# Direct Analysis of Folic Acid in Digestive Juices by LC/TOF-MS

Application

**Food Safety**


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## Abstract

**By use of the high resolving power and accurate mass measurement of the LC/TOF-MS it is possible to measure folic acid without cleanup directly in gastrointestinal juices. The samples are obtained from an *in vitro* digestion model. The method is validated for measuring uptake in this type of model.**


## Introduction

Health benefits associated with the intake of folic acid are well known. Increasing the daily intake can be achieved through medication or through the consumption of enriched food products. In the latter case, it is important to know the fraction of the total intake that is accessible in the gastric tract. To study the accessibility, an *in vitro* digestion model can be used.

Due to the complex nature of gastrointestinal juices (for example, saliva, stomach juice, and chyme), analysis of folic acid in these juices generally requires an extensive cleanup procedure. Traditionally, cleanup procedures consist of immuno affinity chromatography or solid phase extraction.

Using the high-resolution capabilities and unique accurate mass measurement of LC/TOF-MS, these cleanup steps can be eliminated and direct detection of folic acid in gastrointestinal juices is possible. By doing so, the risk of degradation of folic acid during cleanup is minimized and high-throughput analysis becomes possible. This application describes the direct measurement of folic acid in gastrointestinal juices.

## Experimental

### Cleanup Procedure

Saliva, stomach juice, and chyme were prepared as described elsewhere.[1] Sample preparation is straightforward; an aliquot of the juices is taken and directly injected in the LC/TOF-MS system.

### LC/MS Method Details

**LC Conditions**

| | |
|---|---|
| Instrument: | Agilent LC 1200 SL |
| Column: | ZORBAX SB-C8, 100 mm x 2.1 mm, 1.8 µm (p/n 828700-906) |
| Column temp: | 80 °C |
| Mobile phase: | A: 1% formic acid in water<br>B: 26/60/14<br>v/v acetonitrile/water/methanol |
| Gradient: | 5% B at 0 min<br>5% B at 4 min<br>40% B at 8 min<br>40% B at 8.1 min<br>5% B at 9 min |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 10 µL |

**MS Conditions**

| | |
|---|---|
| Instrument: | Agilent 6210 LC/TOF-MS |
| Source: | Positive ESI |
| Drying gas flow: | 9 L/min |
| Nebulizer: | 60 psig |
| Drying gas temp: | 350 °C |
| $V_{cap}$: | 4000 V |
| Fragmentor: | 150 V |
| Skimmer: | 60 V |
| Scan mode: | *m/z* 110—940, 12419 transients/scan, 1.5 scan/sec |
| Reference mass solution: | 100 mg/L solutions of purine 2 ml/L and HP-921 1 ml/L in acetonitrile infused into second sprayer at constant rate |
| Reference mass: | *m/z* 121.020873 and 922.009798 |

## Results and Discussion

### Calibration Standards

The structure of folic acid is shown in Figure 1. Because sensitivity is not a concern in this analysis conditions were only optimized for accurate mass measurement and linearity of response within the concentrations expected for the samples. In Figure 2 a typical chromatogram is shown of a standard of 1 µg/mL (expressed as concentration in matrix). The S/N for this concentration is typically greater then 500.

Calibration curves are produced from seven calibration points in the range between 1 µg/mL to 100 µg/mL. It is demonstrated that the LC/TOF-MS has the necessary linear dynamic range.

