Determination of Aflatoxin B1 in Smokeless Tobacco Products by use of UHPLC-MS/MS

Supporting Information

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# Supplemental Table 1. UHPLC-MS/MS Analysis Parameters for AFB1, AFB2, AFG1, and AFG2

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Analyte*a* | Parent ion (*m*/*z*) | Product ion (*m*/*z*) | Dwell time (s) | Cone voltage (V) | Collision voltage (V) | Retention time window (min) |
| AFB1 (Q) | 313.32 | 241.22 | 0.033 | 48 | 34 | 0.85–1.2 |
| AFB1 (C) | 313.32 | 285.08 | 0.033 | 48 | 22 | 0.85–1.2 |
| 13C17-AFB1 (IS) | 330.37 | 301.09 | 0.033 | 40 | 22 | 0.85–1.2 |
| AFB2 (Q) | 315.30 | 259.13 | 0.033 | 58 | 28 | 0.8–1.0 |
| AFB2 (C) | 315.30 | 287.12 | 0.033 | 58 | 24 | 0.8–1.0 |
| 13C17-AFB2 (IS) | 332.37 | 303.22 | 0.033 | 38 | 26 | 0.8–1.0 |
| AFG1 (Q) | 329.30 | 199.96 | 0.033 | 52 | 38 | 0.7–0.9 |
| AFG1 (C) | 329.30 | 243.10 | 0.033 | 52 | 26 | 0.7–0.9 |
| 13C17-AFG1 (IS) | 346.37 | 257.17 | 0.033 | 30 | 28 | 0.7–0.9 |
| AFG2 (Q) | 331.33 | 189.09 | 0.052 | 54 | 40 | 0–0.8 |
| AFG2 (C) | 331.33 | 245.12 | 0.052 | 54 | 28 | 0–0.8 |
| 13C17-AFG2 (IS) | 348.45 | 200.17 | 0.052 | 64 | 40 | 0–0.8 |

*a*Q: quantitation ion; C: confirmation ion; IS: internal standard.

# Supplemental Table 2. Analytical Performance for AFB2, AFG1, and AFG2

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | Transition | LOD (ppb)*a* | QC sample tested | | Characterization mean and standard deviation (SD)*b* | | | Recovery of target spike (%)*b* |
| Pool | Target (ppb) | Mean (ppb) | Total SD (ppb) | CV (%) |
| AFB2 | 315.3 > 287.1 | 0.01 | Low | 0.20 | 0.203 | 0.017 | 8.6 | 101 |
| Med | 0.60 | 0.610 | 0.050 | 8.1 | 102 |
| High | 2.0 | 2.06 | 0.19 | 9.4 | 103 |
| 315.3 > 259.1 | 0.01 | Low | 0.20 | 0.203 | 0.016 | 8.0 | 102 |
| Med | 0.60 | 0.605 | 0.050 | 8.3 | 101 |
| High | 2.0 | 2.04 | 0.18 | 8.9 | 102 |
| AFG1 | 329.3 > 200.0 | 0.02 | Low | 0.4 | 0.404 | 0.034 | 8.4 | 101 |
| Med | 1.2 | 1.21 | 0.08 | 6.2 | 101 |
| High | 3.5 | 3.55 | 0.24 | 6.8 | 101 |
| 329.3 > 243.1 | 0.05 | Low | 0.4 | 0.404 | 0.101 | 25.1 | 101 |
| Med | 1.2 | 1.25 | 0.123 | 9.8 | 104 |
| High | 3.5 | 3.54 | 0.239 | 6.8 | 101 |
| AFG2 | 331.3 > 189.1 | 0.1 | Low | 0.8 | 0.805 | 0.088 | 10.9 | 101 |
| Med | 2.4 | 2.45 | 0.156 | 6.3 | 102 |
| High | 3.5 | 3.59 | 0.206 | 5.7 | 102 |
| 331.3 > 245.1 | 0.1 | Low | 0.8 | 0.783 | 0.147 | 18.8 | 98 |
| Med | 2.4 | 2.46 | 0.195 | 7.9 | 103 |
| High | 3.5 | 3.59 | 0.264 | 7.4 | 102 |

*a*Measured over 15 d (1 replicate/d). *b*Measured over 20 d (2 replicates/d).

