RESTEK Pure Chromatography

Highly Efficient LC-MS/MS Analysis of Multiple Mycotoxins Utilizing Biphenyl Column Selectivity with Inert Column Technology

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Introduction

With an ever-growing list of mycotoxins to analyse, multi-analyte methods are an attractive alternative; affording time and cost savings to laboratories. However, with a range of chemistries across the broad group of mycotoxins this does prove a challenge. When analysed using a standard C18 column, high pH conditions are required for accurate analysis of Alternaria toxins and Ergot Alkaloids. These conditions typically are problematic for silica based HPLC columns, often causing significantly reduced column lifetime. Another factor at play with some groups of mycotoxins, are non-specific adsorption (NSA) or binding (NSB) with the metal surfaces of HPLC systems. These interactions further cause problems with peak shape, analyte sensitivity, and reproducibility from injection to injection.

In this work, Restek look to establish the benefits of coated column technologies by comparing methods developed on Inert and standard hardware to remove high pH requirements, along with matrix or chemical based passivation techniques for a wide panel of mycotoxin analytes.

Materials and Method

Table I: Method developed by Restek to analyze Mycotoxins under MS-compatible, acidic conditions being advantageous for column lifetime.

Columns	Raptor (Inert) Biphenyl		
Dimensions:	100 mm x 2.1 mm ID		
Particle Size:	2.7 µm		
Temp.:	60 °C		
Standard/Sample			
Diluent:	50:50 Methanol:Water		
Conc.:	10 ng/ mL		
Inj. Vol.:	5 µL		
Detector:	MS/MS		
Ion Mode:	ESI+		
	MRM		
Mobile Phase A:	Water, 0.05% formic acid		
Mobile Phase B:	Methanol, 0.05% formic acid		
Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	75	25
5.00	0.4	50	50
9.00	0.4	0	100
9.01	0.4	75	25
11.0	0.4	75	25

Restek previously developed a method for mycotoxin analysis including Alternaria toxin and ergot alkaloid epimers using standard HPLC conditions with our Raptor Biphenyl phase, which was carried out under **acidic** conditions. In order to assess the effect of inert coating, we compared standard Stainless Steel (SS) Hardware for the wetted flowpath (column body and frit) against Restek's Inert Column technology. These columns have an inert glass-like layer permanently fixed to them using Chemical Vapour Deposition, which should limit NSA and NSB interactions

In order to perform this testing, we carried out side-by-side analysis, analysing a panel of 34 mycotoxins via LC-MS/MS, using a standard Raptor Biphenyl 2.7µm, 100 x 2.1mm column (9309A12), comparing the results against a coated hardware Raptor Inert Biphenyl 2.7µm, 100 x 2.1mm column (9309A12-T). All other instrument conditions were kept identical between analyses.

More information including method and chromatogram here

Results and Discussion

In the data table below, we analyse the area and height ratio between each of the two hardware types. Values greater than 1 describing a positive improvement in response, and less than 1 describing a loss of sensitivity.

Across the full panel of 34 mycotoxins analyzed, **97%** of the analytes showed an **improvement** in sensitivity for both peak area and peak height. Of the panel, **Aflatoxin B1** was the **sole** compound which showed a **slight reduction** in both metrics. All other components showed ratios up to 10.1, meaning that **signal intensity** is up to **10 times higher** using inert columns.

The **fumonisin** (B1, B2, and B3) compounds are typically more problematic to analyse in matrix samples, with a **tendency for NSA/NSB** interactions leading to peak tailing, poor sample recoveries and carry-over issues. The use of inert hardware here resulted in an **average of five-fold improvement** in peak **area**, and **seven-fold** improvement for peak **height** across the three compounds, along with significant improvement on peak symmetry and a reduction of tailing. This demonstrates the dramatic improvement in NSA/NSB interactions when switching to Inert columns.

Whilst the response for aflatoxin B1 was reduced overall using the Inert hardware, this analyte could still readily be quantified, with both column hardwares producing gaussian peaks and demonstrating good resolution from other analytes.

Furthermore, we have compared chemical passivation from the 1st to the 10th injection to an inert column hardware showing that already the first the injection using inert column hardware shows doubled response. Lower detection limits and improved peak shape facilitate analysis.

