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Short Research Article

# Production and use of mycotoxins uniformly enriched with stable isotopes for their dosage in biological samples: (3) Tools for pharmacokinetics and as internal standards†

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Pharmacological studies of exogenous compounds often encounter problems: these compounds are in such infinitesimal amount in their biological matrices, that they require particular detection method. We have implemented an alternative method to the usual radioactivity, based on incorporation of stable isotopes, through the example of biosynthesis of uniformly $^{13}$C-enriched mycotoxins. The isotopic cluster obtained from a 10% $^{13}$C enrichment of several mycotoxins (and their metabolites) can be easily recovered from biological tissue samples by mass spectrometry allowing an easy discrimination from natural non-enriched compounds.

We illustrate such pharmacological approaches by *in vitro* zearalenone metabolism. Such enriched compound can also be used as internal standard with high reliability in order to quantify mycotoxins in contaminated food samples.

## Results and discussion

### Metabolic study of zearalenone (ZEN)

ZEN is known to be metabolized to several metabolites (Figure 1)[1] and some of them have, as ZEN, some endocrine or even carcinogenic effects[2] which make their detection and quantification in biological tissues very important. In particular, one of them may be a catechol, structurally close from that formed on estrogen and involved in estrogen-dependent cancers.

In a 30 min incubation at 37°C with ZEN in the presence of microsomes from rats liver treated with phenobarbital (CYP P450s inducer), we can analyze the metabolites profile by HPLC–MS.

As it is shown in Figure 2, the isotopic pattern is conserved for all the metabolites of ZEN, which makes their identification easier in an HPLC–MS analysis. In a similar approach, such isotopic enrichment can be used for quantitative evaluation of transport across biological membranes, and for discriminating in a complete mycotoxin mixture, the relative ability of each component to cross the cellular epithelium layer.

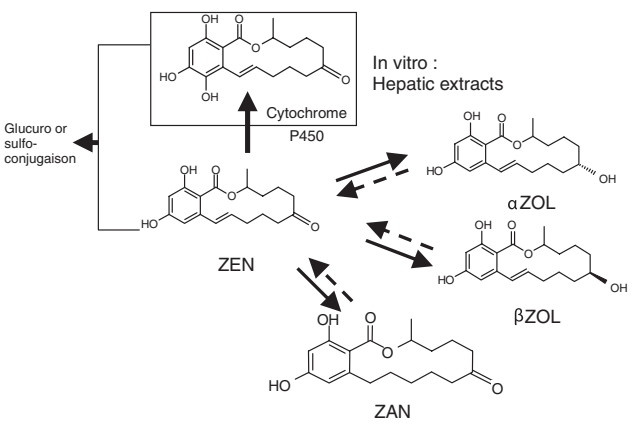


**Figure 1** Zearalenone (ZEN) and its metabolites.[1]

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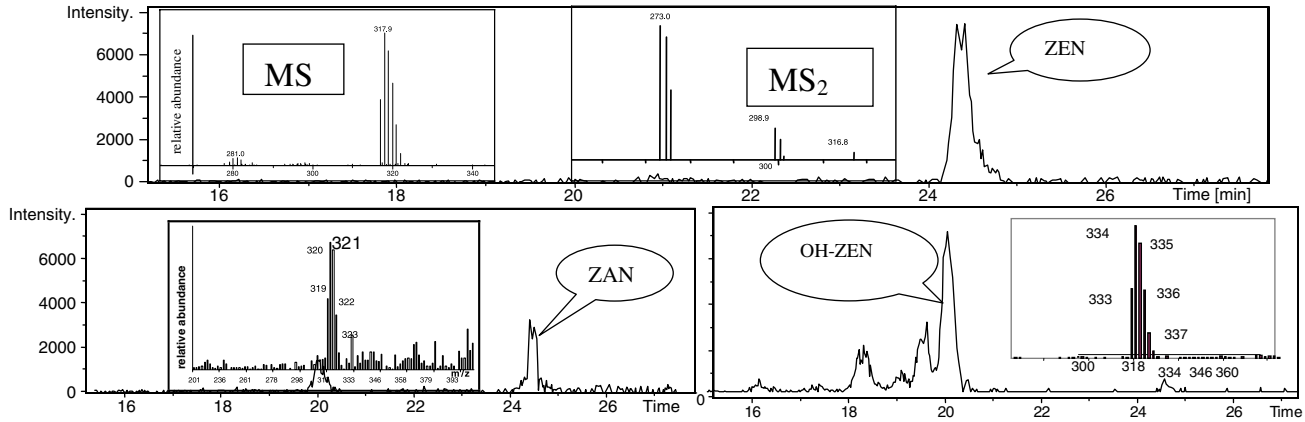


**Figure 2** Ion extracted chromatograms of ZEN ($m/z = 319$) and its metabolites ($m/z = 321$ and 335) formed by rat liver microsomes treated with phenobarbital. Figure available in colour online at www.interscience.wiley.com

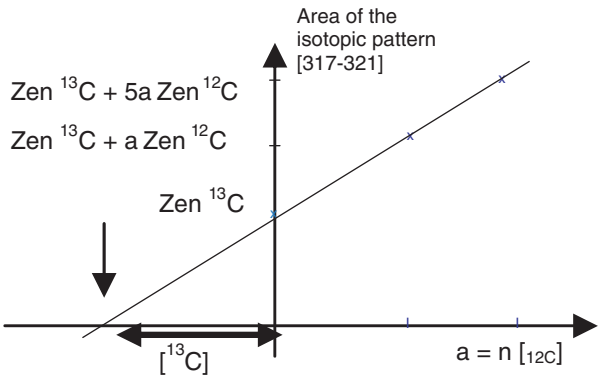


**Figure 3** Typical calibration line of ZEN $^{13}C$ using the additions gauged method (addition of specific quantities of ZEN $^{12}C$). Figure available in colour online at www.interscience.wiley.com

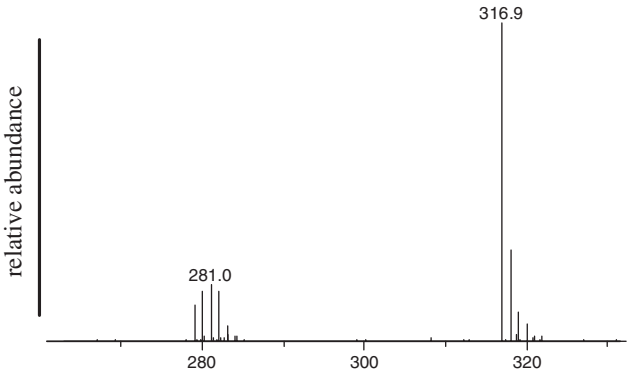


**Figure 4** Mass spectrum of ZEN 10% U-$^{13}C$ from a raw sample spiked with 0.02 μmol of ZEN $^{12}C$.

## Use of U-$^{13}C$ labelled molecules as internal standards (IS)

In order to use these enriched molecules as internal standards, we quantified the 10% U-$^{13}C$ zearalenone using known amounts of commercial $^{12}C$ zearalenone (Figures 3 and 4). The deduced concentration of 10% U-$^{13}C$ ZEN was approximately $4 \times 10^{-4}$ mol/L. This compound can thus be used as an internal standard in complex mixture such as wheat seeds or flour without optimization of the extraction process.

In order to decrease the super-imposition of the MS spectra of the natural and U-$^{13}C$ mycotoxins, we are presently trying enrichments of $^{13}C$ going from 30 to 100%.

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