# Supplemental Table 3. Robustness Testing: Volume of Sample Extract Loaded on IAC Column

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | Transition | QC sample tested | | Method specification:  1 mL loading of IAC*a* | | Test condition 1: 0.5 mL loading of IAC (0.5× SOP specification)*b* | | Test condition 2: 2 mL loading of IAC  (2× SOP specification)*b* | |
| Pool | Target (ppb) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) |
| AFB1 | 313.3  >  241.2 | Low | 0.07 | 0.078 | 6.0 | 0.080 | 10.6 | 0.077 | 4.7 |
| Medium | 0.21 | 0.220 | 5.4 | 0.225 | 10.4 | 0.234 | 8.0 |
| High | 0.70 | 0.738 | 4.4 | 0.753 | 5.2 | 0.764 | 8.0 |
| 313.3  >  285.1 | Low | 0.07 | 0.079 | 9.7 | 0.081 | 14.2 | 0.080 | 6.4 |
| Medium | 0.21 | 0.220 | 5.7 | 0.242 | 11.7 | 0.236 | 8.6 |
| High | 0.70 | 0.730 | 5.1 | 0.706 | 4.0 | 0.741 | 5.1 |
| AFB2 | 315.3  >  287.1 | Low | 0.20 | 0.203 | 6.8 | 0.220 | 12.0 | 0.206 | 6.6 |
| Medium | 0.60 | 0.610 | 6.4 | 0.624 | 6.1 | 0.626 | 3.9 |
| High | 2.0 | 2.06 | 6.7 | 2.12 | 7.4 | 2.04 | 4.7 |
| 315.3  >  259.1 | Low | 0.20 | 0.203 | 7.1 | 0.204 | 6.8 | 0.211 | 5.7 |
| Medium | 0.60 | 0.605 | 6.6 | 0.655 | 6.3 | 0.625 | 4.0 |
| High | 2.0 | 2.04 | 6.3 | 2.07 | 5.1 | 2.03 | 9.4 |
| AFG1 | 329.3  >  200.0 | Low | 0.4 | 0.404 | 6.3 | 0.446 | 8.1 | 0.387 | 8.6 |
| Medium | 1.2 | 1.21 | 6.6 | 1.22 | 8.1 | 1.21 | 5.6 |
| High | 3.5 | 3.55 | 6.8 | 3.81 | 7.7 | 3.67 | 10.2 |
| 329.3  >  243.1 | Low | 0.4 | 0.404 | 15.0 | 0.586 | 42.9 | 0.371 | 13.6 |
| Medium | 1.2 | 1.25 | 7.7 | 1.24 | 16.2 | 1.10 | 5.2 |
| High | 3.5 | 3.54 | 5.3 | 3.58 | 13.8 | 3.39 | 8.9 |
| AFG2 | 331.3  >  189.1 | Low | 0.8 | 0.805 | 12.7 | 0.807 | 19.7 | 0.732 | 13.6 |
| Medium | 2.4 | 2.45 | 5.6 | 2.52 | 4.7 | 2.51 | 9.2 |
| High | 3.5 | 3.59 | 5.6 | 3.63 | 6.4 | 3.79 | 7.1 |
| 331.3  >  245.1 | Low | 0.8 | 0.783 | 11.1 | 0.704 | 35.3 | 0.650 | 13.2 |
| Medium | 2.4 | 2.46 | 6.1 | 2.61 | 23.0 | 2.43 | 7.5 |
| High | 3.5 | 3.59 | 6.5 | 3.81 | 9.2 | 3.65 | 9.4 |

