Advantages of Selective Analyte Clean-up for Mycotoxin Testing of *Cannabis and Hemp Products*

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Introduction

The formation of toxic mycotoxins is very often observed during the storage and drying of plant materials for medical and pharmaceutical use. Therefore, these materials are strictly monitored and regulated for their toxin content, respectively. Hemp and cannabis contain many secondary plant metabolites with medical relevance, which unfortunately severely affect mycotoxin analysis in a negative way. Thus, special clean-up procedures are recommended to gain analytical sensitivity and to reduce matrix interferences, which often lead to miscalculation of analyte concentrations. Generally hemp and cannabis products are investigated according to mycotoxin regulations for food and feed. An antibody based sample clean-up with miniaturized AflaCLEAN™ SMART or OtaCLEAN™ SMART immunoaffinity cartridges in combination with a robotic system FREESTYLE ThermELUTE™ is shown. The clean-up is highly selective and specific, respectively, yielding in a high depriviation of matrix interfering substances as well as perfect analytical results.

Figure 1: SMART columns handled in FREESTYLE ThermELUTE™

Material and Methods

Figure 2: Comparison of AflaCLEAN™ SMART with 3 mL column

10 gram homogenized sample material are extracted by 50 mL methanol/water (v/v), 25 mL n-hexane by vigorous stirring. After filtration and centrifugation at 3000 x g, 2 mL methanolic phase (bottom) was diluted with 12 mL PBS buffer containing 8 % Tween20. 2.8 mL of it was loaded onto Immunoaffinity cartridge AflaCLEAN™ SMART or OtaCLEAN™ SMART respectively by FREESTYLE ThermELUTE™ robotic system. After washing with 2 mL deionised water and thermal denaturation, the eluate was completely injected automatically into the HPLC. HPLC parameters for aflatoxin: flow rate 1.2 mL/min (60/30/15 v/v/v) water/methanol/acetonitrile, LC-column (150 mm RP C-18) temperature 36 °C, high performance post column photochemical derivatisation by UVE, Ex. 365 nm, Em. 460 nm. HPLC parameters for ochtratoxin A: flow rate 0.6 mL/min (40/55/5 v/v/v +1% AA) water/methanol/acetonitrile, LC-column (125 mm RP C-18) temperature 40 °C, FLD: Ex 335 nm, Em 465 nm. Time from sample application to chromatogram takes approx. 15 min.

Results

The applied antibodies show very selective binding and purification of the sample, as well as a massive reduction of matrix interferences as shown in the optical comparison (Fig. 3). This increases the analytical sensitivity significantly. The automated processing and complete transfer of the eluate into the LC allows a gain of sensitivity by a factor of 10 (Fig.4) by high volume injection without excessive peak broadening or significant increase in chromatographic time. The sensitivity was calculated as LOD and LOQ for the different toxins and is in the range of ppt (ng/kg) (Tab.1). The linearity of recovery is given by the tested range from 0,125 ppb total toxin to 20 ppb total toxin with a correlation coefficient of at least 0,99 (Fig. 5). Recovery was calculated from spiked and naturally contaminated material and ranged from ***88 % to 99.7 %*** for the individual toxins (Tab.2).

Analyte	Aflatoxin B1/G1	Aflatoxin B2/G2	Ochratoxin A
LOD (ppb)	**0,05**	**0,013**	**0,04**
LOQ (ppb)	**0,15**	**0,04**	**0,12**

Table 1: Analytical sensitivity of the individual toxins in hemp and cannabis samples. LOD Limit of detection and limit of quantification were calculated from the calibration curve (matrix match) and analytical standard.

Spiking level (ppb)	Aflatoxin B1	Aflatoxin G1	Aflatoxin B2	Aflatoxin G2	Ochratoxin A
2,5	**95,3**	**94,1**	**88,0**	**90,8**	**91,6**
5	**92,4**	**90,1**	**91,2**	**88,3**	**94,6**
10	**99,1**	**96,4**	**97,6**	**96,6**	**96,5**
20	**99,7**	**93,0**	**92,3**	**91,7**	**99,1**

Table 2: Recovery of aflatoxins B/G and ochratoxin A from spiked dried hemp flower samples.

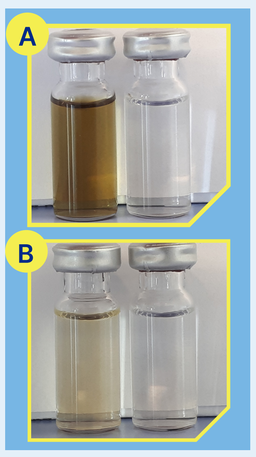

Figure 3: Optical comparison of hemp and cannabis extract before (left) and after immunoaffinity clean-up (right).
Cannabis sample (A)
Hemp seed extract (B).