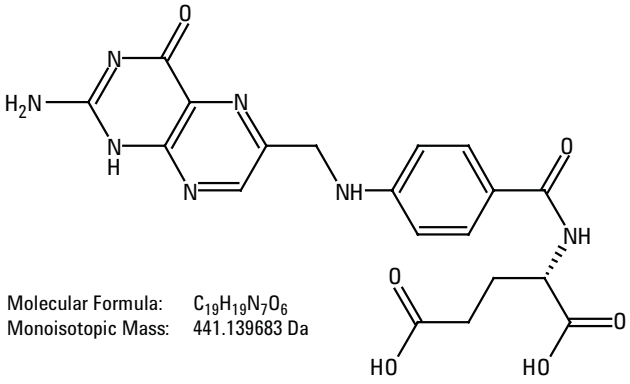


**Figure 1. Structure of folic acid, its exact molecular weight, and its empirical formula.**

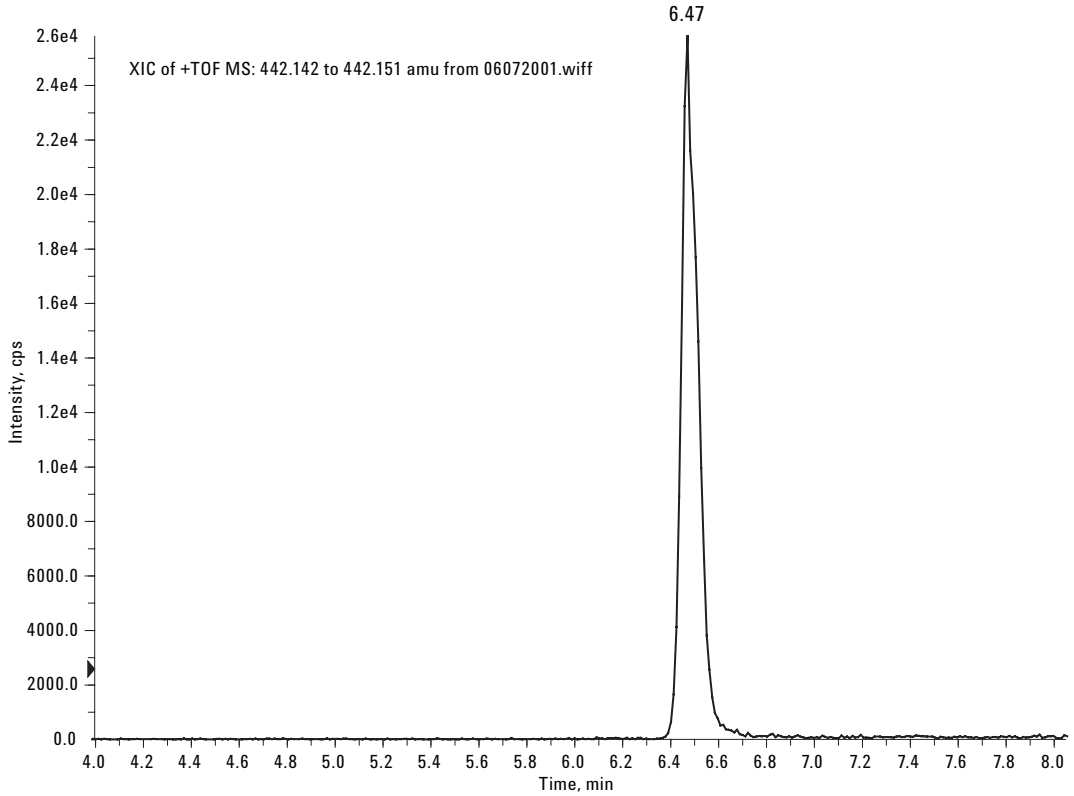


**Figure 2. Extracted ion chromatogram of a standard of folic acid at 1 µg/mL (ion extraction window of ± 10 ppm of exact mass of M+H ion).**