Table II: Analysis of 34 mycotoxins with stainless steel (SS) and Inert Raptor Biphenyl column. Comparison of area and height ratios. Values > 1 = better sensitivity, Values < 1 = worse selectivity *(only Aflatoxin B1, marked in orange).*

Compound	Peak Area			Peak Height		
	Stainless Steel	Inert	Areas Ratio (Inert/SS)	Stainless Steel	Inert	Height Ratio (Inert/SS)
Fumonisin B1	32578	122878	**3.77**	399544	2415567	**6.05**
Fumonisin B2	23427	151719	**6.48**	383130	3869822	**10.1**
Fumonisin B3	29864	143302	**4.80**	472279	3444421	**7.29**
Ergocristine	171197	195562	**1.14**	3865898	4450058	**1.15**
Ergocristinine	1393116	1583053	**1.14**	29212317	32975663	**1.13**
Ergotamine	433635	493003	**1.14**	8149518	9274156	**1.14**
Ergotaminine	397370	462119	**1.16**	7885403	9237991	**1.17**
Ergocryptine	446481	522204	**1.17**	9671753	11360839	**1.17**
Ergocryptinine	658788	778972	**1.18**	13680420	16765348	**1.23**
Ergocornine	370509	387025	**1.04**	7248981	7732744	**1.07**
Ergocorninine	590167	704029	**1.19**	12052359	14389283	**1.19**
Ergosine	445243	486620	**1.09**	8630932	9366602	**1.09**
Ergosinine	439026	496734	**1.13**	7820785	8740527	**1.12**
T-2	43286	56535	**1.31**	1046233	1394735	**1.33**
HT-2	10183	15221	**1.49**	216703	323765	**1.49**
Tentoxin	70973	95175	**1.34**	1577164	2131907	**1.35**
Ochratoxin	173686	190060	**1.09**	4039682	4411953	**1.09**
Diacetoxyscirpenol	47850	68139	**1.42**	846403	1208826	**1.43**
Fusarenone X	3865	7668	**1.98**	60409	121790	**2.02**
15-acetyl-DON	17055	31369	**1.84**	269862	517570	**1.92**
3-acetyldeoxyvinalenol	13353	22613	**1.69**	179204	296396	**1.65**
Aflatoxin G2	171597	262824	**1.53**	3429501	5274354	**1.54**
Aflatoxin G1	224058	304389	**1.36**	4607959	6102959	**1.32**
ZON	25617	37162	**1.45**	656915	927455	**1.41**
Aflatoxin B2	159389	295648	**1.85**	3462489	5724754	**1.65**
Aflatoxin B1	265935	223520	**0.84**	5335576	4425821	**0.83**
Alpha-zearalenol	16202	30224	**1.87**	382092	702420	**1.84**
Deoxynivalenol	6935	17346	**2.50**	117927	281906	**2.39**
Nivalenol	1790	4182	**2.34**	25276	64495	**2.55**
Altenuene	63224	113850	**1.80**	1187958	2059700	**1.73**
Alternariol monomethyl ether	19537	31024	**1.59**	428922	640689	**1.49**
Alternariol	48204	73272	**1.52**	837410	1302192	**1.56**
Citrinin	499900	1007880	**2.02**	5031182	9828890	**1.95**
Tenuazonic acid	21503	47828	**2.22**	89293	197658	**2.21**

