**MYCOTOXIN ANALYSIS: A FOCUS ON RAPID METHODS**

**General Comments:**

This book offers a general overview of mycotoxin analysis with an emphasis on rapid methods. Although it could be useful for developing countries, it also contains inconsistencies which could be indicative of discrepancies between statements or of questionable information. The references tend to be older and although most of the older material is still valid, having predominantly older references suggests that this review will be slightly dated before it is published.

**Specific Comments:**

Pg.1, please check the Omurtag reference.

Pg. 3, the genomic studies they cite are from 2008, which is almost prehistoric given how rapidly that field has moved.

Tables 2&3: certain limits are marked with an asterisk to indicate guidance levels, which begs the question: what do the other values pertain to (Official control levels? Violative levels?).

Pg. 4, a citation for the sampling protocols prescribed by the Codex would be helpful.

Figure 2: the "GC" should be on its own line, below "LC-MS". Also "MIP" is almost always used as a purification strategy, not as a detection strategy.

Pg. 8, the term "Pre-Analysis" is not commonly used. The analysis is a procedure that starts with a commodity/food and ends with actionable information. So analysis includes sampling, etc. What the authors are calling "analysis" is more accurately described as "detection".

Pg. 8, the authors seem to be confusing the extraction steps with the isolation steps. "Extraction" comprises drawing the mycotoxin out of the matrix and into solution. "Isolation" consists of further purifying or concentrating the extract so that it can be detected. LLE is a means by which to help isolate toxins from an extract, as is solid-liquid partitioning. As described in this article, the LLE and solid-liquid extraction are actually isolation steps.

LLE is not so much based on "insolubility of mycotoxins in aqueous phase and organic phase..." as it is based upon the partitioning of the toxin between the two solutions based on polarity. The word “insolubility” suggests that the toxin is not soluble (i.e. is a solid) in a particular solution or solvent. That is usually not the case.

Table 4: why is strength highlighted in green?

Table 5: this is illustrative of why reliance on dated literature is a problem: many years ago the literature indicated that aflatoxins could be extracted with entirely aqueous systems that incorporate detergents like sodium dodecyl sulfate (SDS) to help solubilize them. Since that time, a large number of manufacturers of aflatoxin test kits have developed and are selling aqueous-based extraction systems for aflatoxins that do not involve solvents at all. This table (dated 2006) completely ignores this significant advance in extraction technology for aflatoxins. Since the intended audience may be analysts working in regions were solvent availability or disposal may be a problem, this omission should be addressed.

Pg. 9, check the reference "International, 2000". Is this the AOACI?

Pg, 10, again, this is isolation, not really extraction.

Pg. 10, second column, line 4, indicates IAC is multifunctional. IAC can purify, concentrate and isolate. If this is “multifunctional”, then what about the other SPE? Usually I think of the MycoSep columns as multifunctional. Multifunctional can mean many things. Generally it is taken to mean (as with the MycoSep columns) that the columns can be used for multiple analytes. Here the authors are not making the distinction between two types of SPE: where the analytes are retained or where impurities are retained and the analytes pass through. The distinction is important, since it distinguishes the MycoSep (Romer-type) SPE columns from traditional C18-SPE columns, etc.

Pg. 11, SPME can certainly use MIPs, the MycoSep columns are **not** based on MIPs. The older literature suggests these are based on neutral alumina, silica, charcoal, etc., not polymers. The company Affinisep does use MIPS, however.

Pg. 11, the authors mentioned the strength of IAC was its specificity but gave no explanation for characterizing cross-reactivity as a weakness.

Pp . 11 & 12, perhaps we have different definitions of "state of the art" and "conventional". In my view "conventional" methods are widely used. State of the art methods use the best, most current, technology available but are not necessarily widely available. My suggestion would be to call section 1.2 "chromatographic methods" and section 2.2 "rapid methods".

Pg. 11, this section is actually 2.2.1 not 1.2?

Pg. 11, please cite the Official Method number for the AOACI aflatoxin method being described.

Pg. 11: note that the LOD of this method is the same as the regulatory limit for AFB1 in the EU. This suggests that the method is likely not fit for the purpose of meeting EC requirements. Perhaps a more recent HPLC method can be cited that meets the EC requirements?

Pg. 11, second column, second paragraph, last section “…rapid test methods rather than laboratory based reference method”, do the authors mean to indicate that rapid methods are portable? It seems to me one of the advantages of rapid methods is their being portable.

Pg. 12, Table 7, shows LFD and ELISA are portable but Tables 16, 17, 19, and 21 indicate LFD and ELISA are not portable. Please define “portable”.

Pg. 14, Table 8, it is unclear what the authors mean by “no interfering of the mobile phase”.

Pg. 14, second column, lines 4-5, “pre-conditioned strip is wetted” the authors did not mention with what the strips are wetted. Usually test extract is applied to LFD directly without pre-conditioning.

Tables 16, 17, 19, and 21, parameters of the columns are not uniformly listed. Tables 16 and 17 give no method time but give extraction solvent, while Tables 19 and 22 give method time but no extraction solvent. This difference makes method comparison impossible. Please define “method time”. Does it include extraction, and/or filtration, and dilution?

Table 35, 8-12 hours for HPLC method time is over estimated. The method time of recent methods is much shorter.

Page, 46, benzene and chloroform are no longer commonly used for preparation of standards solutions. Please give information on using toluene and acetonitrile mixture, or acetonitrile, or methanol, as alternatives.

Further suggestions: perhaps the authors should give more up-to-date information and emphasis on method validation and matrix interference for some of the rapid methods.