Supporting Information

myco-DES: enabling remote extraction of mycotoxins for robust and reliable quantification by stable isotope dilution LC-MS/MS

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Comparison of different DES materials

LC-FLD/UV conditions. For preliminary experiments LC-FLD/UV methods were applied instead of the final LC-MS/MS method to analyze aflatoxins (AF), ochratoxin A (OTA) and deoxynivalenol (DON) separately. Chromatographic separation of aflatoxins B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1, AFG_2) was achieved on a LiChrospher RP_{18} column (250 mm × 4 mm, 5 µm; Knauer, Berlin, Germany) applying isocratic elution (water/acetonitrile (MeCN)/methanol (MeOH), 60:20:20, *v/v/v*, 119 mg/mL potassium bromide, 100 µL/L nitric acid) at a flow rate of 1 mL/min at 40 °C with an injection volume of 90 µL. For post-column bromination to enhance signal of fluorescence of AFB_1 and AFG_1 (λ_{ex}: 365 nm, λ_{em}: 435 nm) a Coring cell (Coring, Gernsheim, Germany) was used. Chromatography of OTA was carried out on the same column using isocratic elution (water/MeCN/acetic acid, 49.5:49.5:1, v*/v/v*) at a flow rate of 1 mL/min at 40 °C with an injection volume of 100 µL. Fluorescence detection of OTA was achieved at λ_{ex}: 330 nm and λ_{em}: 460 nm. DON was analyzed by UV detection (λ: 220 nm) after chromatographic separation on a Kinetex® EVO C_{18} column (100 mm × 4.6 mm, 2.6 µm; Phenomenex, Aschaffenburg, Germany) using gradient elution with solvent A (water/MeCN/MeOH, 90:5:5, *v/v/v*) and solvent B (MeCN) at a flow roate of 500 µL/min at 40 °C with an injection volume of 50 µL. The following gradient was applied: min/% B: 0/0, 8/0, 10/80, 12/0.

Absorbance and spreading. 50 µL of an acid blue 3 solution (MeCN) was fixed on every dried extract spot (DES) material for initial evaluation. Subsequently, 10 µL and the suitability of a solvent mixture of MeCN and water (50:50, *v/v*) was evaluated. Results are presented in Figure S1.

Recovery. Recovery rates of the analytes $AFB_{1/2}$, $AFG_{1/2}$, OTA and DON were determined by fixing 10 µL of a standard solution (8 ng/mL AF, 24 ng/mL OTA and 4 µg/mL DON in MeCN) on every DES material followed by re-extraction according to the method of Osteresch *et al.*[1]

The complete spot was cut out and extracted with water/acetone/MeCN (30:35:35, *v/v/v*, 1000 µL) in a 2 mL safe-lock tube under sonication for 30 min. An aliquot (800 µL) was evaporated to dryness at 50 °C under reduced pressure in a separate 2 mL safe-lock tube and the residue reconstituted using water/MeCN/MeOH (90:5:5, *v/v/v*, 330 µL). Following centrifugation (10,000 × *g*, 10 min) the extract was analyzed by LC-FLD/UV and recovery calculated as the ratio of the determined peak area and the peak area of an accordingly diluted standard solution subjected to the same evaporation and reconstitution procedure. Results are given in Figures S2-4.

Stability. Stability was assessed by storing DES samples prepared as described above (Recovery) in paper envelopes at 30 °C and 65% relative humidity (RH). Recovery rates were determined after 1, 3, 7, 14, 21 and 28 days and normalized by freshly prepared recovery samples. Results are shown in Figures S2-4.

Interferences of coextracted compounds of the blank DES materials. Signal suppression and enhancement (SSE) for the different DES materials were studied. Therefore, blank materials were extracted as described above besides one modification: to facilitate a higher throughput the aliquot (800 µL) was transferred to a deactivated glass vial instead of another safe-lock tube, which allows for faster evaporation. As centrifugation of the glass vials was not possible after reconstitution in eluent, suspended solids were separated using polytetrafluoroethylene (PTFE) filter vials. The obtained DES extract was spiked to natural contamination levels at 0.01 ng/mL AF, 0.03 ng/mL OTA and 0.5 ng/mL DON and subjected to LC-MS/MS analysis following DIN EN 17279:2020. Matrix effects were assessed by comparing the peak area of the analytes in DES extract to those obtained in neat solvent and are given in Figure S5. Interestingly, it was observed that the peak area of OTA in the reference solution (filtered by PTFE filter vials as well) was only half of the expected area. It appears that OTA binds at active sites of the PTFE filter, while these binding sites will (partially) be saturated by matrix components from DES

extracts. As a result, the concentration of OTA in spiked DES extracts is higher and the matrix effect of OTA therefore overestimated. In addition, co-eluting matrix peaks for OTA were observed when analyzing blank extracts of DES materials 6614 and 8951.

