Effect of Storage Conditions on Salbutamol Stability in Spiked Swine Urine Samples: ELISA Analysis with Linear Mixed Models

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Abstract

Background: Beta-agonists, specifically salbutamol, are pharmacologically essential for treating respiratory disorders in humans. However, their illicit use in the swine industry as repartitioning agents to enhance leanness and reduce fat poses significant risks to food safety and public health. The Department of Livestock Development utilizes LC-MS/MS as the gold standard for confirmatory residue testing in urine and animal products. While, field test and regional laboratory centers use ELISA as it remains a critical high-throughput screening tool due to its cost-effectiveness and speed. Positive samples from screening test are stored and then sent for confirmatory testing. Whether these conditions affect sample stability have not been clarified. Therefore, this study focuses on the effect of storage conditions on sample stability, a critical factor for the accuracy of analytical results prior to confirmatory testing.

Objectives: To evaluate the effect of storage temperature, duration, and initial concentration on the stability of salbutamol in swine urine.

Methods: Urine samples from swine, previously confirmed to be free of salbutamol, were spiked with salbutamol at three concentrations (2.5, 5, and 10 ng/mL; n=3 per level) and stored under three conditions: room temperature (RT, 25–30 °C), refrigeration (RE, 2–8 °C), and freezing (FR, below −20 °C). Salbutamol concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) with a commercial test kit (Kwinbon Biotech, China) at 0, 7, 14, 21, and 28 days. Data were analyzed using a Linear Mixed Model (LMM) to evaluate main effects (temperature, time and initial concentrations) and their interactions on the stability and concentration of salbutamol. Sample ID was specified as a random effect to account for the correlation within repeated measurements (within-subject variance), with statistical significance set at $p \leq 0.05$.

Results: Salbutamol concentrations decreased significantly with increasing storage duration compared to baseline (day 0) under all temperature conditions ($p < 0.001$). The most degradation occurred at RT, where concentrations fell to 89.70% by day 28—significantly lower than the 93.98% retention observed under FR conditions ($p = 0.02$, 95% CI = -7.89, -0.66). Samples stored under RE conditions retained 91.49% of the initial concentration. These findings indicate that salbutamol exhibited the highest stability under FR conditions. Notably, the initial spike concentration did not significantly influence the degradation rate ($p > 0.05$), indicating a consistent percentage-based decline.

Conclusion: Storage temperature is a critical determinant of long-term salbutamol stability in swine urine. For samples requiring storage exceeding three weeks, freezing is recommended to ensure analytical accuracy. Prolonged exposure to room temperature must be avoided to prevent erroneous results that could compromise the efficacy of food safety surveillance and subsequent confirmatory processes. These findings support the development of standardized protocols for sample handling prior to confirmatory analysis.

Keywords: Salbutamol stability, Swine urine, Sample storage conditions, ELISA, Linear mixed models

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