CASE STUDY

Food Fraud

Milk Authenticity – Organic vs Non-organic

With increasing concerns over contaminants in milk, both intentionally and unintentionally added, a growing number of people are switching to organic milk (sales of whole organic milk were up 17% between January and October of 2011 in the U.S. with reduced fat organic milk up 15%).[1] This surge in popularity, coupled with high food and fuel prices, has caused shortages in the supply of organic milk.[2] With demand therefore outstripping supply, and a gallon of organic milk costing anywhere from 25% to 100% more than conventional milk, the selling of conventional milk as organic is an attractive proposition to fraudsters. In the U.S. and E.U., the labelling of organic products has meant stricter policing of farming practices but this is not the case with all countries. Furthermore, with the growing export of organic milk powders, these fake organic milk powders can find their way into the West through distributors or through processed foods, such as chocolates, which will also command a higher price if claiming to be organic. While these substitutions invariably do not cause health problems it is still fraud, with consumers not getting what they paid for and hardworking organic farmers losing business and having profit margins eroded.

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What is the Difference Between Organic and Non-organic Milk?

When producing organic milk, farmers must adhere to the following rules (These vary by country. Below are those for the U.S.):

- Grazing time – Farmers have to ensure that at least 30% of their cows' diet comes from pasture grass during a mandatory grazing season (no less than 120 days).[3]
- Antibiotic use – Organic dairy cows are not to be treated with antibiotics as a routine. If a cow should require an antibiotic, it's not allowed back in the milk production rotation until 12 months of antibiotic-free certification have passed.
- Bovine Growth Hormone (BGH) – Dairy cows from organic farms are not allowed shots of BGH.
- Pesticide use – The use of pesticides on an organic dairy farm is forbidden. The organic cow cannot consume pesticide-treated feed.[4]

How can we detect this?

Chromatography techniques, such as LC/MS and HPLC, can be used for detection if there are traces of pesticides, antibiotics or even growth hormones in the milk or animal feed. Measuring if the cows have been fed a predominately commercial feed diet, rather than fresh grass or silage, is more difficult. One of the techniques attempted to characterize organic vs. non organic milk is isotopic ratio mass spectrometry (IRMS). This technique has been shown to identify the type and even origin of feed used but requires large databases and has not been explored in enough depth to make definite conclusions. Recent work has been looking at levels of minor acids in the milk. One such study used GC/MS to measure levels of phytanic acid in organic and non-organic milk. As organic cows eat more fresh green matter, they consume more phytol (part of chlorophyll) which is broken down in ruminant's stomachs to phytanic acid. The study found that, on average, organic milk had double the phytanic acid levels than conventional (circa. 300 mg/100 g of milk as opposed to 150 mg/100 g of milk).[5] Another study proposed using hippuric acid which, again, was suggested to be found in higher levels if more grass and silage were consumed, though this study focused on goat's milk.[6]

Speeding up the Analysis

Invariably, most of these techniques involve some waiting time for separation to take place. A technique such as ambient ionization mass spectrometry provided by the AxION™ Direct Sample Analysis (DSA™) system integrated with the AxION 2 Time of Flight (TOF) mass spectrometer does not, as samples can be directly ionized and drawn straight into the MS. This means that some traditionally chromatography based applications, that did take up to an hour to analyze, can now take less than 30 seconds.

Experimental

Three organic and three conventional milk samples were purchased from a local supermarket. All milk samples were subjected to the same preparation in that 1 mL of milk was mixed with 2 mL of acetonitrile and 1 mL of methanol to carry out a protein precipitation. The samples were then centrifuged for 10 minutes at 7800 RPM. Finally, 1 mL of supernatant was diluted and then spiked with internal standard, d5-hippuric acid, to give final concentrations of 5 mg/L of internal standard in each sample. Ten µL of each protein precipitated sample were then pipetted directly onto the stainless steel mesh of the AxION DSA system ready for ionization and analysis. The DSA/TOF experimental parameters are shown in Table 1.

***Table 1.* Experimental parameters for the analysis of hippuric acid in milk.**

DSA Parameters	Value
Heater Temperature	350 °C
Auxiliary Gas Pressure	80 psi
Drying Gas Flow Rate	3 L/min
Drying Gas Temperature	25 °C
Corona Current	-5 uA
TOF Parameters	**Value**
Mode	Pulse (Negative)
Mass Range	100-700 m/z
Capillary Exit Voltage	-100 V

Results

Figure 1 shows the mass spectra of an organic milk sample. It is clear that the dominate signals are from the hippuric acid and the deuterium substituted hippuric acid standard. If these peaks are examined in more detail and then overlaid, Figure 2, we can see that, from the area of the peaks, that the response from the hippuric acid is 1.692 times that of the deuterated standard. If this is repeated for conventional milk, Figure 3, we see that the ratio is closer to 1 at 0.932.

