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## DEVELOPMENT OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF FOUR STEROID HORMONES IN DIFFERENT MATRICES

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Since animal products are a major source of human exposure to steroids. The extensive use of these hormones resulted in a critical request for accurate, sensitive, simple and applicable methods for the determination of these steroid hormones in pure form and in different matrices as hen eggs, chicken liver and Tilapia farming pond water. The aim of this work is to develop a reliable liquid chromatography coupled with mass spectrometric method for the determination of selected steroid hormones in complicated matrices as hen egg, chicken liver and Tilapia farming pond water. Using solid phase and liquid liquid extraction as methods of sample preparation. In the present study LCMS/MS method was demonstrated for the simultaneous separation and quantification of 4 steroid hormones Ethinlyestradiol, 17 alpha Methyl testosterone, testosterone, and progesterone. Using mobile phase of methanol and 0.1% Formic in ratio (70:30) at different m/z ratios. The method validation was carried out on each hormone showing: linearity for Ethinlyestradiol 0.5 µg/ml to 30 µg/ml  $r^2=0.9997$ , 17 $\alpha$ -methyl testosterone 0.5 µg/ml - 20 µg/ml  $r^2=0.9999$ , Testosterone 0.5 µg/ml - 20 µg/ml  $r^2=0.9999$  and progesterone 0.5 µg/ml- 20 µg/ml  $r^2=0.9999$ . LOD and LOQ of (0.7 and 2.12), (0.23 and 0.69), (0.35 and 1.08), and (0.36 and 1.11) for Ethinylestradiol, 17  $\alpha$  methyl testosterone, testosterone and progesterone respectively. The method was validated using the ICH guidelines and successfully applied on egg, chicken liver and Tilapia pond freshwater samples from Egypt.

## BIOGRAPHY

Areeg Z Alkarali has completed her bachelor's degree in pharmaceutical sciences in 2012 from Misr International University; after that she finished her master's degree in pharmaceutical analytical chemistry in 2018 from Suez Canal University.

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