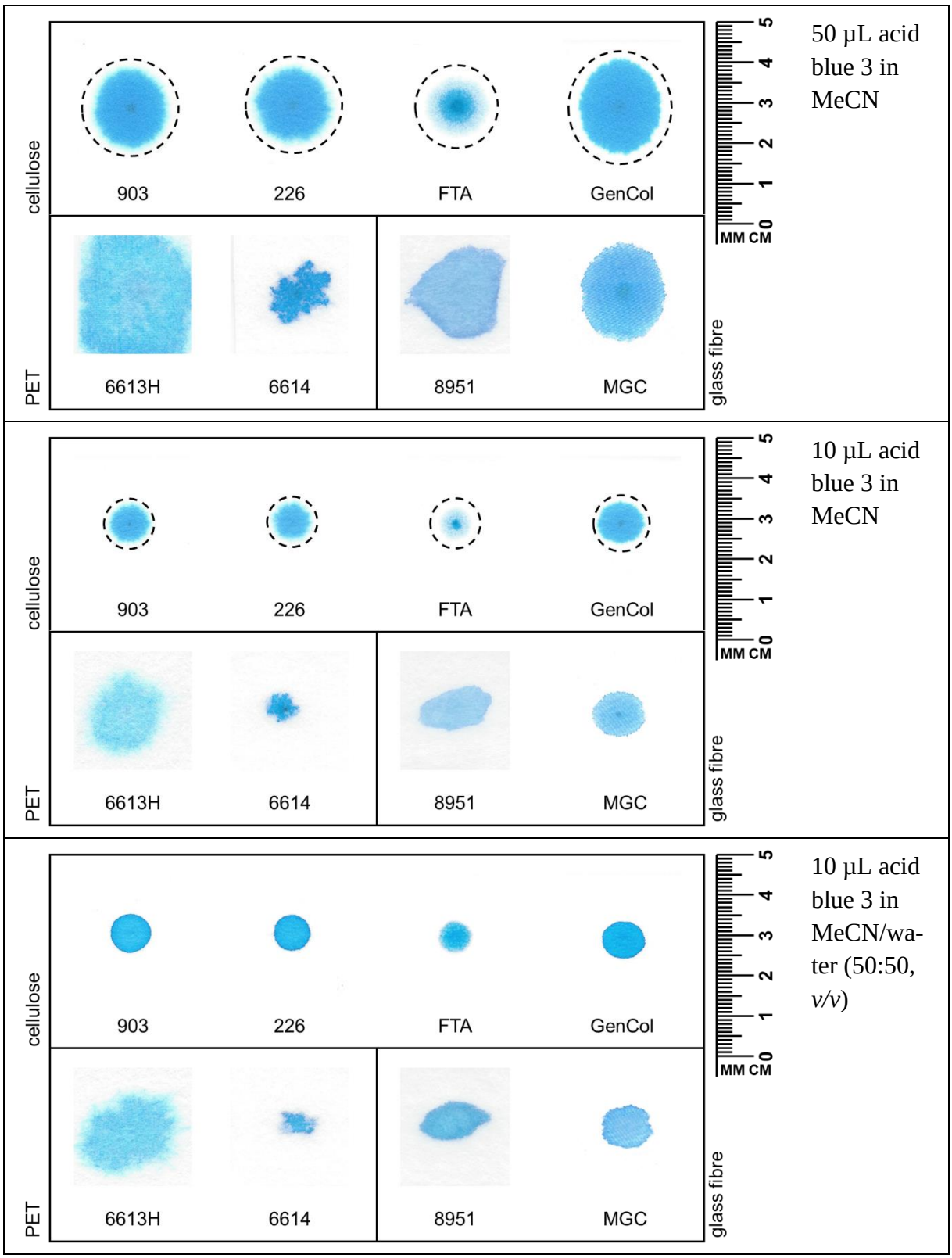

Figure S1 Spots obtained by fixing an acid blue 3 solution on different DES materials. Spot size and shape were influenced by the composition of the DES materials (main components cellulose, polyethylene terephthalate (PET) and glass fiber are indicated) as well as the solvent and volume (dashed circles: solvent front of spots).

