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Comment on New Reagent for Trace Determination of Protein-Bound Metabolites of Nitrofurans in Shrimp Using Liquid Chromatography with Diode Array Detector

A recently published paper described a new derivatizing reagent for the simultaneous analysis of four nitrofuran antibiotics in shrimp (*1*). An advantage claimed for the new reagent is that it permits the use of relatively inexpensive HPLC with diode array detector (HPLC-DAD) instrumentation, rather than HPLC-MS/MS.

A key principle for such analysis must surely be that, for each of the four nitrofurans, the marker metabolites being derivatized with the new reagent ought to be specific; that is, they should not be compounds known to occur in foods that have not been subject to illegal nitrofuran treatment, yet the authors made no mention of this principle.

For one of the four nitrofuran antibiotics, nitrofurazone, the choice of semicarbazide as the marker for analysis is particularly problematical in that it is well documented to occur in several types of foods that have not been exposed to nitrofurazone abuse (*2*−*6*). Of particular relevance to the analysis of shrimp is that wild crayfish are known to contain semicarbazide in cases when nitrofurazone abuse cannot have occurred (*6*). It is not known why wild crayfish should contain semicarbazide, but it has been observed that semicarbazide forms from the biological molecule hydroxyurea under acidic, neutral, and, to a greater extent, basic conditions (*7*). Like crayfish, shrimp are decapod crustaceans and are likely to have similar biochemistry. Inattention to this problem will impair the credibility of any test method because of the likelihood of false-positive results.

The authors state, incorrectly, that, under European Union (EU) legislation, semicarbazide remains the marker residue. However, what the EU regulation (*8*), which is ref 12 of that publication, actually establishes is a "minimum required performance limit" of 1 μg/kg for nitrofuran metabolites in poultry meat and aquaculture products—but no directives are given as to the specific identity of the nitrofuran metabolites to be analyzed.

Nitrofurans have short half-lives in muscle and liver, in part because of biological reduction of the nitro group (*9*), making their analysis difficult. Nevertheless, specific detection of abuse can be accomplished by analysis for intact nitrofurazone in nonmeat tissues, such as eyes (specifically the retina) (*10*). For some foods, including eggs, detecting nitrofurazone abuse can be accomplished by analysis for intact nitrofurazone (*11*). For other foods for which this is impractical because of rapid depletion, there is a need for alternative approaches to be developed. 5-Nitro-2-furaldehyde was detected after its hydrolysis from an intact nitrofuran extracted from meat of treated poultry (*12*) and may prove to be a useful, if generic rather than specific, analyte for nitrofurans. Other metabolites examined for nitrofurazone analysis include semicarbazide (*13*) and possibly 5-(*N*-carbamoylamino)imino-4-oxo-3-sulfhydrylpentanenitrile sulfur-bound to glutathione (*14*) (shown in **Figure 1**).

During method development for detecting nitrofurazone abuse, it is important that laboratories consider the likelihood of false positives ensuing from using semicarbazide as a metabolite. If laboratories wrongly claim foods have been treated with nitrofurazone, the confusion they create will undermine their authority to help eliminate this illegal practice.

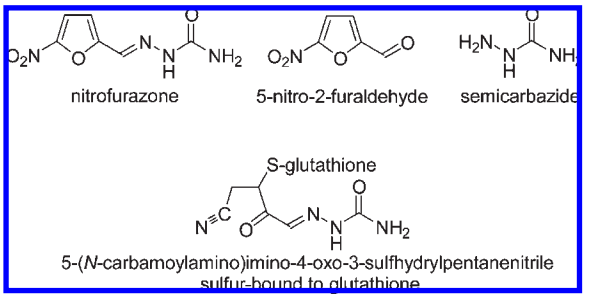

Figure 1. Metabolites used for nitrofurazone testing.

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