# Christmas Time – Biscuit Time: Heavenly Smell All Over the House!

But Who Thinks About Mycotoxins? Invisible, Odourless, yet, an Unwanted Ingredient

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Most of us love sweets! In Christmas time, our demand even grows. It's hard to resist the cosy smell of gingerbread, mulled wine and furthers – especially being homemade. As tasty they are – as dangerous they can be. Most ingredients are potential sources of mycotoxins. Thus, the less quality management, the higher the risk to grab an intoxicated item. Let's leave the worst case scenario and come to the facts. Chocolate, mulled wine, almond biscuits, nuts, and marzipan are typical sources of mycotoxins. Unsurprisingly, the toxins are resistant to temperature and "survive" the baking process. Hence, thorough analysis of raw materials yet processed food is of utmost importance. Especially for Christmas sweets, matrix-analyte interactions are highly complex and demand comprehensive sample preparation. Application of immunoaffinity columns offers appropriate solutions for any initial matrix.


Figure 1: Aflatoxins B/G in Marzipan
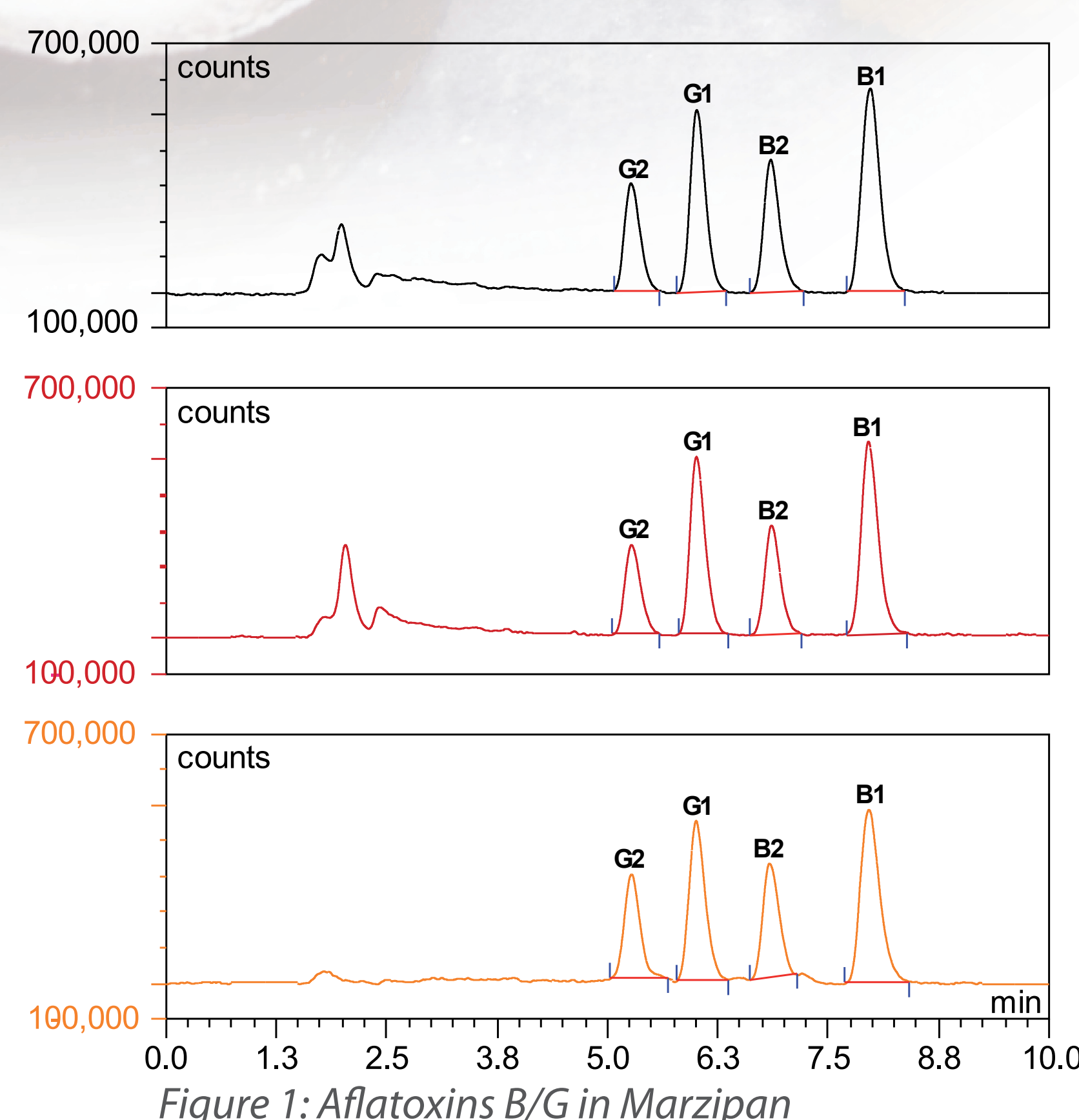
Marzipan
Clean-Up with AflaCLEAN Column