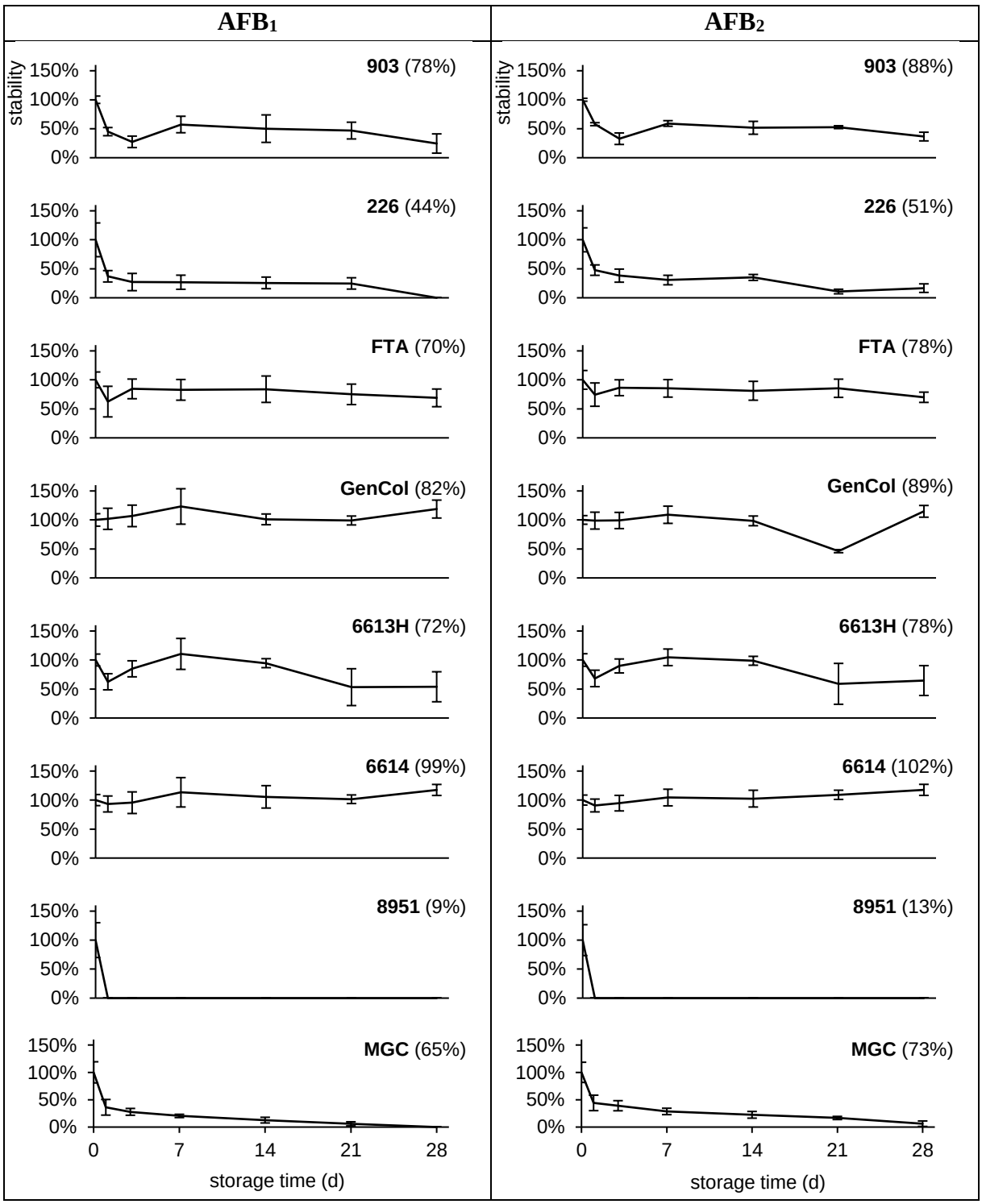

Figure S2 Stability of $AFB_{1/2}$ fixed on different DES materials (application of 10 µL standard solution containing 8 ng/mL AF, 24 ng/mL OTA and 4 µg/mL DON in MeCN). Storage at 30 °C and 65% RH for four weeks; packed in paper envelopes; recovery rates for instant re-extraction with water/acetone/MeCN (30:35:35, *v/v/v*) (given in brackets) were set at 100%. Determination of $AFB_{1/2}$ was performed using LC-FLD (n = 6).

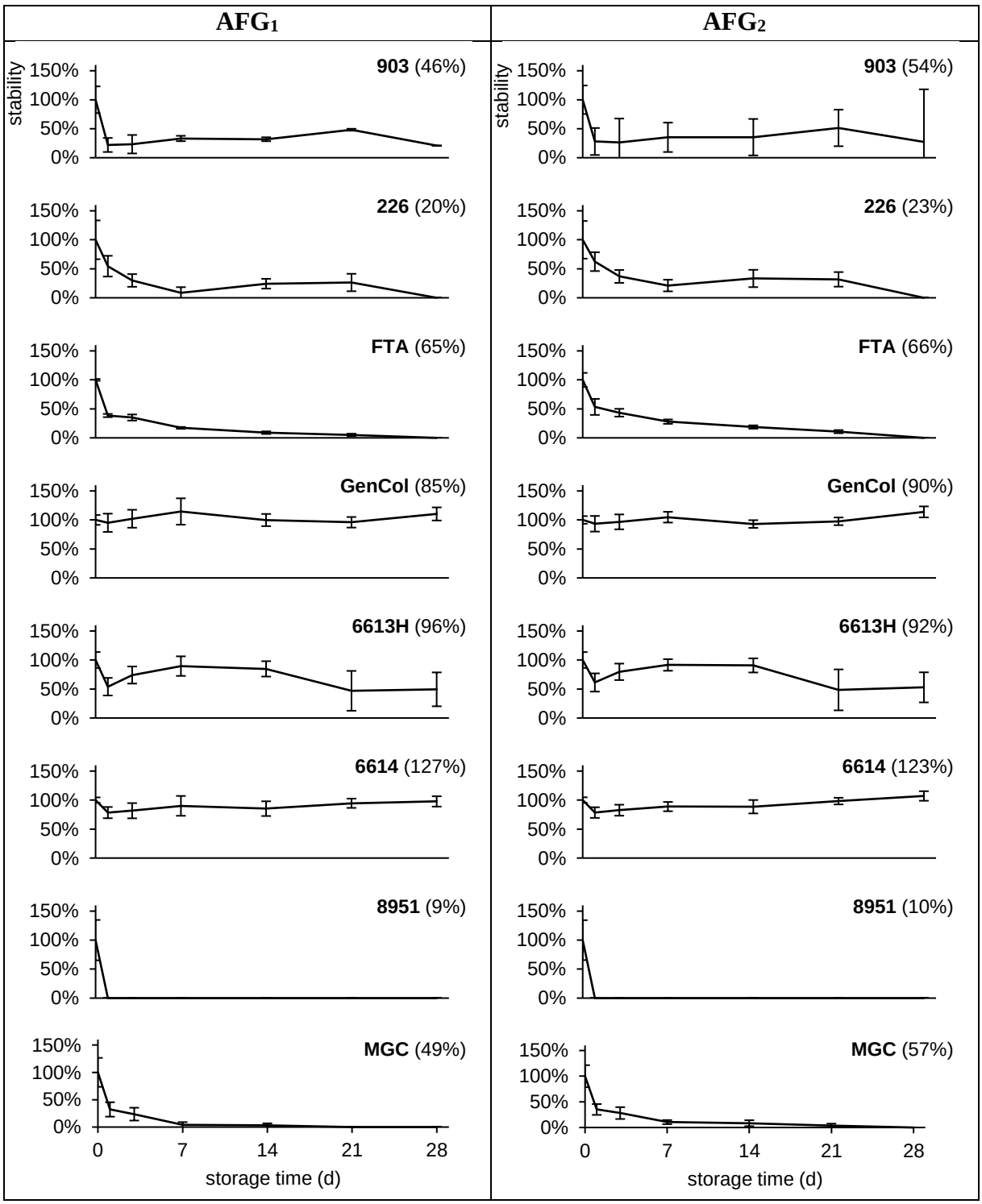

Figure S3 Stability of $AFG_{1/2}$ fixed on different DES materials (application of 10 µL standard solution containing 8 ng/mL AF, 24 ng/mL OTA and 4 µg/mL DON in MeCN). Storage at 30 °C and 65% RH for four weeks; packed in paper envelopes; recovery rates for instant re-extraction with water/acetone/MeCN (30:35:35, *v/v/v*) (given in brackets) were set at 100%. Determination of $AFG_{1/2}$ was performed using LC-FLD (means ± standard deviation, $n = 6$).