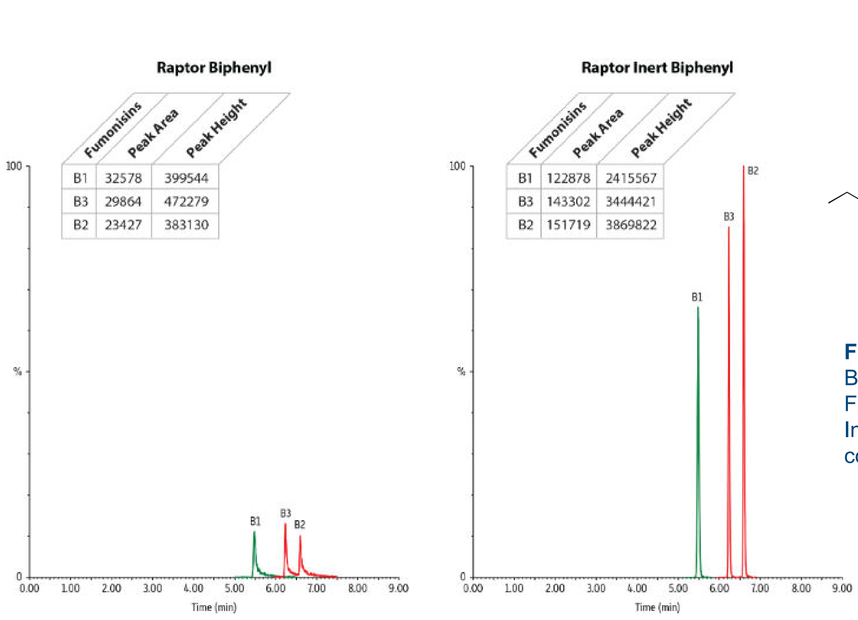

Figure 1: Comparison of Fumonisins B1-B3 analyzed on Raptor Inert Biphenyl and traditional SS Raptor Biphenyl. Right: Chemical Structure of Fumonisin B2.

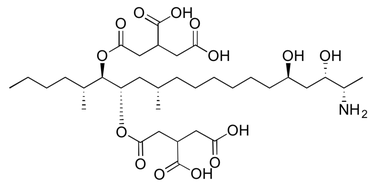

Figure 2: Structure of Fumonisin B2. The highly acidic nature of Fumonisins responds well to the Inert Hardware of the Biphenyl column.

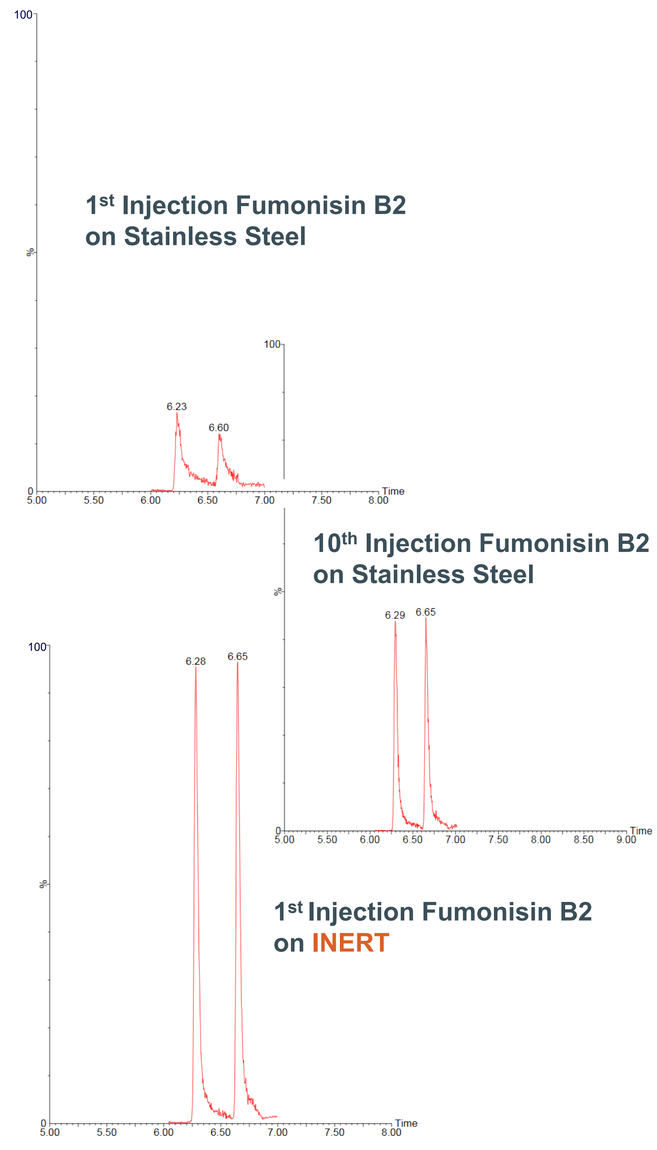

Figure 3: Comparison of chemical passivation 1-10 injections) on a conventional SS column to the first injection on an INERT Raptor Biphenyl column.

Conclusion

The unique selectivity of the Biphenyl stationary phase run under acidic conditions, made a multi-mycotoxin method possible combining Alternaria toxins, ergot alkaloid epimers and other major mycotoxins in one method. The chosen conditions lead to increased column lifetime and was beneficial for MS detection.

The combination with the inert column hardware technology enabled low detection limits, improved peak shapes and high response rates. Laborious conditioning and complicated passivation are not necessary with these coated inert columns, saving time and enables to measure more samples/time.

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