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Liquid chromatographic method development for steroids determination (corticoids and anabolics). Application to animal feed samples

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Abstract-

A LC isocratic separation study of a complex mixture containing 18 steroids (corticoids and anabolics), used potentially as growth promoters, was carried out. For this purpose, using a Hypersil ODS column at controlled temperature, mobile phases (from binary to quaternary) prepared from water and MeOH, ACN or THF as organic modifiers and UV detection at 245 nm, were employed (dehydroepiandrosterone was detected at 200 nm). The optimum separation was achieved using water/acetonitrile (65:35, v/v) as mobile phase at 30 degrees C, allowing the separation of 16 out of 18 steroids in about 30 min. The retention scale using optimized binary mobile phases was related with steroids hydrophobicity and structure, allowing a classification into three groups for these compounds. To improve the separation several alkyl-silica packings were tested: Type A (Lichrospher C8) and Type B (Luna C18, Kromasil C18, Purospher C18 and Synergy C12). Taking into account resolution, number of separated compounds and run time analysis the Hypersil column was selected as the best choice for further applications. Calibration graphs were obtained using fluorocortisone, fluoxymesterone or methylprednisolone as internal standard. The optimized separation was applied to the analysis of piglet feed samples spiked with steroids. The sample preparation process included solvent extraction using diethyleter and solid phase extraction using silica cartridges. The recoveries were in the range 70-92\%. Decision limits and detection capability were in the range 34-198 and 41-249 microg/kg, respectively. Repeatability was also evaluated.

Index Terms- Steroids; Reversed-phase columns; Animal feed

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