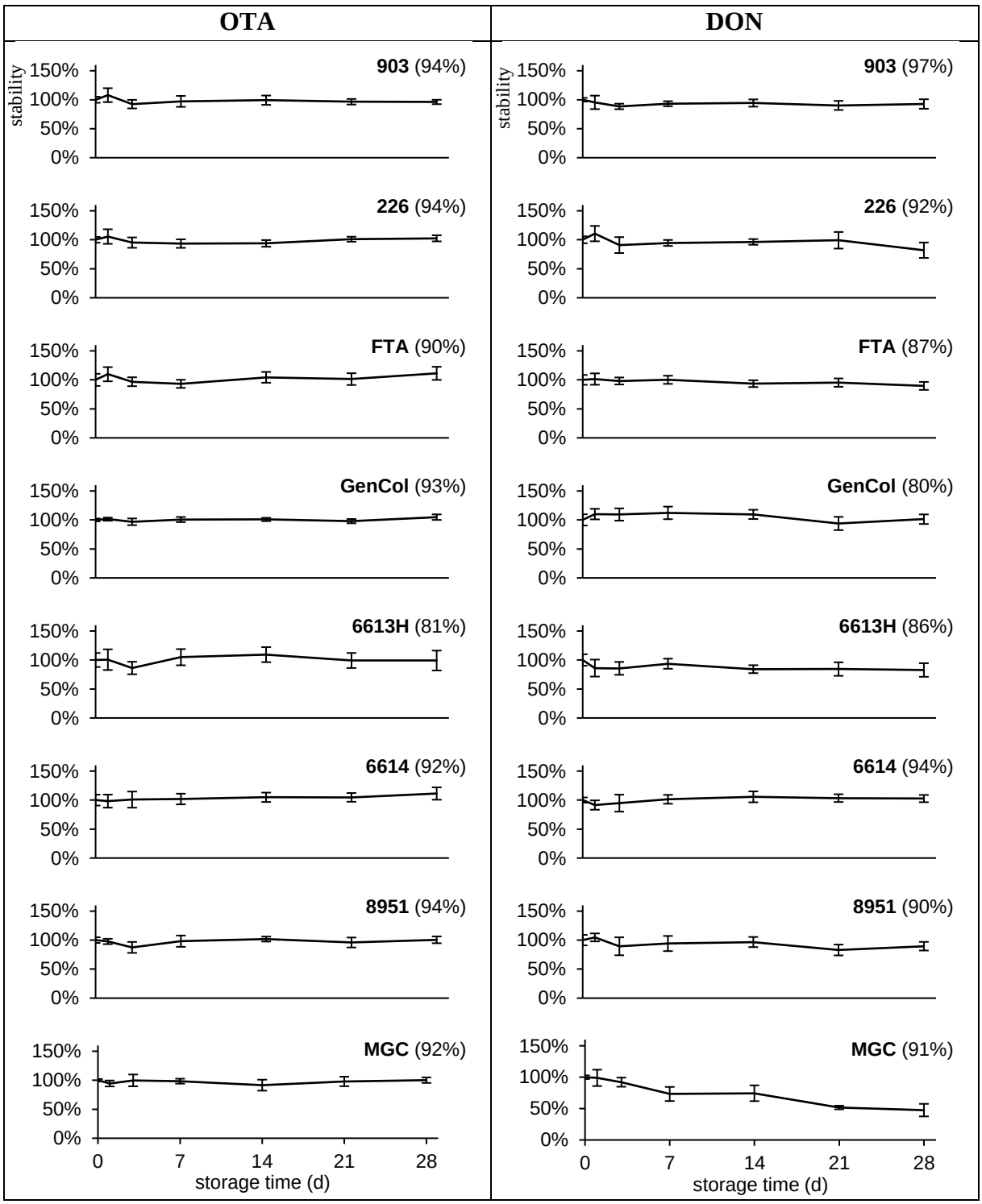

Figure S4 Stability of OTA and DON fixed on different DES materials (application of 10 µL standard solution containing 8 ng/mL AF, 24 ng/mL OTA and 4 µg/mL DON in MeCN). Storage at 30 °C and 65% RH for four weeks; packed in paper envelopes; recovery rates for instant re-extraction with water/acetone/MeCN (30:35:35, *v/v/v*) (given in brackets) were set at 100%. Determination of OTA and DON was performed using LC-FLD/UV (means ± standard deviation, $n = 6$).