*a*Measured over 20 d (2 replicates/d). *b*Measured over 1 d (7 replicates)*.*

# Supplemental Table 4. Robustness Testing: Extraction Composition

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | Transition | QC sample tested | | Method specification:  80:20 methanol:water*a* | | Test condition 1: 70:30 methanol:water*b* | | Test condition 2: 90:10 methanol:water*b* | |
| Pool | Target (ppb) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) |
| AFB1 | 313.3  >  241.2 | Low | 0.07 | 0.078 | 6.0 | 0.073 | 7.1 | 0.082 | 4.1 |
| Medium | 0.21 | 0.220 | 5.4 | 0.228 | 4.4 | 0.228 | 6.1 |
| High | 0.70 | 0.738 | 4.4 | 0.721 | 2.7 | 0.77 | 4.3 |
| 313.3  >  285.1 | Low | 0.07 | 0.079 | 9.7 | 0.077 | 6.9 | 0.074 | 8.4 |
| Medium | 0.21 | 0.220 | 5.7 | 0.225 | 4.2 | 0.224 | 3.9 |
| High | 0.70 | 0.730 | 5.1 | 0.750 | 2.4 | 0.75 | 5.2 |
| AFB2 | 315.3  >  287.1 | Low | 0.20 | 0.203 | 6.8 | 0.208 | 6.7 | 0.206 | 7.4 |
| Medium | 0.60 | 0.610 | 6.4 | 0.632 | 6.1 | 0.602 | 7.0 |
| High | 2.0 | 2.06 | 6.7 | 2.004 | 4.4 | 1.93 | 4.0 |
| 315.3  >  259.1 | Low | 0.20 | 0.203 | 7.1 | 0.198 | 7.1 | 0.213 | 9.0 |
| Medium | 0.60 | 0.605 | 6.6 | 0.621 | 4.8 | 0.62 | 4.6 |
| High | 2.0 | 2.04 | 6.3 | 2.00 | 5.5 | 1.99 | 5.3 |
| AFG1 | 329.3  >  200.0 | Low | 0.4 | 0.404 | 6.3 | 0.402 | 5.7 | 0.413 | 7.6 |
| Medium | 1.2 | 1.21 | 6.6 | 1.22 | 5.2 | 1.19 | 5.4 |
| High | 3.5 | 3.55 | 6.8 | 3.735 | 4.3 | 3.68 | 4.0 |
| 329.3  >  243.1 | Low | 0.4 | 0.404 | 15.0 | 0.400 | 6.6 | 0.419 | 10.2 |
| Medium | 1.2 | 1.25 | 7.7 | 1.21 | 6.4 | 1.25 | 5.5 |
| High | 3.5 | 3.54 | 5.3 | 3.62 | 6.5 | 3.77 | 4.0 |
| AFG2 | 331.3  >  189.1 | Low | 0.8 | 0.805 | 12.7 | 0.797 | 3.8 | 0.842 | 7.3 |
| Medium | 2.4 | 2.45 | 5.6 | 2.45 | 3.6 | 2.48 | 4.4 |
| High | 3.5 | 3.59 | 5.6 | 3.68 | 6.4 | 3.71 | 6.6 |
| 331.3  >  245.1 | Low | 0.8 | 0.783 | 11.1 | 0.817 | 3.7 | 0.880 | 8.5 |
| Medium | 2.4 | 2.46 | 6.1 | 2.46 | 6.9 | 2.51 | 5.5 |
| High | 3.5 | 3.59 | 6.5 | 3.61 | 5.5 | 3.61 | 6.7 |

*a*Measured over 20 d (2 replicates/d). *b*Measured over 1 d (7 replicates)*.*

# Supplemental Table 5. Robustness Testing: Mass of Tobacco Extracted

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | Transition | QC sample tested | | Method specification: 2 g dried, milled tobacco*a* | | Test condition 1: 1 g dried, milled tobacco*b* | | Test condition 2: 3g dried, milled tobacco*b* | |
| Pool | Target (ppb) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) |
| AFB1 | 313.3  >  241.2 | Low | 0.07 | 0.078 | 6.0 | 0.075 | 7.1 | 0.077 | 6.1 |
| Medium | 0.21 | 0.220 | 5.4 | 0.218 | 1.6 | 0.224 | 3.9 |
| High | 0.70 | 0.738 | 4.4 | 0.724 | 3.4 | 0.762 | 3.1 |
| 313.3  >  285.1 | Low | 0.07 | 0.079 | 9.7 | 0.071 | 9.0 | 0.075 | 11.1 |
| Medium | 0.21 | 0.220 | 5.7 | 0.218 | 3.5 | 0.227 | 5.3 |
| High | 0.70 | 0.730 | 5.1 | 0.753 | 3.5 | 0.757 | 2.0 |
| AFB2 | 315.3  >  287.1 | Low | 0.20 | 0.203 | 6.8 | 0.205 | 3.1 | 0.200 | 7.4 |
| Medium | 0.60 | 0.610 | 6.4 | 0.598 | 6.8 | 0.629 | 3.4 |
| High | 2.0 | 2.06 | 6.7 | 2.04 | 6.3 | 2.05 | 4.7 |
| 315.3  >  259.1 | Low | 0.20 | 0.203 | 7.1 | 0.199 | 6.4 | 0.205 | 5.1 |
| Medium | 0.60 | 0.605 | 6.6 | 0.625 | 4.8 | 0.642 | 2.3 |
| High | 2.0 | 2.04 | 6.3 | 2.09 | 6.6 | 2.10 | 5.0 |
| AFG1 | 329.3  >  200.0 | Low | 0.4 | 0.404 | 6.3 | 0.423 | 5.1 | 0.411 | 5.0 |
| Medium | 1.2 | 1.21 | 6.6 | 1.235 | 4.4 | 1.20 | 3.9 |
| High | 3.5 | 3.55 | 6.8 | 3.64 | 3.2 | 3.48 | 1.2 |
| 329.3  >  243.1 | Low | 0.4 | 0.404 | 15.0 | 0.414 | 11.1 | 0.421 | 11.1 |
| Medium | 1.2 | 1.25 | 7.7 | 1.278 | 2.2 | 1.20 | 3.6 |
| High | 3.5 | 3.54 | 5.3 | 3.62 | 4.2 | 3.51 | 6.5 |
| AFG2 | 331.3  >  189.1 | Low | 0.8 | 0.805 | 12.7 | 0.377 | 1.6 | 0.820 | 6.3 |
| Medium | 2.4 | 2.45 | 5.6 | 2.58 | 3.9 | 2.49 | 6.0 |
| High | 3.5 | 3.59 | 5.6 | 3.76 | 7.4 | 3.57 | 3.2 |
| 331.3  >  245.1 | Low | 0.8 | 0.783 | 11.1 | 0.387 | 11.8 | 0.848 | 5.2 |
| Medium | 2.4 | 2.46 | 6.1 | 2.59 | 3.8 | 2.54 | 9.1 |
| High | 3.5 | 3.59 | 6.5 | 3.71 | 7.0 | 3.45 | 1.6 |

