**Determination of mycotoxins with dSPE and HPLC-MS/MS in food and feed**

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**Introduction**

Mycotoxins are important contaminants widespread in food and feed. They are metabolites produced by certain types of molds [1]. Mycotoxins frequently found in food and feed are aflatoxins, ochratoxins, fumonisins, trichotheccenes and zearalenone. Their toxicity and potential health hazards are well known and leads to a demand for sensitive and efficient sample preparation methodologies [2].

The QuEChERS-methodology is a well-known technique for extraction of contaminants like pesticide residues in food and feed [3]. This methodology provides advantages allowing rapid and cheap cleanup of strongly matrix-contaminated samples. Each compound of QuEChERS cleanup-mix shows different effects on matrix reduction. The Diamino phase (PSA) removes, e.g., sugars and organic acids. Magnesium sulfate removes water. C₁₈ ec removes nonpolar interferences such as fats and so on. For optimizing cleanup step, mix-composition can be adapted to sample matrix.

**Dispersive solid phase extraction (dSPE)**

**Extraction**
- Weigh 4 g homogenized sample into a 50 mL centrifuge tube
- Add 25 μL mycotoxin standard mixture (β = 0.1 μg/mL each analyte in acetonitrile)
- Add 10 mL 0.1% formic acid in water, shake vigorously and wait 10 min
- Add 10 mL acetonitrile and agitate
- Add CHROMABOND® QuEChERS Mix M1, shake vigorously for 1 min and cool the mixture down in an ice bath
- Centrifuge at 4500 rpm for 20 min at 20 °C
- Take organic phase for cleanup procedure

**Cleanup**
- Add 6 mL of organic phase into centrifuge tube with CHROMABOND® QuEChERS Mix M1 or Mix M2
- Shake vigorously for 1 min
- Centrifuge at 4500 rpm for 20 min at 20 °C
- Evaporate 2 mL extract to dryness at 80 °C under a stream of nitrogen and redissolve in 0.5 mL acetonitrile

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**Subsequent analysis HPLC-MS/MS**

**Chromatographic Conditions**
- **Column 1**: EC 100/3 NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm, (REF 763734.36)
- **Column 2**: EC 50/3 NUCLEOSHELL® Bluebird RP 18, 2.7 µm, (REF 763432.30)
- **Eluent A**: 0.1% formic acid in water
- **Eluent B**: 0.1% formic acid in acetonitrile
- **Gradient**: hold 5% B for 1 min, from 5% B to 40% B in 5.0 min, from 40% B to 95% B in 1.0 min, hold 95% B for 1 min, from 95% B to 5% B in 5.0 min, hold 5% B for 3.0 min
- **Flow rate**: 0.90 mL/min
- **Temperature**: 40 °C
- **Injection volume**: 3 μL
- **MS conditions**: API 5500, ion source ESI, positive ionization mode, scan type MRM
- **Flow rate**: 20 ng/2 μL, mrin spray voltage 5500 V, temperature 550 °C, nebulizer gas 45 psi, turbo gas 45 psi, CAD medium

**MRM Transitions**

**Table 1: MRM transitions for mycotoxins**

**Chromatograms**

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**Recovery rates**

**Matrix reduction**

**Conclusion**

The results show that the determination of mycotoxins could be carried out successfully with all the tested analytes. Figure 2 and 3 show the analysis of QuEChERS extracts of wheat flour and rye flour on NUCLEOSHELL® Bluebird RP 18 column leads to better signal response, better peak shape and separation of matrix interferences.

The recovery rates for cleanup with QuEChERS methodology for the different products and matrices is shown in figure 4 and 5. In particular, the recovery rates for wheat flour are better than for the matrix rye flour. The better reproducibility and the homogenous recovery rates on NUCLEOSHELL® Bluebird RP 18 column are caused by better chromatographic performance.

Figure 6 and 7 present the matrix reduction for the cleanup procedure. By using CHROMABOND® QuEChERS clean-up Mix M2 a highest matrix reduction could be achieved for both sample matrices. The reduction of dry mass and of UV-VIS-absorption is significant for both tested products. In summary, the presented application describes a quick and convenient method for the determination of mycotoxins with dSPE using CHROMABOND® QuEChERS Mix M1 and CHROMABOND® QuEChERS Mix M2 for sample clean-up.

**References**


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**Product information**

The following MACHEREY-NAGEL products have been used in this application:

REF 763734.30, EC 100/3 NUCLEOSHELL® Phenyl-Hexyl, 2.7 μm
REF 763432.30, EC 50/3 NUCLEOSHELL® Bluebird RP 18, 2.7 μm
REF 730649, CHROMABOND® QuEChERS extraction Mix M1
CHROMABOND® QuEChERS Mix M1, order on request
CHROMABOND® QuEChERS Mix M2, order on request