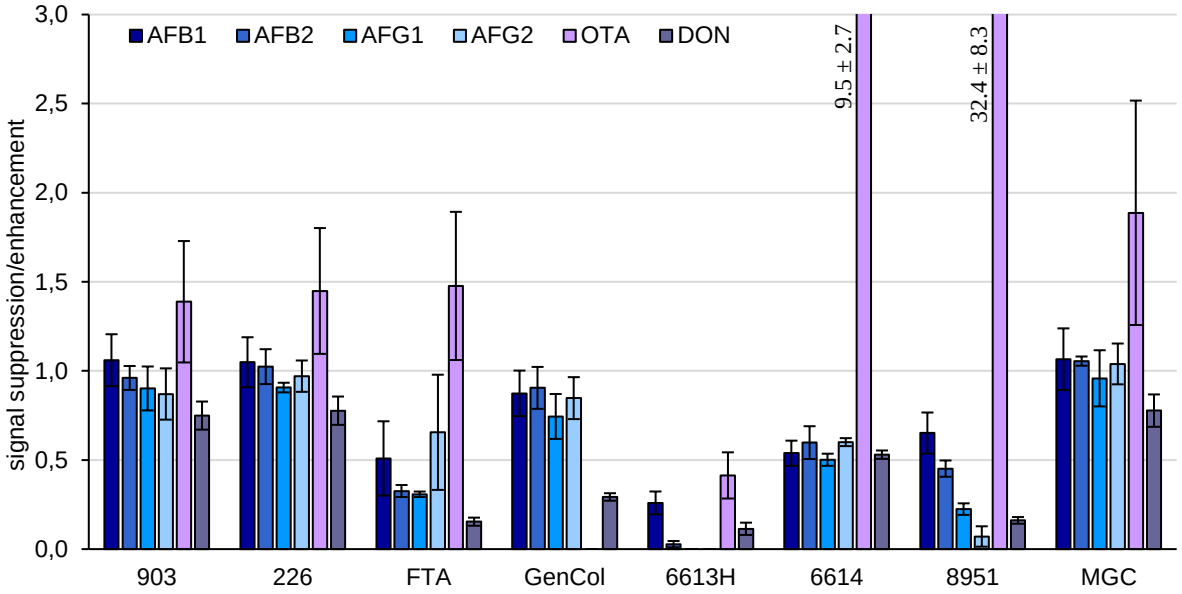

Figure S5 Signal suppression and enhancement (SSE) induced by matrix components co-extracted from DES materials; assessed by calculating the ratio of the peak area of the analytes in solvent and DES extract (0.01 ng/mL AF, 0.03 ng/mL OTA and 0.5 ng/mL DON). After extraction of the DES materials and evaporation to dryness, the residues were reconstituted in eluent for LC-MS/MS detection (means ± standard deviation, $n = 6$). SSE of OTA is overestimated due to the used PTFE filter vials binding OTA. For materials 6614 and 8951 co-eluting matrix peaks were observed.

Reduction of matrix load

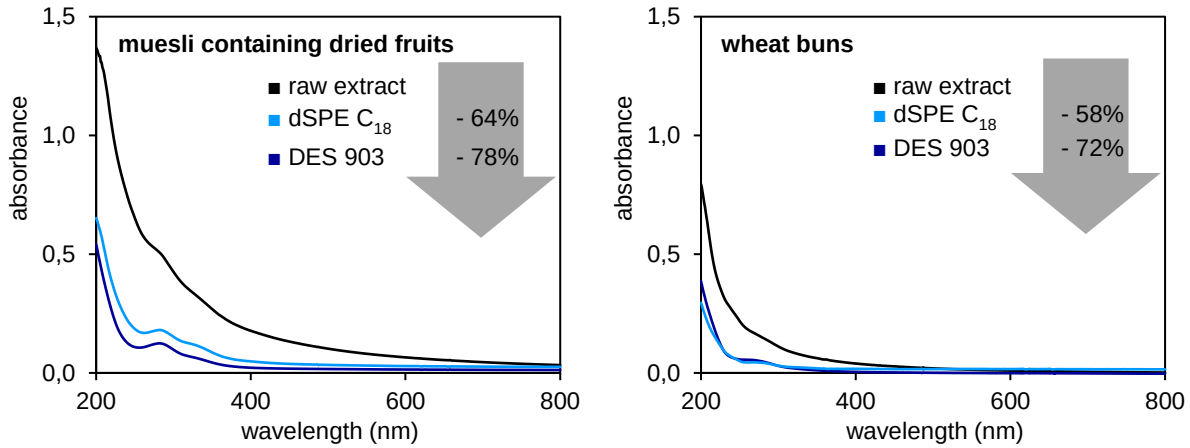

Figure S6 Reduction of matrix components from the extraction solution of muesli containing dried fruits and wheat buns through clean-up by dSPE (C_{18}) or DES material 903. Comparison to dilute raw extract by integration of the UV/vis spectra.

Evaluation of chromatographic effects

To identify the part of the spot containing the highest concentration of the analytes of interest a mixed standard solution (80 µL; 1.5 ng/mL AF und OTA, 100 ng/mL DON in extraction solvent) was applied on DES material 903 to obtain a spot of 2.8 cm in diameter just big enough to allow for five consecutive 3 mm disks, which were punched out and analyzed separately (Figure S7). The obtained peak area of each disk was related to the sum of all disks to calculate the distribution on the spot.

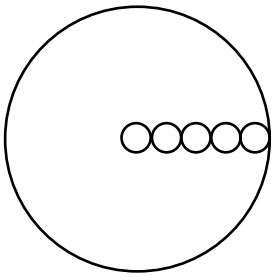

Figure S7 Analyzed disks of the spot to determine the distribution of analytes.