*a*Measured over 20 d (2 replicates/d). *b*Measured over 1 d (7 replicates)*.*

# Supplemental Table 6. Robustness Testing: Volume of Elution Solvent Used in IAC Step

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | Transition | QC pool tested | | SOP specification:  1 mL acetonitrile*a* | | Test condition 1: 0.5 mL acetonitrile (0.5× SOP specification)*b* | | Test condition 2: 2 mL acetonitrile  (2× SOP specification)*b* | |
| Pool | Target (ppb) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) |
| AFB1 | 313.3  >  241.2 | Low | 0.07 | 0.078 | 6.0 | 0.077 | 7.2 | 0.078 | 13.0 |
| Medium | 0.21 | 0.220 | 5.4 | 0.231 | 7.3 | 0.231 | 9.9 |
| High | 0.70 | 0.738 | 4.4 | 0.747 | 5.4 | 0.760 | 9.0 |
| 313.3  >  285.1 | Low | 0.07 | 0.079 | 9.7 | 0.077 | 8.9 | 0.076 | 15.0 |
| Medium | 0.21 | 0.220 | 5.7 | 0.245 | 10.1 | 0.234 | 9.1 |
| High | 0.70 | 0.730 | 5.1 | 0.743 | 3.4 | 0.741 | 9.3 |
| AFB2 | 315.3  >  287.1 | Low | 0.20 | 0.203 | 6.8 | 0.222 | 12.3 | 0.207 | 6.8 |
| Medium | 0.60 | 0.610 | 6.4 | 0.633 | 9.3 | 0.621 | 5.3 |
| High | 2.0 | 2.06 | 6.7 | 2.15 | 6.5 | 2.08 | 4.9 |
| 315.3  >  259.1 | Low | 0.20 | 0.203 | 7.1 | 0.218 | 14.0 | 0.210 | 8.0 |
| Medium | 0.60 | 0.605 | 6.6 | 0.635 | 13.5 | 0.632 | 4.7 |
| High | 2.0 | 2.04 | 6.3 | 2.13 | 6.5 | 2.08 | 3.0 |
| AFG1 | 329.3  >  200.0 | Low | 0.4 | 0.404 | 6.3 | 0.413 | 8.6 | 0.396 | 12.9 |
| Medium | 1.2 | 1.21 | 6.6 | 1.17 | 5.9 | 1.15 | 8.1 |
| High | 3.5 | 3.55 | 6.8 | 3.4 | 10.6 | 3.59 | 5.3 |
| 329.3  >  243.1 | Low | 0.4 | 0.404 | 15.0 | 0.464 | 17.3 | 0.404 | 10.8 |
| Medium | 1.2 | 1.25 | 7.7 | 1.14 | 20.9 | 1.23 | 10.9 |
| High | 3.5 | 3.54 | 5.3 | 3.45 | 9.2 | 3.36 | 8.3 |
| AFG2 | 331.3  >  189.1 | Low | 0.8 | 0.805 | 12.7 | 0.765 | 18.3 | 0.778 | 8.1 |
| Medium | 2.4 | 2.45 | 5.6 | 2.67 | 9.4 | 2.49 | 5.7 |
| High | 3.5 | 3.59 | 5.6 | 3.74 | 10.4 | 3.62 | 4.9 |
| 331.3  >  245.1 | Low | 0.8 | 0.783 | 11.1 | 0.738 | 33.0 | 0.739 | 13.0 |
| Medium | 2.4 | 2.46 | 6.1 | 2.57 | 16.2 | 2.51 | 7.4 |
| High | 3.5 | 3.59 | 6.5 | 3.70 | 15.4 | 3.58 | 6.1 |

