

Photochemical Derivatization of Aflatoxin B1 and G1

Q: Is the technology a new and still unknown technology?

A: No, the technology of photochemical derivatization of aflatoxins is known for decades. It is also used successfully for other analytes.

Q: Is it approved and already used, respectively?

A: The AOAC has accepted this methodology as an analytic techniques and the comparability of this method with the Cobra cell was confirmed and published by an EU central lab (Papadopoulou-Bouraoui, A., Stroka, J., Anklam, E. (2002) *J. AOAC Int.* **85**, 411 – 416). Furthermore, routine labs work successfully with this methodology.

Q: How is the comparability to other derivatizing reagents such as (iodine e. g. with the Pickering Laboratories post-column derivatization system) or bromine (Cobra cell)?

A: As a rule the photochemical derivatization gives slightly increased signals compared to the derivatization with iodine; compared to the electrochemical derivatization with bromine the signals for B2 and G2 are slightly better, and G1 slightly lower signal intensity, the observed signals fulfill the regulatory requirements even for baby food.

Q: How does the photochemical derivatization work?

A: By the use of UVC irradiation with a wave length of 254 nm the HPLC eluent, especially the water, is photochemically excited. This for example derivatizes aflatoxin B1 to B2a specifically, reproducibly, and irreversibly, by hydroxylation of a double bond (technically one molecule of water is attached to one aflatoxin molecule). In turn, aflatoxin B2a may be excited to fluoresce by light with a wave length of 365 nm and so is getting measurable. Aflatoxin G1 behaves analog.

Q: Is the derivatization only temporary or stable?

A: The photochemical derivatization of aflatoxins is permanent, that is the fluorescence excitation can be conducted easily in the fluorescence detector.

Q: Do I need reagents or reagent pumps?

A: No, the eluent, especially the water, is already the reagent. The generation of the active species is done photochemically in situ. Nothing has to be pumped to the eluent, therefore neither complex reagent pumps are necessary, nor is the column eluate diluted by reagent addition.

Q: Are there quality requirements for the HPLC water?

A: Yes, the HPLC water must be suitable for fluorescence detection; using water of inferior quality may result in significantly lower signals for the aflatoxins.

Q: At which wave lengths are the aflatoxins measured?

A: The excitation wave length is 365 nm; the B/G-type aflatoxins show fluorescence optimum at 460 nm, the specification of fluorescence detector allow further signal optimisation in terms of sensitivity.

Q: Does the derivatization with UVC irradiation disturb the detection of the other aflatoxins?

A: No, the other aflatoxins such as B2, G2, M1 and M2 are not affected and can be measured at the same time.

Q: Is the system susceptible to wear?

A: No, the reactor loop and UVC lamp are designed for many thousand hours of operation.

Q: Do I have to change my HPLC system?

A: From technical points of few nothing has to be changed. The photochemical reactor is simply connected between the outlet of the HPLC column and the detector inlet. The settings of the whole system remain the same.

Q: Do I have to change my sample preparation?

A: No, you can proceed as usual, e. g. cleanup with immuno affinity column.

Q: What are the main advantages of this method in comparison to the traditional post-column derivatization e. g. with the Pickering system or the Cobra cell?

The system is attractive for several reasons. No reagents are pumped to or have to be added into the eluent, thereby reducing cost, processing steps, and problems with the 'chemistry'. Also aged solutions or clogged tubes are a thing of the past. The manual handling is reduced to a minimum, meaning you only have to switch on the mains in the days routine. Finally, the purchase as well as the maintenance cost is low. This method for post column derivatisation of aflatoxins is more robuste and has higher stability in the daily analytical work Possible prone to failure is reduced due to the point that no extern derivatisation reagents are needed.