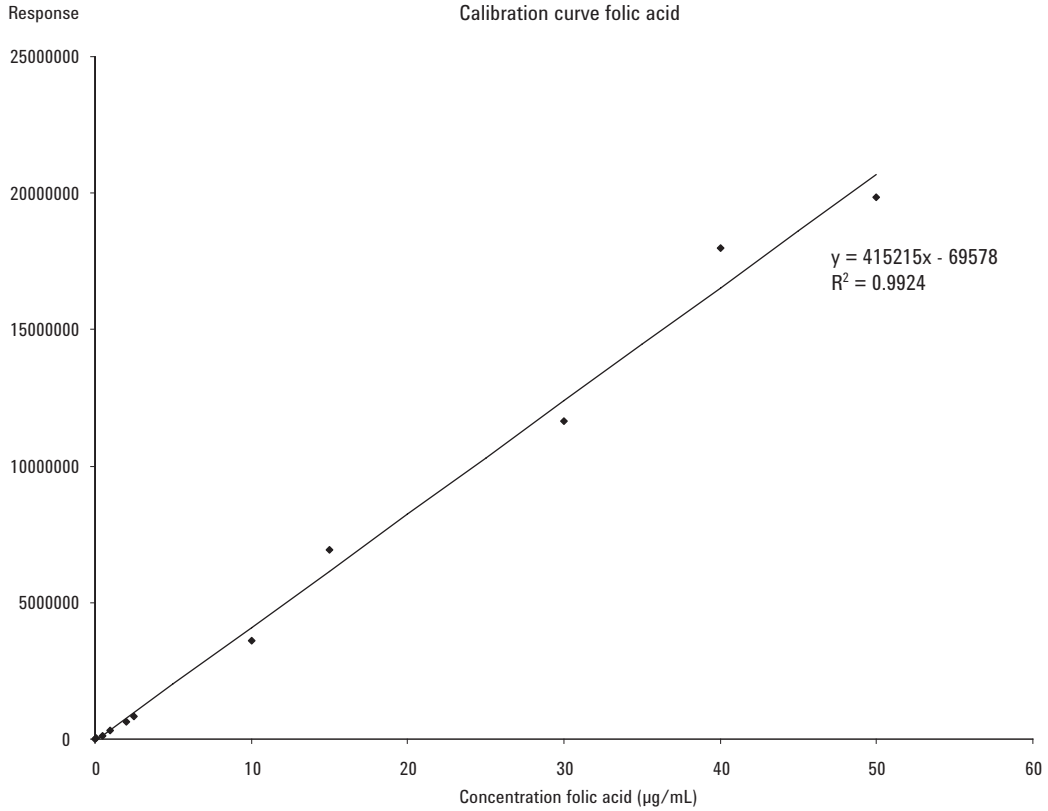


**Figure 3. Calibration curve of folic acid standards from 1 to 100 µg/mL.**

### Validation of the Method

To obtain reliable validation results it is important to obtain clean chromatograms without matrix interference. Figures 4 to 6 show examples of total ion chromatograms, extracted ion chromatograms (EIC), and spectra of spiked gastrointestinal juices (1 µg/mL). In all samples mass accuracy was better then 3 ppm. EIC of each were extracted with a mass window of 442.1425 to 442.1515 (± 10 ppm) to obtain chromatograms with no matrix interference.

### Validation of the Sample Data

Validation of the method was done by spiking saliva, gastric juice, and chyme at the following concentrations: 1, 2.5, 5, 10, 25, 50, 75, and 100 µg/ml. The results would indicate whether there were ion-suppression effects in these matrices. Figure 7 shows the response for each of these matrices. The data show that chyme produces the least effects, whereas saliva and gastric juice produce slightly higher ion suppression effects.

