VALIDATION OF AN LC/MS/MS METHOD TO QUANTIFY MULTIPLE STEROID HORMONES IN HUMAN SERUM

Viviane do Nascimento, Helio A. Martins-Júnior, Carlos Augusto Rodrigues, José J. M. Xavier, Eduardo Kinio Sugawara, Lívia Rentas Sanches, Carlos Eduardo Santos Ferreira, Adriana Caschera Faulhaber, Marcelo Batista Cidade

The determination of a wide steroid panel in different biological fluids has been used in clinical research studies and supported the diagnostic of several nosological conditions. The steroid hormones have been analyzed at clinical laboratories by radioimmunoassay, immunoassay with chemiluminescense and electrochemiluminescense. Nowadays, these techniques have been replaced by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) due to its high reliability, sensitivity and easy-of-use features. In this study an LC-MS/MS method was validated to quantify 11-deoxycortisol, 17-α-hydroxyprogesterone, androstenodione, corticosterone, cortisol, estradiol, progesterone and testosterone using Atmospheric Pressure Chemical Ionization (APCI) in positive ion mode. The method is based on a protein precipitation with zinc sulphate followed by centrifugation and injection into LC-MS/MS. The analytes were separated using a C18 column and mobile phase composed by methanol and water without any modifier. The total chromatography run time was 13 minutes and an innovative peak modeling algorithm was used to increase the linear range for cortisol and testosterone. The method provided suitable sensitivity for all analytes and the limit of quantitation was 0.025 ng mL^{-1} for androstenodione and 11-deoxycortisol; 0.10 ng mL^{-1} for estradiol, progesterone, 17-OH-progesterone and testosterone; 0.05 ng mL^{-1} for corticosterone and 5.0 ng mL^{-1} for cortisol. The method validation included linearity, dinamic range, precision, accuracy, selectivity, limit of detection and limit of quantitation, all of them in conformity with the RE/899. This methodology has been applied at the hospital laboratory routine to quantify those steroids in human serum.