

## **GC-MS method development and validation for anabolic steroids in feed samples**

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### **Abstract-**

A GC-MS method for the determination of AAS used as growth promoting agents using SIM in piglet feed samples has been developed and validated, using testosterone as internal standard. The formation of volatile steroid derivatives was carried out by derivatization with N-methyl-N-(trimethylsilyl)trifluoroacetamide. The optimum separation was achieved using a Zebron ZB-5 column under a gradient temperature elution, allowing the separation of steroids in 18 min. The required sample treatment process was discussed. A leaching using ACN, saponification using a binary NaOH/MgCl<sub>2</sub> solution, and LLE using ethyl acetate were finally selected. Method validation has been carried out according to the Commission Decision 2002/657/EC criteria established for quantitative confirmatory methods. The extraction efficiencies, CC<sub>alpha</sub> and CC<sub>beta</sub> for these compounds were in the ranges 78-98%, 10-21 and 18-35 mug/kg, respectively. The repeatability and the within-laboratory reproducibility at 1, 1.5, and 2 CC<sub>beta</sub> concentration levels were smaller than 8.2, 7.5, and 5.8% and 12.2, 9.5, and 7.5%, respectively. Accuracy was in the 99-103% range. The robustness was evaluated using the Youden robustness test. The proposed method was applied to the analysis of steroids spiked in different kinds of animal feed samples with satisfactory results.

**Index Terms-** Anabolic steroids; Animal feed; Derivatization; Growth promoting agents

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