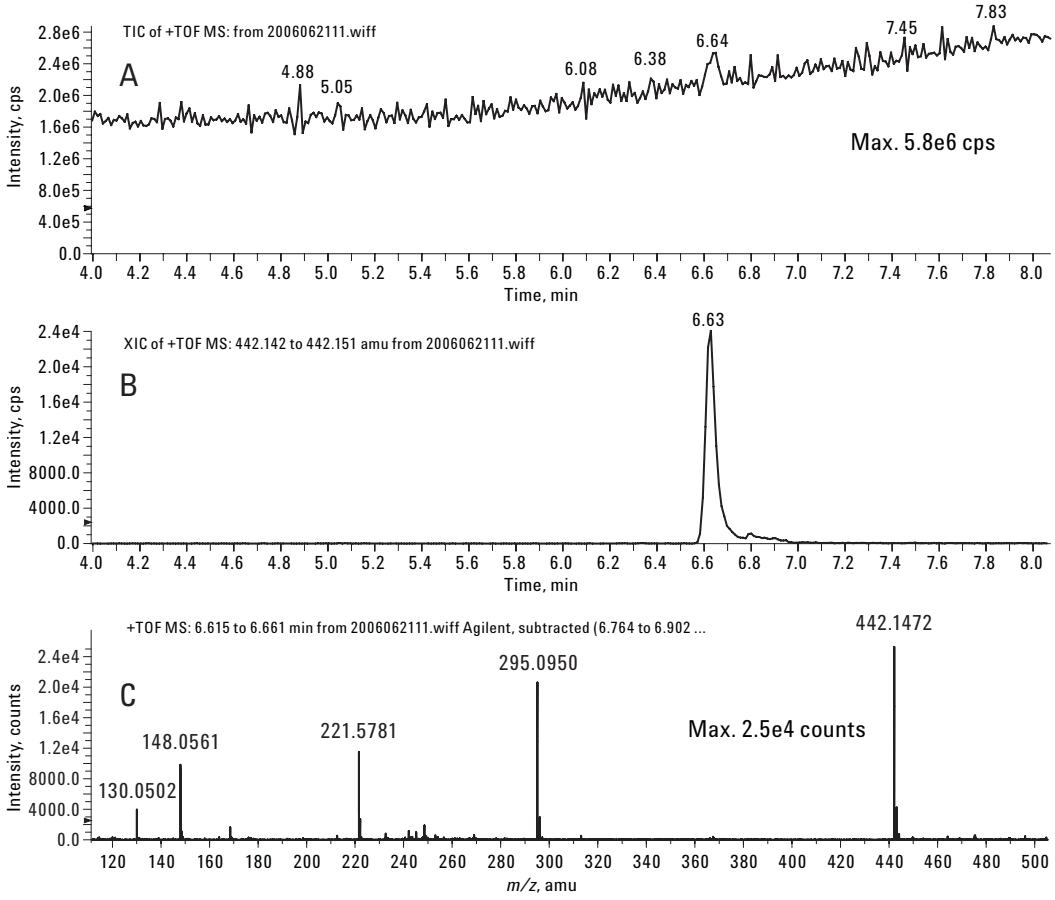


**Figure 4. TIC (A), EIC (B), and spectra (C) for folic acid in saliva spiked at 1µg/mL.**

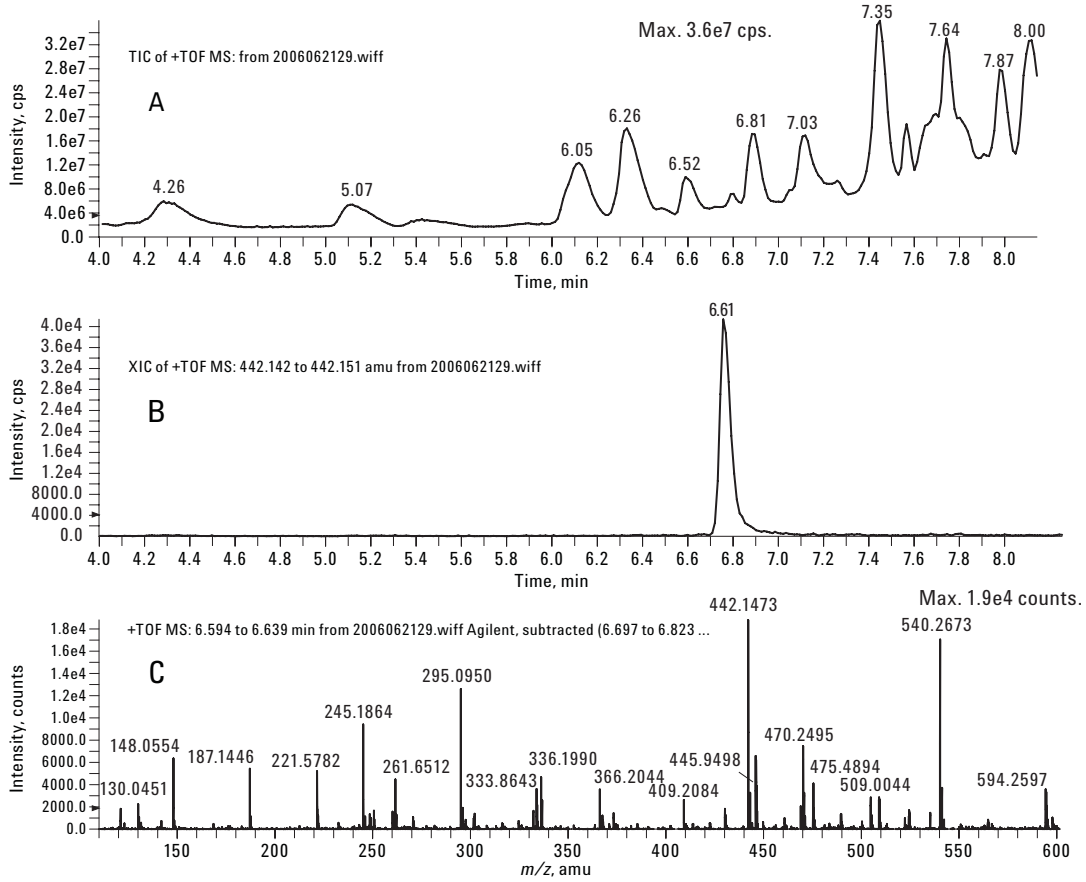


**Figure 5. TIC (A), EIC (B), and spectra (C) for folic acid in gastric juice spiked at 1 µg/mL.**

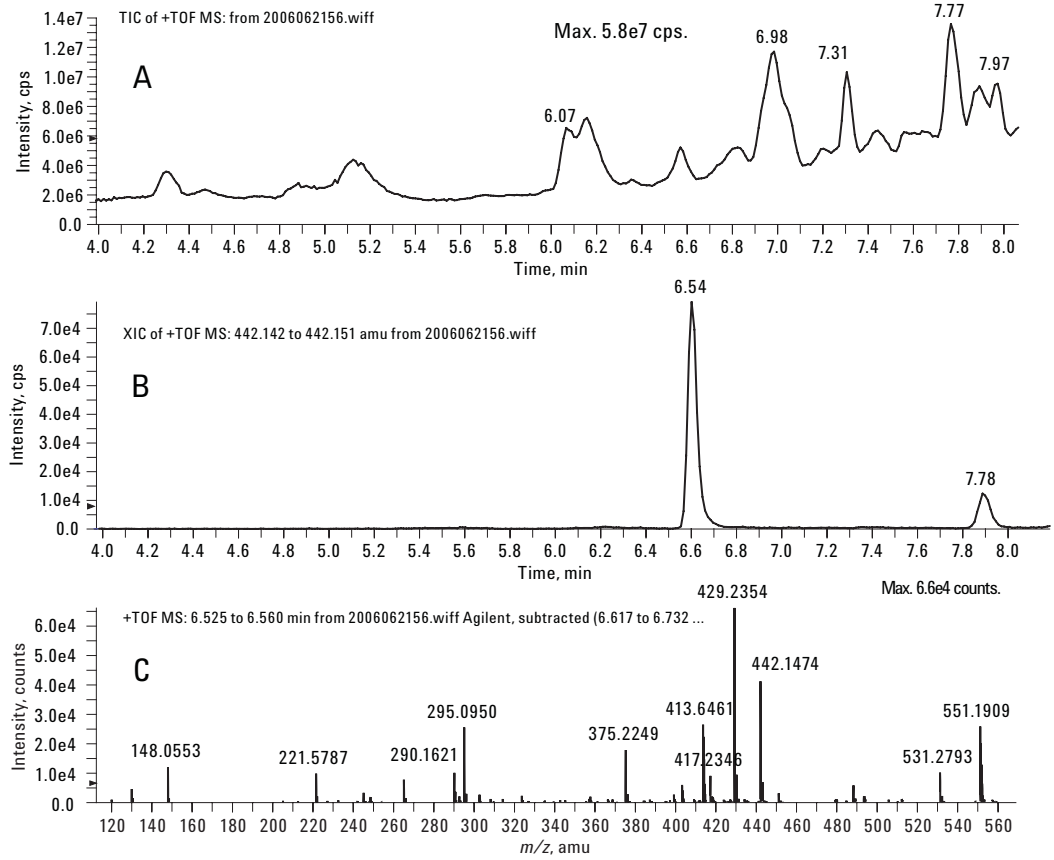


**Figure 6. TIC (A), EIC (B), and spectra (C) for folic acid in chyme spiked at 1µg/mL.**

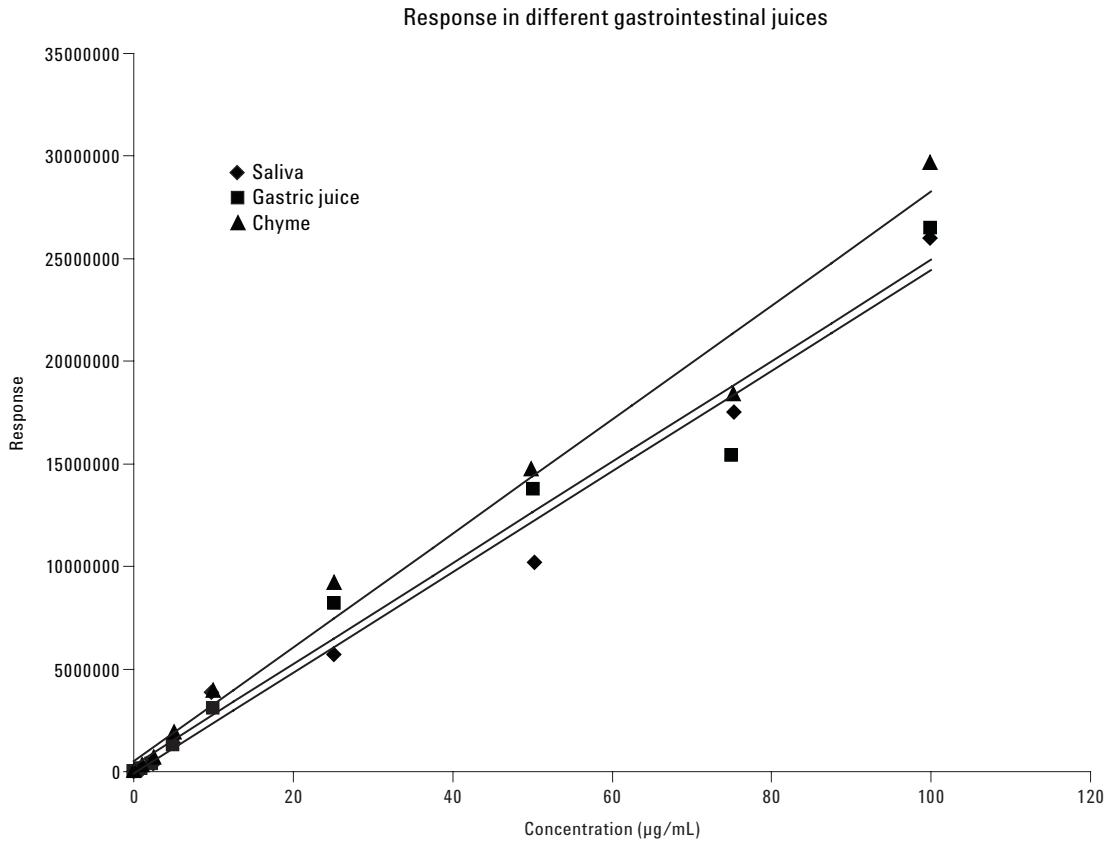


**Figure 7. Calibration curve of folic acid spiked in different juices.**

To determine the accuracy and covariance of the measurement, gastric juice and chyme were spiked (n = 4) at 50 µg/mL and analyzed on four separate days, with a one-week interval. These results are shown in Table 1.

