

**SEPARATION CHARACTERISTICS OF C₂₁ VERTEBRATE
CORTICOSTEROIDS**

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Abstract

A simple, direct, and comprehensive assay for vertebrate corticosteroids was developed using electrokinetic chromatography (EKC). Separation characteristics of analytes were noted for affinities to two different hydrophobic carriers: sodium cholate and sulfated β -cyclodextrin. With sodium cholate there was an inverse correlation between run order and number of oxidations of the analytes, and no evidence of hydrogen bonding with analytes. Sulfated β -cyclodextrin run order was similar to sodium cholate's run order. A combination of the carriers enabled the separation of all analytes.

Concurrent with development of this assay, a sample-concentration method was discovered that increases the sensitivity of EKC by at least an order of magnitude over previous efforts. The hydrophobic-based separation technique combined with the concentration method could be adapted to determine long-term corticosteroid stress response and validated for cetacean sample analysis. Direct detection of 1α -hydroxycorticosterone in elasmobranchs should also be possible using this assay.

A second separation method based on steroid/borate interaction was also described. The mechanism of interaction was determined to be diol bonding (chelation). Ability to chelate is dependent on one of two specific hydroxyl geometries that appear through endogenous biosynthesis. Chelation is discussed with respect to biosynthesis, bioactivity, relationship to adrenal medullary compounds, and ocean pH.