Black: Marzipan spiked after extraction, Red: Marzipan spiked before extraction, Orange: Standard (represents 10 ppb)

Best results and high recovery rates due to high performance clean-up columns even with difficult matrices like marzipan.


Figure 2: Ochratoxin A in Bittersweet Chocolate
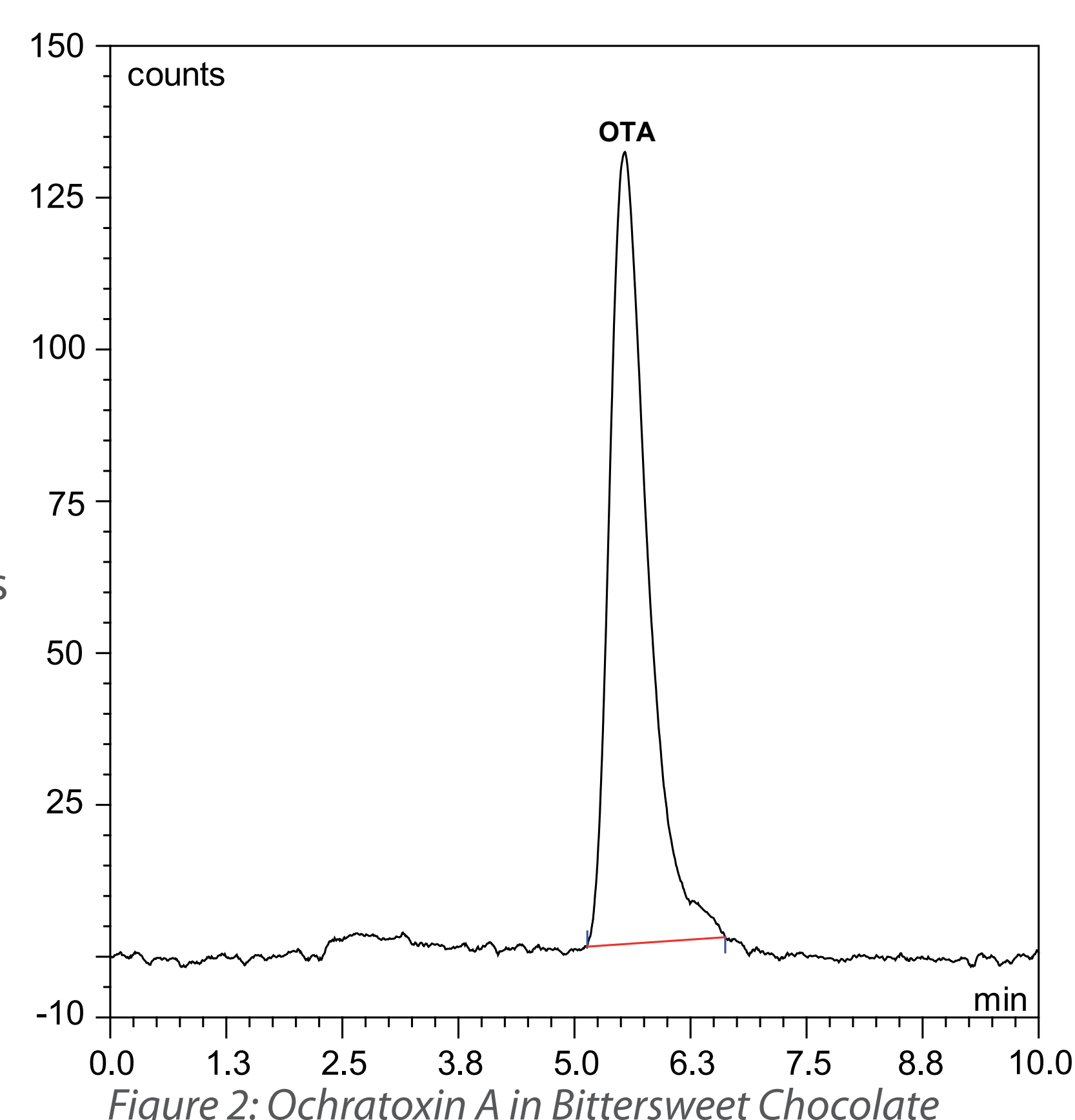
Bittersweet Chocolate
Clean-Up with OtaCLEAN Column