The Limit of Detection (LOD) was determined by preparing a standard curve in saliva, gastric juice, and bile. From this calibration curve the Y-intercept and slope were calculated. The LOD is the corresponding concentration at the Y-intercept plus three times the standard deviation of the Y-intercept. The results of these determinations are given in Table 2.

**Table 1. Accuracy (%) and Covariance (%)**

| | Week 1 | | Week 2 | | Week 3 | | Week 4 |
|---|---|---|---|---|---|---|---|
| | Accuracy (%) | CV (%) | Accuracy (%) | CV (%) | Accuracy (%) | CV (%) | Accuracy (%) |
| Gastric | 91.9 | 1.2 | 99.3 | 4.2 | 82.9 | 6.0 | 91.7 |
| Chyme | 99.9 | 2.1 | 102.5 | 3.7 | 91.4 | 4.2 | 93.3 |

**Table 2. Determination of the Limit of Detection (LOD)**

| | Saliva (µg/mL) | Stomach (µg/mL) | Chyme (µg/mL) |
|---|---|---|---|
| LOD | 0.4 | 0.7 | 0.6 |

## Conclusions

The aim of this work was to develop a method with a minimal cleanup to detect folic acid in gastrointestinal juices. By using the high resolving power of the Agilent LC-TOF, it was possible to measure directly in these juices without any cleanup. The measurements proved to be reproducible with a high accuracy in complex matrices. The response was linear for the different digestive fluids tested with little matrix effects, and the method was validated within the concentrations required for folic acid uptake studies.

## Reference

1. C. H. Versantvoort, A. G. Oomen, E. Van de Kamp, C. J. Rompelberg, and A. J. Sips. "Applicability of an *in vitro* digestion model in assessing the bioaccessibility of mycotoxins from food." *Food Chem Toxicol,* 2005 Jan, 43(1).

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