*a*Measured over 20 d (2 replicates/d). *b*Measured over 1 d (7 replicates)*.*

# Supplemental Table 7. Robustness Testing: Substituting IAC Products

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | Transition | QC pool tested | | SOP specification:  Vicam Afla Test WB*a* | | Test condition 1: Romer Aflastar Fit*b* | | Test condition 2:  Romer Aflastar R*b* | |
| Pool | Target (ppb) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) | Mean (ppb) | CV (%) |
| AFB1 | 313.3  >  241.2 | Low | 0.07 | 0.078 | 6.0 | 0.081 | 11.4 | 0.107 | 4.3 |
| Medium | 0.21 | 0.220 | 5.4 | 0.220 | 3.1 | 0.249 | 6.0 |
| High | 0.70 | 0.738 | 4.4 | 0.711 | 3.5 | 0.785 | 3.5 |
| 313.3  >  285.1 | Low | 0.07 | 0.079 | 9.7 | 0.080 | 7.9 | 0.114 | 5.1 |
| Medium | 0.21 | 0.220 | 5.7 | 0.226 | 4.5 | 0.254 | 6.1 |
| High | 0.70 | 0.730 | 5.1 | 0.715 | 7.9 | 0.772 | 6.3 |
| AFB2 | 315.3  >  287.1 | Low | 0.20 | 0.203 | 6.8 | 0.201 | 6.1 | 0.185 | 6.6 |
| Medium | 0.60 | 0.610 | 6.4 | 0.575 | 6.8 | 0.552 | 3.7 |
| High | 2.0 | 2.06 | 6.7 | 1.97 | 3.0 | 1.85 | 3.1 |
| 315.3  >  259.1 | Low | 0.20 | 0.203 | 7.1 | 0.193 | 9.9 | 0.188 | 6.6 |
| Medium | 0.60 | 0.605 | 6.6 | 0.595 | 8.0 | 0.555 | 8.6 |
| High | 2.0 | 2.04 | 6.3 | 1.94 | 7.2 | 1.90 | 2.5 |
| AFG1 | 329.3  >  200.0 | Low | 0.4 | 0.404 | 6.3 | 0.414 | 4.6 | 0.443 | 3.2 |
| Medium | 1.2 | 1.21 | 6.6 | 1.29 | 5.6 | 1.22 | 5.0 |
| High | 3.5 | 3.55 | 6.8 | 3.64 | 3.8 | 3.73 | 2.5 |
| 329.3  >  243.1 | Low | 0.4 | 0.404 | 15.0 | 0.415 | 6.5 | 0.423 | 8.5 |
| Medium | 1.2 | 1.25 | 7.7 | 1.31 | 3.2 | 1.24 | 7.6 |
| High | 3.5 | 3.54 | 5.3 | 3.78 | 5.0 | 3.70 | 4.0 |
| AFG2 | 331.3  >  189.1 | Low | 0.8 | 0.805 | 12.7 | 0.811 | 4.2 | 0.827 | 4.9 |
| Medium | 2.4 | 2.45 | 5.6 | 2.39 | 3.9 | 2.53 | 5.5 |
| High | 3.5 | 3.59 | 5.6 | 3.42 | 4.0 | 3.59 | 6.1 |
| 331.3  >  245.1 | Low | 0.8 | 0.783 | 11.1 | 0.794 | 7.5 | 0.802 | 3.2 |
| Medium | 2.4 | 2.46 | 6.1 | 2.38 | 4.2 | 2.47 | 2.0 |
| High | 3.5 | 3.59 | 6.5 | 3.48 | 3.5 | 3.57 | 4.3 |

*a*Measured over 20 d (2 replicates/d). *b*Measured over 1 d (7 replicates)*.*