The clean-up of the sample is so good that a chromatography in less than 10 minutes is possible without any carry-over or interferences.

High throughput analysis and high recovery rates due to the excellent matrix clearance efficiency.

Figure 3: Aflatoxins B/G and Ochratoxin A in Spiced Cookies
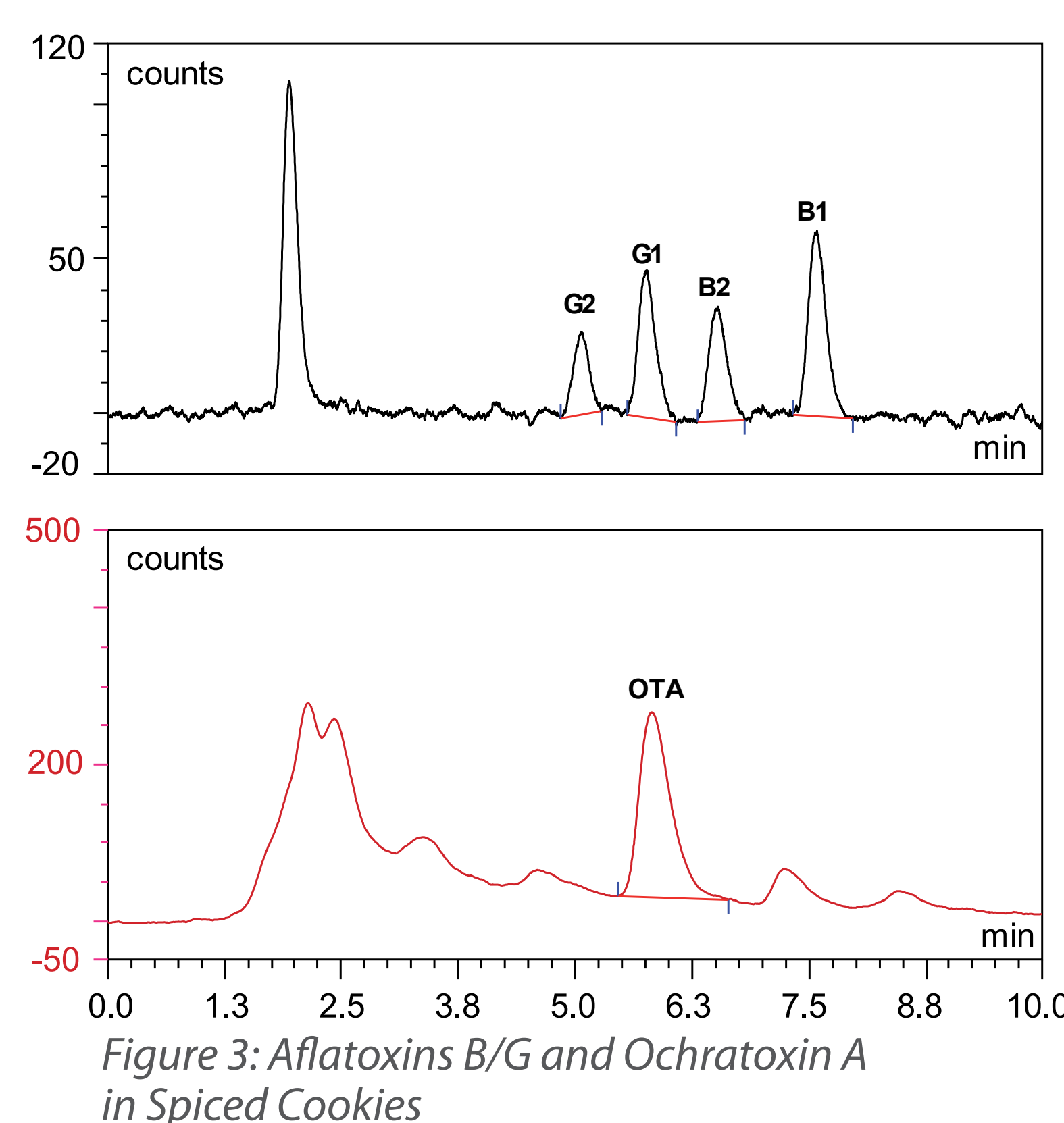
Spiced Cookies
Clean-Up with Afla-OtaCLEAN Column