Stability testing

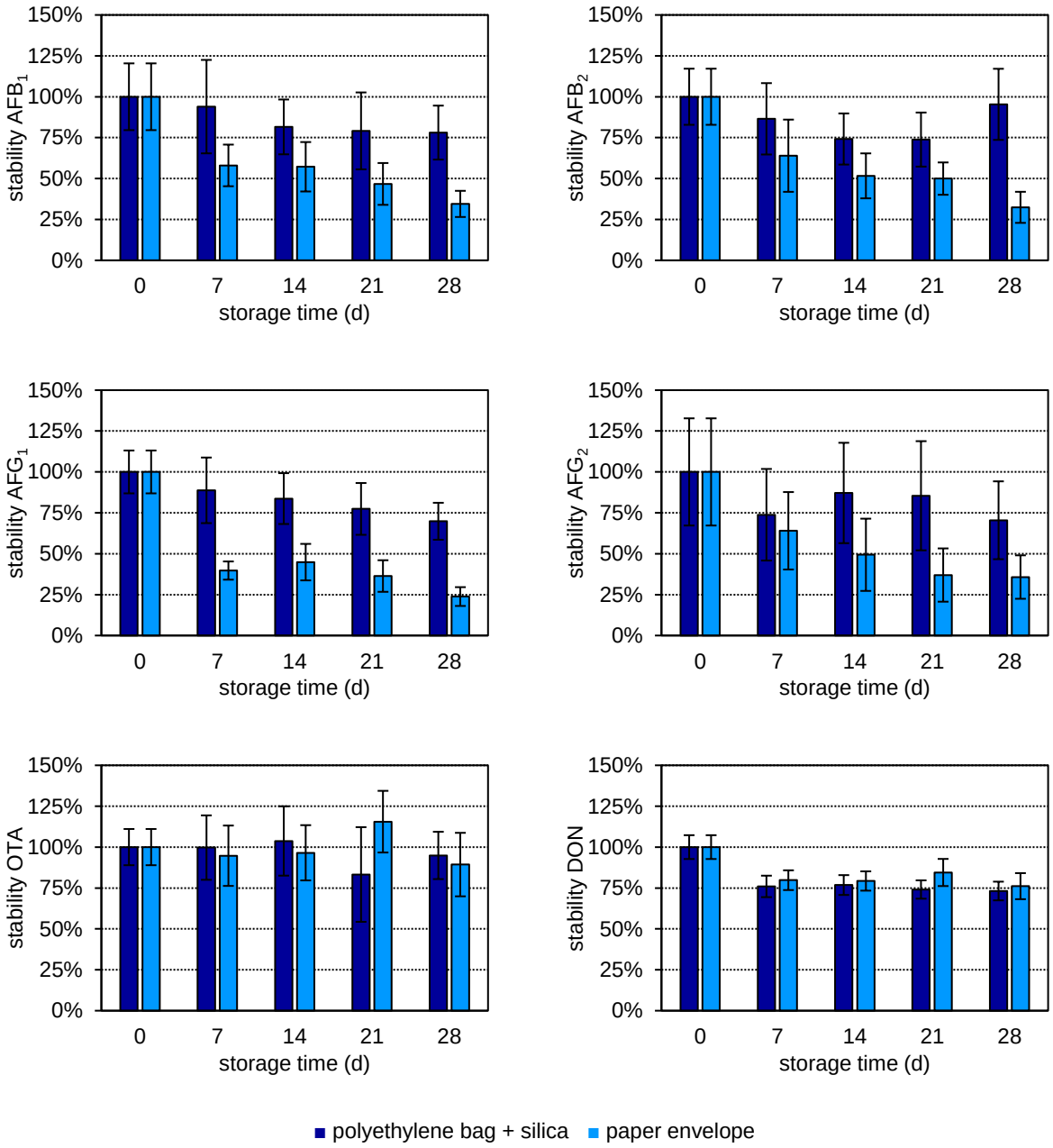

Figure S8 Impact of packaging type on stability of the analytes (1.5 µg/kg AF, 3 µg/kg OTA 450 µg/kg DON) in DES samples obtained from muesli containing dried fruits during storage at 30 °C and 65% RH (n = 6).

