

Article

Simplified Synthesis and Stability Assessment of Aflatoxin B₁-Lysine and Aflatoxin G₁-Lysine

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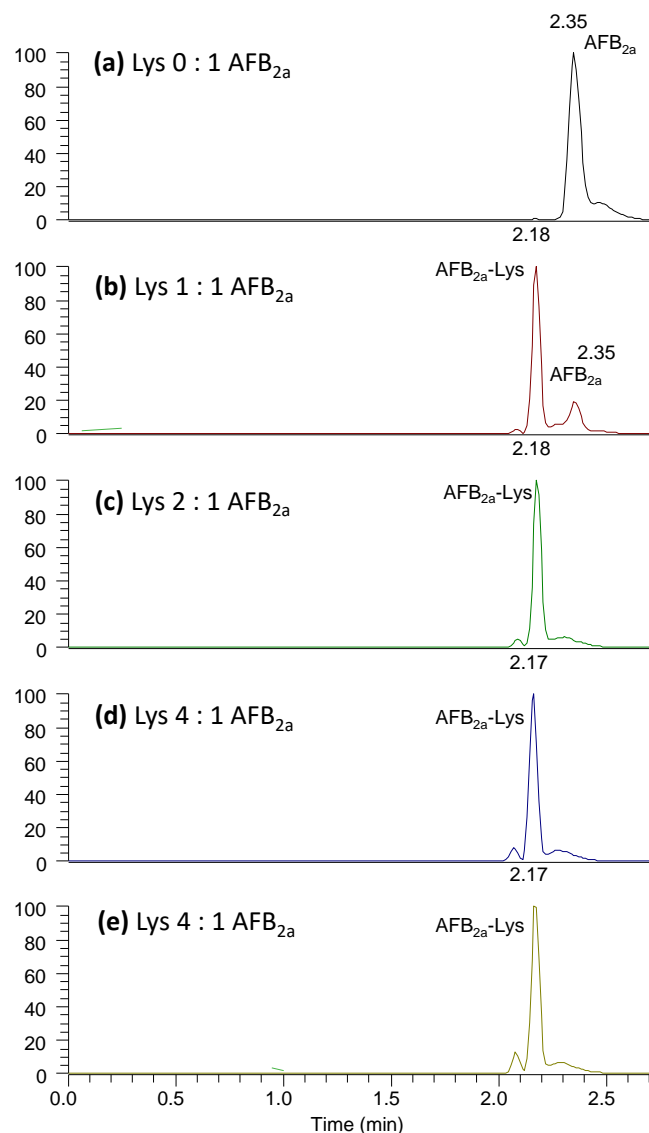


Figure S1. LC-MS/MS chromatogram of AFB_{2a} reacted with a molar ratio of 1 to (a) 0, (b) 1, (c) 2, (d) 4, (e) 10. At a molar ratio above 2, no unreacted AFB_{2a} was detected.

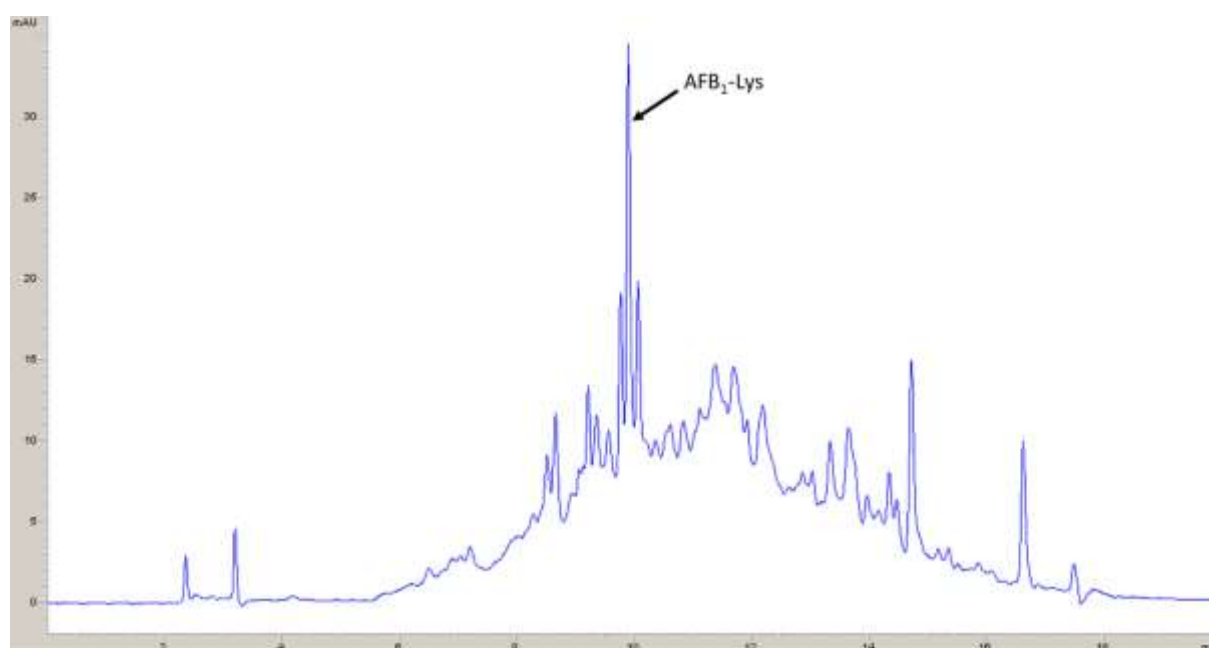


Figure S2. Chromatogram of isolation AFB₁-Lys was obtained using method described in section 4.5. AFB₁-Lys peak of interest is highlighted and was collected using time-based fraction collected. All fractions were screened by LC-MS to confirm identification. Chromatogram obtained at 399 nm referenced to 460 nm.