Black: Chromatogram Aflatoxin B/G
Red: Chromatogram ochratoxin A

Perfect chromatograms using only one column for both analytes.

Recovery Rates

Matrix	Analytes	Recovery Rates for Aflatoxins B/G - Ochratoxin A in %
Brasil Nut	Aflatoxins B/G	92/95/99/90
Marzipan	**Aflatoxins B/G**	**89/93/84/90**
Nutmeg	Ochratoxin A	91
Bittersweet Chocolate	**Ochratoxin A**	**92**
Ginger Bread Spice Mix	Ochratoxin A	88
Mulled Wine	Ochratoxin A	92
Spiced Cookies	**Aflatoxins B/G - OTA**	**99/90/96/89 - 89**
Almonds	Aflatoxins B/G - OTA	91/96/96/97 - 97

Materials and Experimental Procedures

Solid materials (20 g) were homogenised and 2 g of sodium chloride was added. The sample was extracted by MeOH/water (80:20 (v/v)), 50 mL n-hexane was added during extraction to remove fat and oil from the crude extract. The extract was centrifuged by 3000 x g for the separation of n-hexane and MeOH phase. The MeOH phase was used for further sample dilution. For all samples samples, the crude extract was diluted according to extraction and clean-up procedures of the manufacturer. A max. of 50 mL (or 14 mL for samples containing lots of fat) was applied onto AflaCLEAN, OtaCLEAN or Afla-OtaCLEAN columns. After loading, the column was washed by 10 mL water and dried by air flush. The toxins were eluted with MeOH. All eluates were adjusted to HPLC solvent concentration. Standards were treated accordingly.

Aflatoxin B/G Analysis

HPLC (Thermo Scientific ultimate 3000): Methanol/water/acetonitril (v/v/v: 0/15/60), column temp. 36 °C, flow rate 1.2 mL/min, separation column with gard column (LCTech) excitation 365 nm / emmission 460 nm. UV derivatisation of aflatoxin B1/G1 by UVE.

Ochratoxin A Analysis

HPLC (Thermo Scientific ultimate 3000): Methanol/water/acetonitril (v/v/v: 55/05/40) column temp. 40 °C, flow rate 0.6 mL/min, separation column RP EC 125/3 nucleosil 120-3 C18 with gard column (Macherey&Nagel), excitation 335 nm / emmission 465 nm.

