

## **Liquid chromatographic method development for anabolic androgenic steroids using a monolithic column Application to animal feed samples**

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### **Abstract-**

An isocratic HPLC method for the determination with screening purposes of anabolic androgenic steroids (AASs: fluoxymesterone, boldenone, nortestosterone, metandrostenolone, norethindrone, methyltestosterone and bolasterone), used as growth promoting agents, in finishing pig feed samples has been developed and validated. The separation was achieved by using a reversed-phase Chromolith RP-18e column at controlled temperature, UV-detection at 245nm and epitestosterone as internal standard. The method development involved optimization of different aqueous-organic mobile phases using methanol or acetonitrile as organic modifiers, flow-rate and temperature. The optimum separation for these compounds was achieved at 40 degrees C using ultrapure water:acetonitrile (71:29, v/v) as mobile phase and 3mLmin<sup>-1</sup> flow-rate, allowing the separation of AASs with baseline resolution in about 15min. The optimized method was applied to the analysis of AASs in finishing pig feed samples. Prior to HPLC, sample preparation procedure was used by leaching using acetonitrile, saponification in a basic medium and solid-phase extraction using polymeric Absolut Nexus cartridges. Method validation has been carried out according to the European Commission Decision 2002/657/EC. The extraction efficiencies, decision limits (CC<sub>alpha</sub>) and detection capabilities (CC<sub>beta</sub>) for these compounds were in the range 83-96%, 27-37 and 32-47microgkg<sup>-1</sup> range, respectively. The within-laboratory reproducibility at 1, 1.5 and 2 CC<sub>beta</sub> concentration levels were smaller than 13, 10 and 8%, respectively. Finally, the proposed method was successfully applied to nine different kinds of animal feed.

**Index Terms-** Anabolic androgenic steroids; Growth promoting agents; Animal feed; Monolithic column

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