Table S1. Synthesis of Aflatoxin B₁-lysine (AFB₁-lysine).

Note: this procedure can also be undertaken with a AFG₁ without modification. Similarly, different isotopically labelled lysines may also be used in place of the native lysine used herein.

- 1) Weigh out 1 mg of AFB₁ in an amber glass vial with a cap
 - a. NOTE: using plastic vials could result in the appearance of anti-static agents and plasticizers.
- 2) Dissolve 1 mg of AFB₁ in 400 µL of LC-MS grade acetonitrile.
 - a. Vortex for 15 s and sonicate in a water bath at 25 °C water bath for 3 minutes.
- 3) Add 500 µL of LC-MS grade H₂O and ensure that all the aflatoxin residue is fully dissolved.
- 4) Add 100 µL of 2M HCl into the solution, cap the vessel.
- 5) Place the glass vial at 45 °C and shaking at 300 rpm for up to 48 hours
 - a. Prior to proceeding to the next step, remove a 10 µL aliquot and transfer to 990 µL of 50% ACN. Check the reaction mixture by LC-MS for *m/z* 331 (AFB_{2a}) and *m/z* 313 (AFB₁) and ensure that the majority of AFB₁ has been converted. If not, continue incubation for 12-24 hours
- 6) Split the solution into 100 µg aliquots by transferring approximately 100µL into fresh amber HPLC vials
- 7) Carefully dry down the solution under nitrogen without heat.
 - a. NOTE: Adding heat could result in oxidation to occur
- 8) Add 200 µL of LC-MS grade acetonitrile to wash the dried residue and remove any residual acid.

- a. NOTE: pH control during the coupling step is important and small amounts of acid can alter the pH of the dilute pH 10 buffer used
- 9) Resolubilize the dried residue (100 µg AFB₁ equiv.) in 100 µL of 0.05M sodium bicarbonate (pH 10.0).
- 10) Add 10 µL of lysine (19 mg/mL) dissolved in sodium bicarbonate (pH 10.0).
- 11) Incubate the solution at room temperature for at least 30 minutes.
 - a. Ensure that all the dried residue is mixing with the lysine solution by aspirating it with a pipette
 - b. Note: the intermediate AFB_{2a}-lysine is stable in this solution for up to 3 weeks when stored at 4 °C

FOR THE SYNTHESIS OF AFB_{2a}-lysine, END HERE

- 12) Prior to proceeding to the next step, remove a 10 µL aliquot and transfer to 990 µL of 50% ACN. Check the reaction mixture by LC-MS for *m/z* 441 (AFB_{2a}-lys) and *m/z* 331 (AFB_{2a}) and ensure that the majority of AFB_{2a} has coupled with the lysine. If not, add additional lysine to the solution.
- 13) Dry the solution down using a gentle stream of nitrogen with slight heat
 - a. NOTE: avoid high heat at this step as the pyrrole moiety can become easily oxidized.
- 14) Add 100 µL of MeOH to wash the dried AFB_{2a}-lysine residue and dry again under a gentle stream of nitrogen, without heat.
- 15) Resolubilize the AFB_{2a}-lysine residue (100 µg AFB₁ equiv.) in 200 µL of MeOH.
 - a. Vortex vigorously to ensure that the residue is completely resolubilized.

NOTE: Only continue with a 100 µg sub-aliquot to ensure that the concentration of H₂O₂ is optimal prior to adding H₂O₂ to all sub-aliquots

- 16) Dilute a stock solution of fresh H₂O₂ into water for a final concentration of 18.3% (v/v).
 - a. NOTE: Store the H₂O₂ solution on ice at all times.
- 17) Add 3 µL of the 18.3% to the AFB_{2a}-lysine methanol solution.
- 18) Incubate with shaking at room temperature for 1 hour.
- 19) Dry down the sample with a stream of nitrogen and gentle heat.
- 20) Resolubilize with 100 µL of 50% ACN, remove 10 µL and dilute into 990 µL of 50% ACN. Check the reaction mixture by LC-MS for *m/z* 457 (AFB₁-lys) and *m/z* 441 (AFB_{2a}-lys) and ensure that the majority of AFB_{2a}-lys has been oxidized. If greater than 50% of AFB_{2a}-lys remains, add an additional 2 µL of H₂O₂ and incubate for another hour. NOTE: Adding excess H₂O₂ could result in significant loss of product and it may be advisable to isolate the product that is there instead of attempting to generate more.
- 21) Dry down the solution with a gentle stream of nitrogen without heat in order to remove excess H₂O₂. The residue can be stored at -20 °C dry or reconstituted in 50% acetonitrile and stored at -20 °C for at least 3 weeks with limited degradation.