On-line derivatization strategies for the sensitive fluorescent detection of aflatoxins in food and feed by liquid chromatography

Ana M. GARCIA-CAMPAÑA, Natalia ARROYO-MANZANARES,
José F. HUERTAS-PÉREZ, Ahmed M. HAMED,
David MORENO-GONZÁLEZ, Laura GÁMIZ-GRACIA
Dept. Analytical Chemistry, Faculty of Sciences, University of Granada, Av. Fuente Nueva s/n, 18071 Granada, Spain.

Introduction

Aflatoxins (AFB1, AFB2, AFG1 and AFG2) are highly carcinogenic fluorescent mycotoxins produced by *Apergillus* genera. The emission from AFB1 and AFG1 is strongly quenched in the aqueous mixtures used as mobile phase in liquid chromatography, which is usually overcome by a derivatization step. Several strategies can be applied, being the most common the off-line pre-column derivatization with trifluoroacetic acid (TFA). As alternative, two on-line methods based on high performance liquid chromatography (HPLC) with photoinduced (PI) derivatization and ultra-high performance liquid chromatography (UHPLC) with post-column chemical reaction by adding pyridinium bromide perbromide (PBPB), are proposed in this work.

Materials and Methods

UHPLC-FLD method: a mobile phase consisting on water, acetonitrile and methanol in gradient mode was used and a "T" connector to mix the PBPB solution with the eluate from the chromatographic column. A reaction coil was placed in an oven at a 25 ºC joining the outlet of the "T" connector with the inlet of the FLD cell.

HPLC-PI-FLD method: a UV- derivatization module previous to the fluorescence detector was used. The excitation and emission wavelengths were 365 and 460 nm, respectively.

Results and Discussion

The UHPLC-FLD method was characterized in rice, while the HPLC-PI-FLD method was validated in different feedstuffs. Solid-liquid extraction was used as sample treatment in all the cases. The HPLC-PI-FLD method was also applied for the determination of AFs in vegetables milks and yogurt, using dispersive liquid-liquid extraction as sample treatment.

Conclusion

Different derivatization strategies were evaluated for the fluorescent detection of aflatoxins by UHPLC and HPLC, obtaining low LOQs that allowed their determination at levels established by current legislation.

Acknowledgement: Project AGL2015-70708-R.