Ergocristinine | **2** | 1.5 | 0.18 | 11.69 | 80.7 |
| **10** | 8.2 | 0.83 | 10.02 |
| **50** | 43.9 | 3.78 | 8.60 |
| **150** | 108.3 | 7.33 | 6.77 |
| **250** | 199.5 | 19.41 | 9.73 |
| **600** | 515.2 | 55.73 | 10.82 |
| Ergocryptinine | **2** | 1.7 | 0.14 | 7.56 | 79.8 |
| **10** | 8.0 | 0.61 | 7.53 |
| **50** | 41.1 | 4.27 | 10.38 |
| **150** | 105.0 | 4.91 | 4.67 |
| **250** | 195.9 | 16.91 | 8.63 |
| **600** | 466.6 | 50.25 | 10.77 |
| Ergosinine | **2** | 1.7 | 0.13 | 7.62 | 91.2 |
| **10** | 9.0 | 0.47 | 5.21 |
| **50** | 46.4 | 3.96 | 8.53 |
| **150** | 126.6 | 8.71 | 6.88 |
| **250** | 237.2 | 22.02 | 9.28 |
| **600** | 594.6 | 39.68 | 6.67 |
| Ergometrinine | **2** | 1.6 | 0.12 | 7.01 | 91.1 |
| **10** | 8.7 | 0.52 | 6.01 |
| **50** | 41.4 | 2.92 | 7.04 |
| **150** | 129.6 | 10.10 | 7.79 |
| **250** | 231.5 | 14.91 | 6.52 |
| **600** | 717.8 | 52.08 | 7.55 |
| Ergotaminine | **2** | 1.6 | 0.14 | 8.54 | 87.2 |
| **10** | 8.9 | 0.74 | 8.33 |
| **50** | 45.1 | 3.56 | 7.89 |
| **150** | 116.9 | 7.63 | 6.52 |
| **250** | 214.2 | 14.81 | 6.91 |
| **600** | 580.0 | 40.39 | 6.96 |
| 1 Standard Deviation; 2 The Coefficient of Variation (CV%) of the repeatability was calculated by analyzing blank samples in six replicates at the six fortified levels. |

**Table S9.** Validation Parameters for EAs sum

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Samples | Spiking Levelµg/kg | Mean conc µg/kg | Sd1 | RSDr2 | Mean Recovery % |
| EAs sum | **24** | 19 | 1.77 | 9.2 | 83.7 |
| **120** | 101 | 7.35 | 7.3 |
| **600** | 512 | 37.28 | 7.3 |
| **1,800** | 1,392 | 72.85 | 5.2 |
| **3,000** | 2,574 | 204.20 | 7.9 |
| **7,200** | 6,473 | 388.05 | 6.0 |

1 Standard Deviation; 2 The Coefficient of Variation (CV%) of the repeatability was calculated by analyzing blank samples in six replicates at the six fortified levels.

* 1. Figures

**Figure S1.** Chromatograms of ergocristine, ergocryptine and corresponding epimers at 3 ng/mL



**Figure S2.** Chromatograms of ergosine, ergocornine and corresponding epimers at 3 ng/mL



**Figure S3.** Chromatograms of ergotamine, ergometrine and corresponding epimers at 3 ng/mL