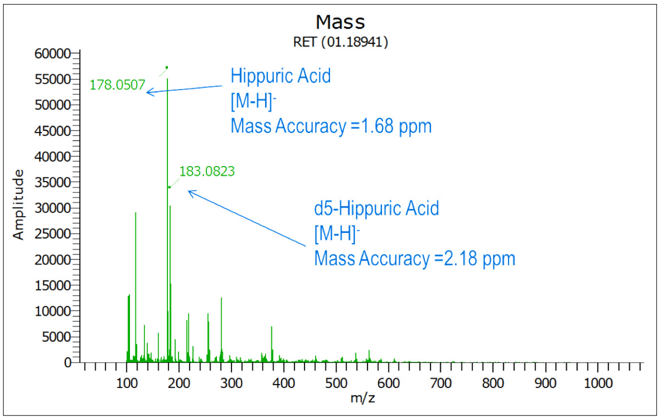

Figure 1. Representative mass spectra of the organic milk sample spiked with 5 ppm internal standard.

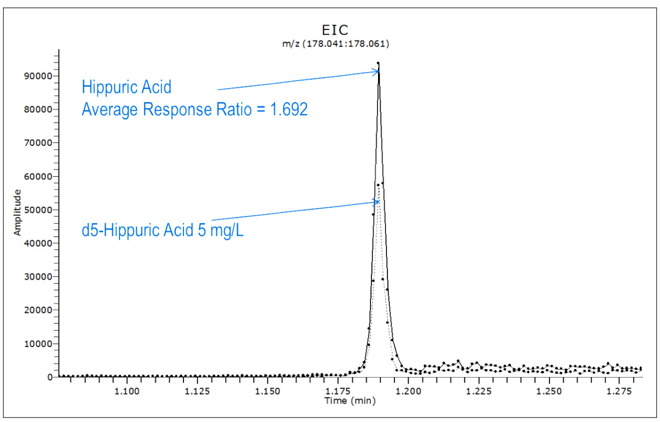

Figure 2. Representative Extracted Ion Chromatogram (EIC) of Hippuric Acid and d5-Hipopuric Acid (5 mg/L) for the organic milk sample.

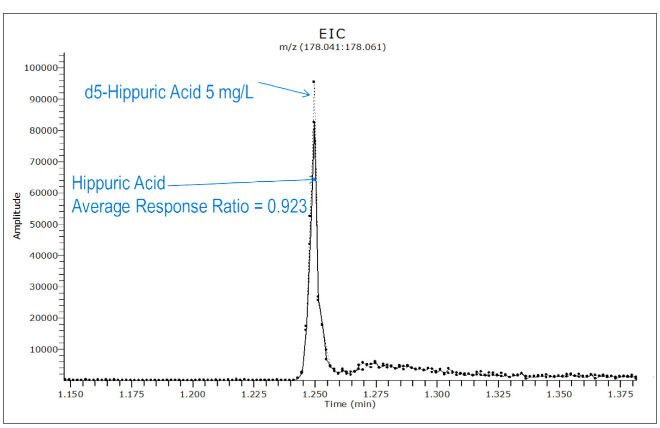

Figure 3. Representative EIC of Hippuric Acid and d5-Hipopuric Acid (5 mg/L) for the conventional milk sample.

Discussion

This was repeated for all 6 samples with the results shown in Table 2. It can be seen that for conventional milk, for all three samples, the hippuric acid concentration was, on average, a 1 to 1 ratio with the reference standard. Factoring in dilution, this means that the levels in the conventional milk were around 20 mg/L. For the organic milk samples 1 and 2, it was clear that the levels of hippuric acid were higher as hypothesized. These samples had around 35 mg/L which is approximately 1.75 times that of conventional milk. One organic milk sample had lower levels than even the conventional milk. An explanation for this could be that this particular sample was the supermarket 'home' or generic brand and was therefore 'not as organic as it suggests on the label'. To support this theory, a wider study would be needed, ideally splitting a herd of cows into two groups, feeding one an organic diet over a year and one a conventional feed based diet and measuring the hippuric acid levels in the resulting milk over the year similar to that studied for goats in a similar study.[6]

***Table 2.* Levels of hippuric acid in organic and conventional milk samples.**

Type of Milk	Response Ratio with respect to 5 mg/L d-5 Hippuric Acid	Hippuric Acid in Milk
Organic Milk Brand 1	1.692	33.48 mg/L
Organic Milk Brand 2	1.837	36.73 mg/L
Generic Organic Milk Brand 3	**0.864**	**17.28 mg/L**
Conventional Milk Brand 1	0.923	18.45 mg/L
Conventional Milk Brand 2	0.998	19.96 mg/L
Conventional Milk Brand 3	1.075	21.51 mg/L

Conclusion

This work has shown that it is possible to measure hippuric acid levels in milk by DSA/TOF MS using a reference standard to ascertain relative concentrations (rather than using a calibration curve). There is also evidence that the hippuric acid levels could be used to ascertain whether cows have been feed an organic diet however a much wider study would be needed to prove this conclusively. This opens up the possibly to study for pesticides, growth hormones, antibiotics and organic diet in one instrument and therefore have a definite check for organic compliance.

References

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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