# LC-MS Analysis of Mycotoxins - Unique Stationary Phases for Alternative Selectivity

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**Abstract**: Mycotoxins are common agricultural contaminants that are heavily regulated worldwide. These regulations require analysis of not individual, but panels of toxins. The high number of analytes required per test leads to the common problem of unresolved peaks in HPLC and HPLC-Mass Spectrometry (LC-MS) analyses. While LC-MS remains a powerful technique for the deconvolution of overlapping signals, there exists alternative strategies to improve these analytical methods. In this work, HPLC columns with unique chemistries were assessed for their ability to differentiate a panel of 12 mycotoxins. Each column demonstrated significantly different selectivity and elution order, making this strategy a viable route for peak deconvolution. Depending on the given panel of mycotoxins, a change in column chemistry may result in greater resolution, and facilitate new methods by which laboratories can adhere to strict regulations on mycotoxins.

## Methods

**Methods**: Three column chemistries were chosen for the simultaneous analysis of 12 mycotoxins. The Cholester column stationary phase consisted of a cholesterol moiety, the πNAP column contained a napthylene functional group as the stationary phase, and the PBr column contained a phenyl bromide stationary phase.

**Chromatographic Conditions-** Flow rate 0.5mL/min, Temperature 40°C, Injection Volume 5µL, Mobile Phase A (Water, 2mM ammonium formate, 0.1% formic acid), Mobile Phase B (Methanol,2mM ammonium formate, 0.1% formic acid). Cholester Column- Gradient; 0-7min 30-53%B, 7-11.5min 53-56%B. πNAP Column- Gradient; 0-5min 30-70%B, 5-10min 70-85%B. PBr Column- 0-1min 30-60%B, 1-3min 60-70%B, 3-8min 70-80%B

### Equipment

Mass Spectrometer: Shimadzu LC-MS 8060
LC Pump A: Shimadzu Nexera X2 LC-30AD
LC Pump B: Shimadzu Nexera X2 LC-30AD
Autosampler: Nexera X2 SIL-30AC
Column Oven: CTO-20AC
Solvent Degasser: DGU-20ASR
Acquisition Software: Lab Solutions

### Instrument Conditions

Ionization Mode: ESI
Nebulizing Gas: Nitrogen 3L/min
Heating Gas Flow: Air 10L/min
Interface Temp: 300C
DL Temp: 250C
Heat Block Temp: 400C
Drying Gas Flow: Nitrogen 10L/min
Collision Gas: Argon 270kPa

### Compound MRM Transitions Collision Energy

| | | |
|---|---|---|
| Deoxynivalenol | 297.3 — 249.3 | -10 |
| Aflatoxin G2 | 331.2 — 313.3 | -25 |
| Aflatoxin G1 | 329.2 — 243.2 | -10 |
| Aflatoxin B2 | 315.3 — 287.3 | -25 |
| Aflatoxin B1 | 313.3 — 285.2 | -25 |
| HT-2 | 447.3 — 345.3 | -20 |
| T2 | 489.3 — 245.2 | -25 |
| Fumonisin B1 | 722.5 — 352.4 | -35 |
| Fumonisin B3 | 706.5 — 336.4 | -35 |
| Fumonisin B2 | 706.5 — 336.4 | -35 |
| Ochratoxin A | 404.2 — 239.3 | -25 |
| Zearalenone | 319.3 — 283.3 | -15 |