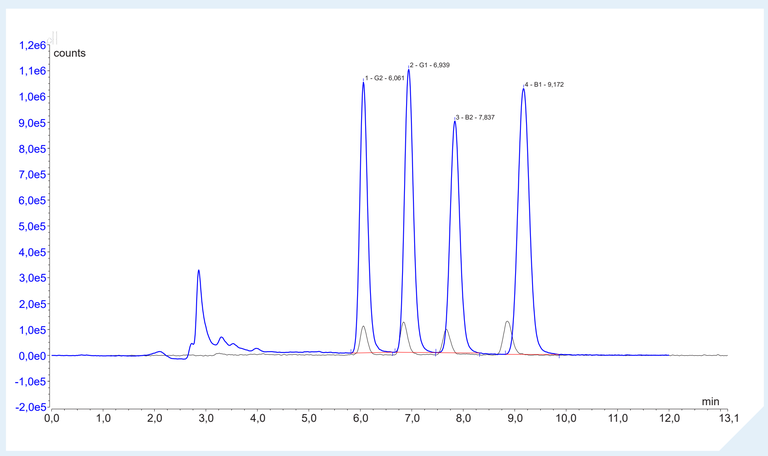

Figure 4: Chromatographic comparsion of a manually processed (black) or FREESTYLE ThermELUTE processed sample (blue).

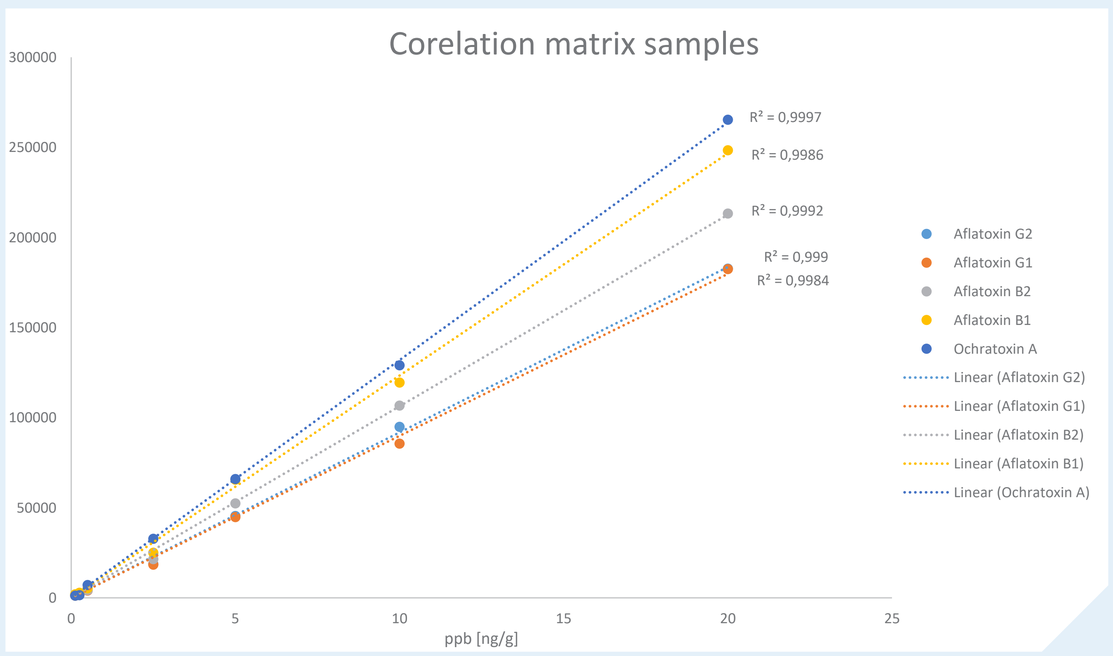

Figure 5: Linearity and correlation of aflatoxin or ochratoxin A, covering the limits of regulation (n=5).

Conclusion

The combination of antibody based clean-up and fully automated processing allows a highly efficient and unattended 24/7 sample preparation. The achieved gain of sensitivity could either be used to investigate smaller sample volumes or to increase the speed of clean-up and analysis. This could be facilitated by the FREESTYLE ThermELUTE™ in combination with high capacity binding and matrix tolerant antibody clean-up cartridges, which are known as AflaCLEAN™ SMART or OtaCLEAN™ SMART cartridges.

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