Analysis of Tetracycline and Sulfamethazine Antibiotics in Ground Water and Animal-Feedlot Wastewater by High-Performance Liquid Chromatography/Mass Spectrometry Using Positive-Ion Electrospray

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Two classes of antibiotics used in animal feed (tetracyclines and sulfamethazines) are analyzed from ground-water and wastewater samples by high-performance liquid chromatography/mass spectrometry (HPLC/MS) using positive-ion electrospray with a detection limit of 0.2 microgram per liter (μg/L). The method consists of filtering 40 milliliters (mL) of water sample through a 0.45-micron glass-fiber filter followed by acidification with phosphoric acid to pH 2. The sample is passed through a solid-phase extraction (SPE) cartridge (ENV+, polymeric resin) and dried under vacuum. The cartridge then is eluted with 4N NH_4OH in methanol, vortexed, and filtered. SPE recovery is approximately 80%. The eluate then is injected into the HPLC/MS system, which is running a methanol/water gradient from 10 to 80% methanol. The addition of the ammonium hydroxide is critical in the hydrolysis of the various epimers of chlortetracycline. The hydrolysis occurs rapidly, giving one chromatographic peak rather than the six epimeric forms of chlortetracycline. The ions monitored by selected-ion monitoring are 479, 481, and 501 (a sodium adduct) for chlortetracycline and 279, 281, and 301 (sodium adduct) for sulfamethazine. Internal standards are used for quantitation, including tetracycline for chlortetracycline and $^{13}C_6$ sulfamethazine for sulfamethazine. Analysis of several ground-water samples collected near waste lagoons and wastewater-lagoon samples show that the antibiotics are detected readily at microgram-per-liter concentrations.

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