Conclusion

Ochratoxin A analysis of various spices and spice mixtures lead to good recoveries (88 – 91 %) and chromatographical results show hardly matrix interference (*Figure 3*). For single ingredients of difficult composite recoveries over 90 % could be achieved for both toxin groups. For composite matrices like chocolate or gingerbread recoveries higher than 85 % and chromatographic results are striking (*Figure 2*)! Hence, the application allows the analysis even of composite matrices, which contain high sugar content or higher amounts of possible interfering matrix (*Figure 1*). Subsequently, it allows full control of all ingredients and quality management even on those matrices which underwent most complex modifications or mixing of individual components. The column clean-up and chromatographical results indicate, that even far below the regulated level of contamination toxins can be quantified and analysed easily. However, even a little poison is poison and the cumulative long-term consequence is unclear. But don't worry the risk of eating a contaminated biscuit in Europe is quite low – so no need to worry about this years' Christmas.
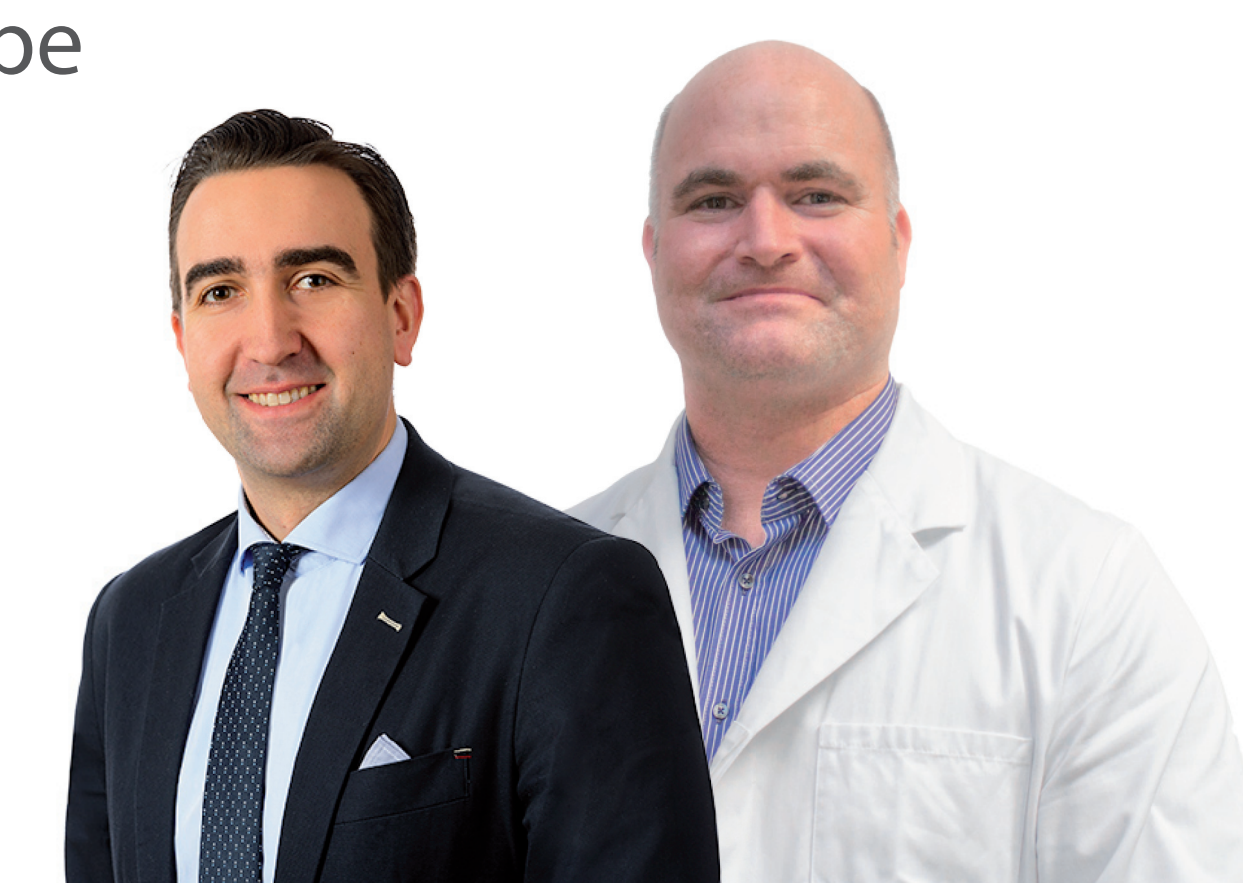
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