## Results

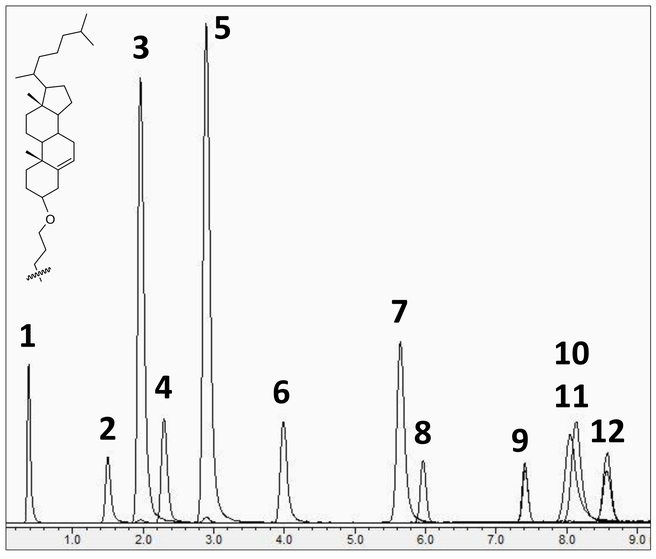


**Figure 1:** Cholester Column

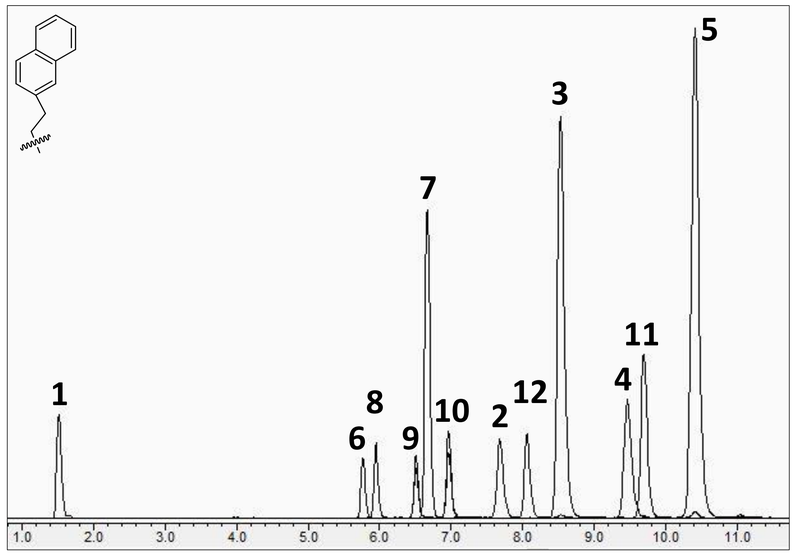


**Figure 2:** πNAP Column

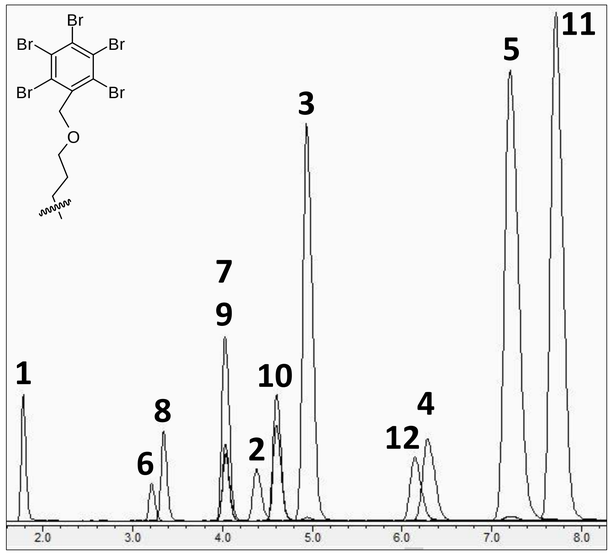


**Figure 3:** PBr Column

**1-Deoxynivalenol**
**2-Aflatoxin G2**
**3-Aflatoxin G1**
**4-Aflatoxin B2**
**5-Aflatoxin B1**
**6-HT-2**
**7-T2**
**8-Fumonisin B1**
**9-Fumonisin B3**
**10-Fumonisin B2**
**11-Ocratoxin A**
**12-Zearalenone**

## Conclusions

**Conclusions**: Each of the three columns tested exhibited significant differences in selectivity and retention. Overall, the πNAP Column demonstrated superior resolution for the 12 mycotoxins tested. These results can be used an inference for each column's affinity for particular structures, and a selection guide for future applications which may contain differing toxins or other small molecules.

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