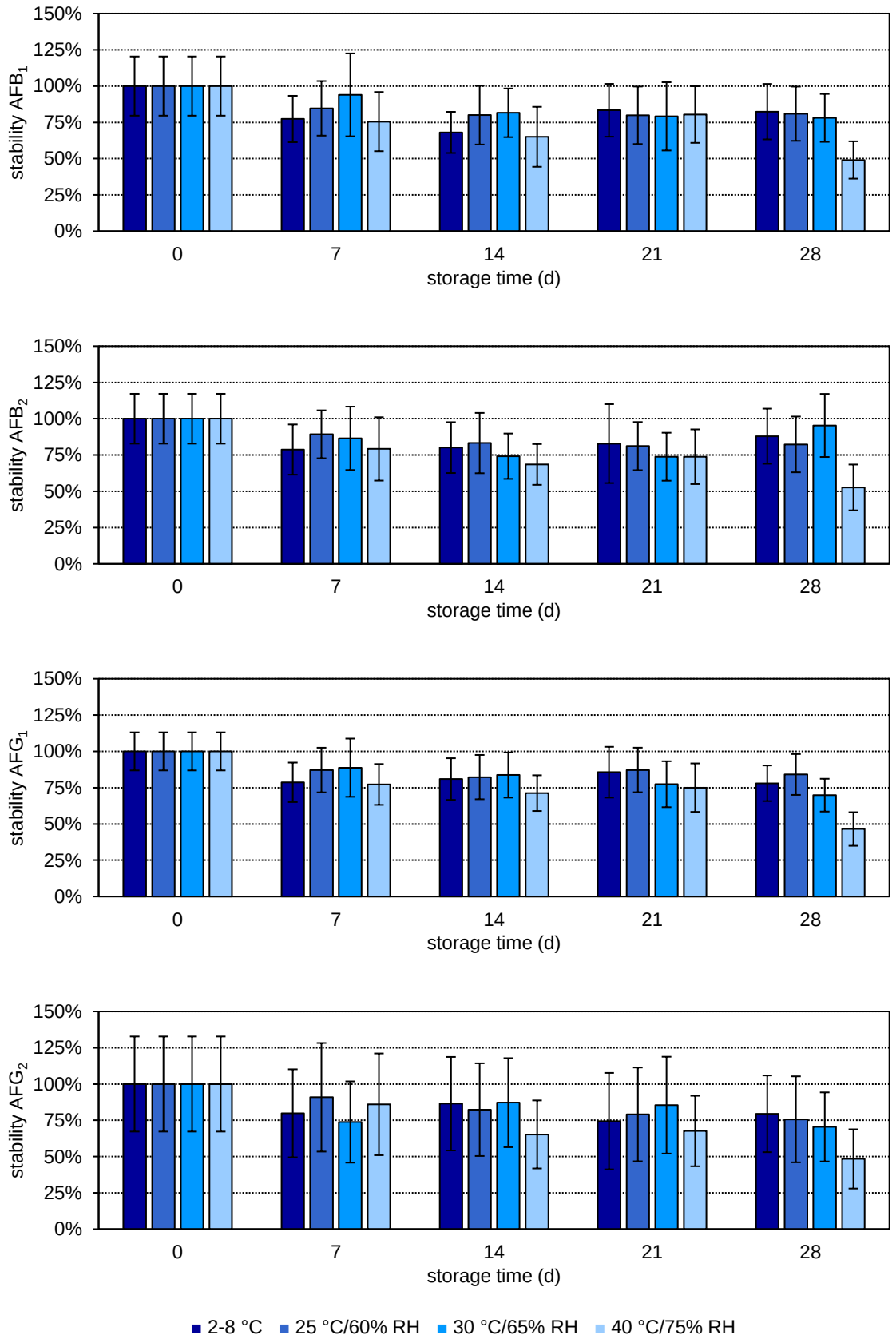

Figure S9 Impact of climatic conditions on stability of AF (1.5 µg/kg) in DES samples obtained from muesli containing dried fruits during storage packed in PE bags with silica (n = 6).

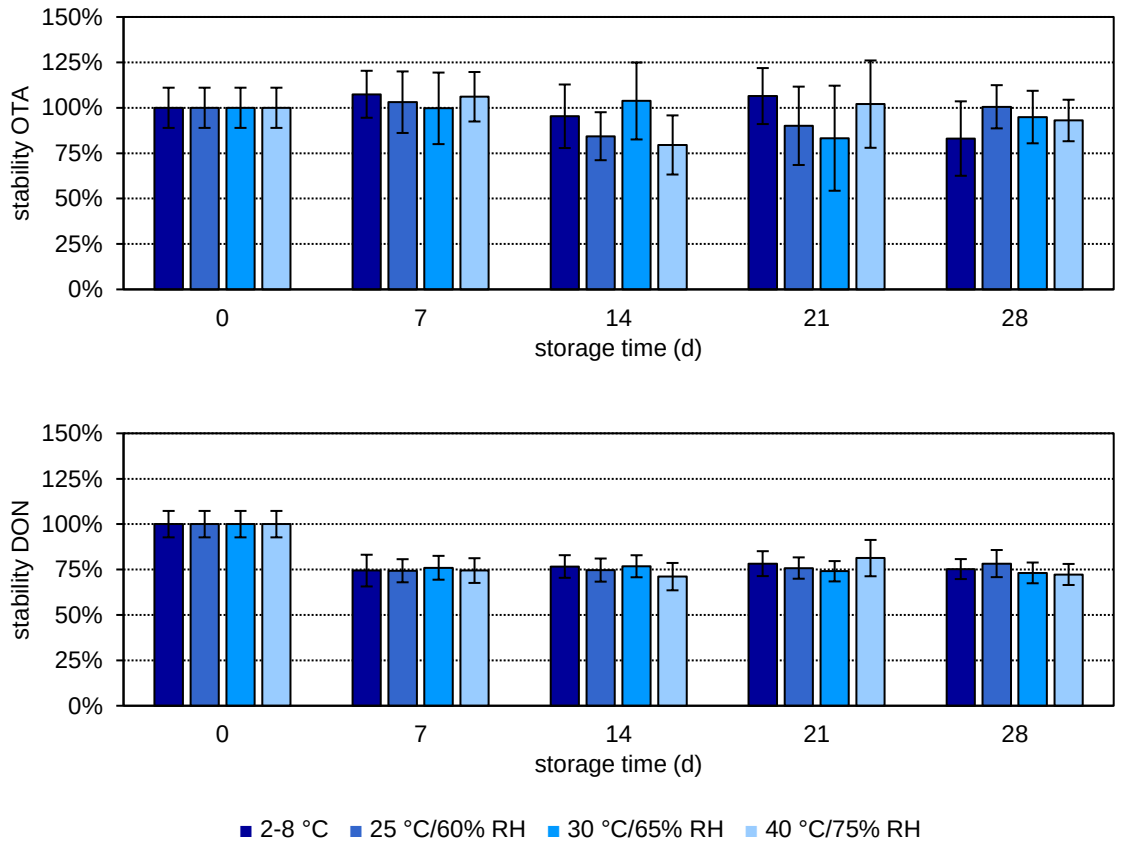

Figure S10 Impact of climatic conditions on stability of OTA (3 µg/kg) and DON (450 µg/kg) in DES samples obtained from muesli containing dried fruits during storage packed in PE bags with silica (n = 6).

