

GOVERNMENT AND SOCIETY

Measuring antibiotics in milk

Because of rigorous testing from the cow to the dairy to the manufacturer of dairy products, the furor over residues of veterinary antibiotics being found in milk has subsided. But the issue has never truly gone away, and the dairy industry always needs new and better tests to detect these residues.

Antibiotic residues can give cheese a pasty body and low acidity and can prevent the fermentation of yogurt. Antibiotics in milk also concern many consumers, whose increasing awareness of what's in their food is changing the way they shop. And milk is one of the most heavily regulated food products worldwide, with special attention focused on the presence of antibiotics.

For all of these reasons, farmers and others in the dairy industry have strong financial incentives to prevent milk that is contaminated with antibiotics from entering the food chain. They dream of quick, safe, simple, specific, quantitative, and qualitative methods to test for antibiotics in milk, because the current methods are very involved. At the moment, a single analysis method cannot adequately detect the numerous drug classes and molecules that might be present.

"Analysis of antibiotic residues follows a strict strategy," explains Michael Petz, who is the head of the department of food chemistry at the University of Wuppertal (Germany) and an expert in detecting antibiotics in milk and other foodstuffs. The first step is a screening test to find evidence of antibiotic content. "It is usually a so-called brilliant black test—a reduction test where the change of color signals the presence of antibiotics," he says. Microbiological tests also are used, although they need several hours to show results, he adds.

If the initial test is positive, microbial inhibitor tests are the next step. They

target a broad spectrum of medicines and can, therefore, simultaneously detect substances such as tetracyclines or β -lactams, which are frequently used to treat mastitis in cows. However, inhibitor tests are not specific for antibiotics, and there are occasional reports of



positive reactions associated with other inhibitors, such as lactoferrin or lysozyme, that occur naturally in milk. Nowadays, immunoassays, which are more specific for certain antibiotic groups or even particular substances, also are available.

With ELISAs, many samples can be processed very specifically in a short time, and thus, a broad spectrum of antibiotics can be analyzed. "But the method is not very quantitative," says Fred Braun from the MUVA Food Quality and Laboratory Center in Kempten (Germany). "To verify the ELISA results, we still have to go further, to HPLC."

HPLC and MS are the gold standards for finding a specific drug and measuring its concentration. But these methods are very laborious and time-consuming and, thus, expensive. The main disadvantages are the complex sample-preparation steps, the small number of samples analyzed per unit time, and the need for trained personnel. Research groups around the world have been looking for far simpler, less tricky approaches to these problems. In the November 15 issue of *Analytical*

Chemistry (pp 7665–7673), Ana M. García-Campaña and her group from the University of Granada (Spain), in collaboration with a Spanish company that produces milk and dairy products, took up the challenge and revitalized an old analytical concept.

"Considering these problems, we were interested in demonstrating the possibilities of monitoring these compounds by a separation technique," says García-Campaña. "We chose capillary electrophoresis coupled with mass spectrometry as the detection technique, a coupling that is scarcely used for the moment, due to the difficulties encountered in the development of the interface."

But she developed a new strategy to solve the main problem—the sample treatment when acidic and zwitterionic antibiotics, such as those in the family of the quinolones, are simultaneously determined. The researchers tested their method by spiking 8 quinolones that are commonly used in veterinary medicine into raw cow's milk at 50, 100, and 200 ng/mL.

CE can be a very useful alternative to HPLC for several reasons. It is a miniaturized technique that does not require organic solvents; the sample volume is quite small; and it can separate a relatively large number of compounds in a short period of time with good resolution and satisfactory sensitivity. Coupling CE with MS offers another interesting advantage: "The method can be used as a standard in relation to the application in milk, because it has been fully validated in this complex matrix, including a previous sample pretreatment step, demonstrating its validity in terms of trueness and precision," says García-Campaña.

Even Petz is in favor of the new method. "Capillary electrophoresis is a good, reliable method," he says. "One should give it a chance." ■

—Hanns-J. Neubert