Automated re-extraction

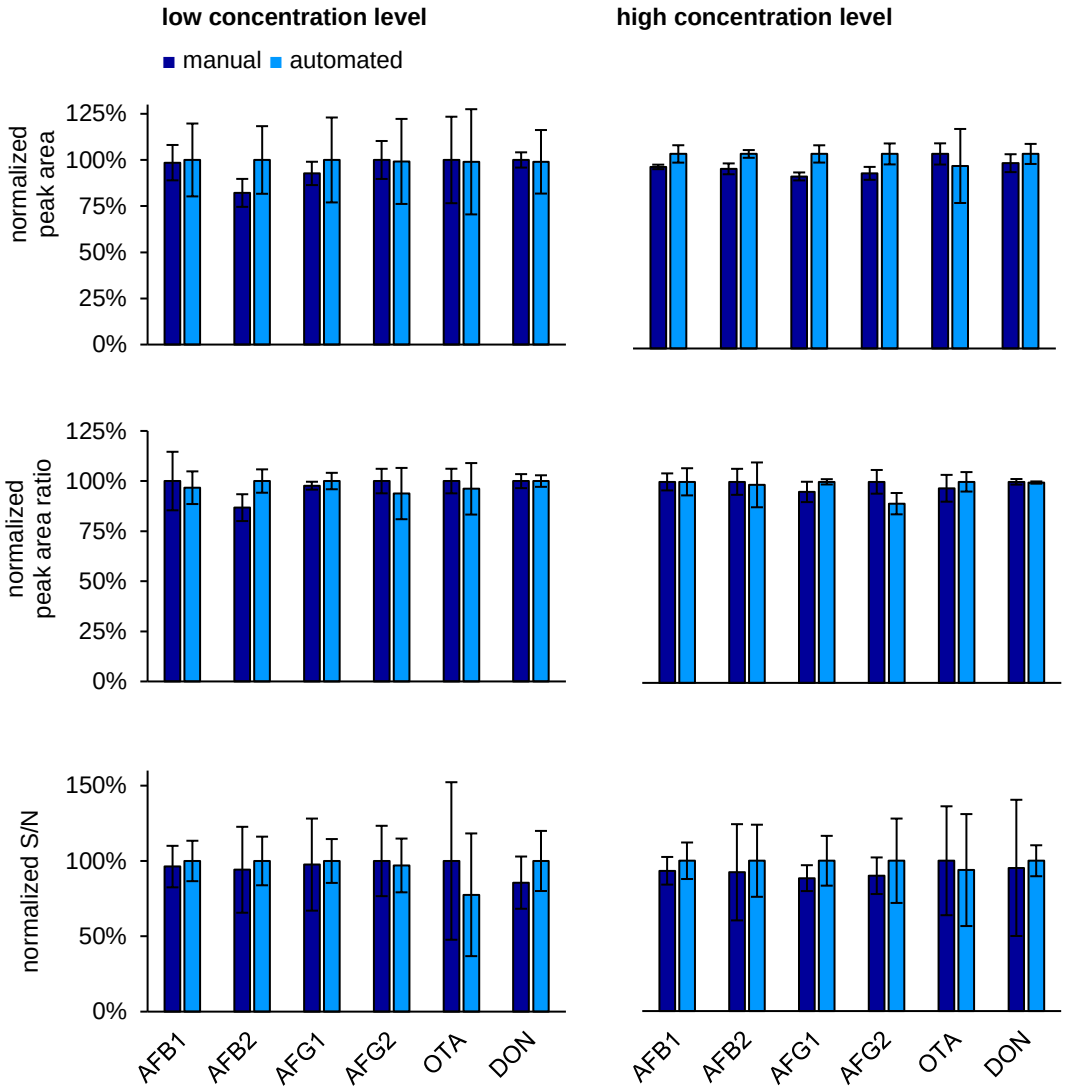

Figure S11 Comparison of manual and semi-automated re-extraction on the basis of spiked muesli DES samples at low (LOQ) and high (15 µg/kg AF, 30 µg/kg OTA, 2.1 mg/kg DON) concentration regarding absolute peak area, peak area ratio and signal/noise ratio (S/N), normalized to 100% respectively (n = 6).

Chromatographic and MS conditions

For some experiments during method development the applied chromatographic and MS/MS conditions differed from the final LC-MS/MS method. The modified parameters are given in the table below.

experiment	**modified parameter**
- SSE of different DES materials - recovery for facilitated re-extraction using the eluent of the LC-method on the basis of a standard solution* - evaluation of chromatographic effects	- injection volume: 30 µL - MS/MS transition for AFG_2 - quantification: *m/z* 331.1 → 313.0 (collision energy: 28 V) - confirmation: *m/z* 331.1 → 189.0 (collision energy: 48 V) - no MS/MS transitions for ^{13}C-labeled analogues
- stability testing	- injection volume: 30 µL - MS/MS transition for AFG_2 - confirmation: *m/z* 331.1 → 285.0 (collision energy: 48 V)

* Recovery on the basis of spiked raw extracts from muesli containing dried fruits and wheat buns was evaluated using the final LC-MS/MS method.

Naturally contaminated samples

To evaluate accuracy and precision also naturally contaminated samples were analyzed. For comparison the following reference values were applied:

- AF in rice flour as specified by proficiency testing (FAPAS 04374): aflatoxin B_1 3.20 µg/kg, aflatoxin B_2 1.94 µg/kg, aflatoxin G_1 2.31 µg/kg, aflatoxin G_2 1.88 µg/kg
- OTA in muesli containing dried fruits as analyzed following DIN EN ISO 15141 (internal reference material): ochratoxin A 1.49 µg/kg
- DON in wheat pasta following DIN EN 15891 (internal reference material): 662 µg/kg

References

(1) Osteresch, B.; Viegas, S.; Cramer, B.; Humpf, H.-U. Multi-Mycotoxin Analysis Using Dried Blood Spots and Dried Serum Spots. *Anal. Bioanal. Chem.* **2017**, *409* (13), 3369–3382. https://doi.org/10.1007/s00216-017-0279-9.