Steroid hormones are neutral and do not ionize at a high level using the electrospray ionization technique: such steroids were usually analysed using GC-MS/MS with derivatization techniques. We are reporting a LC-MS/MS method for the simultaneous quantification of seven steroidal compounds, i.e. E1, E2, DHT, 5-diol, 4-dione, DHEA and Testo. Their quantification limits are 4 pg/mL, 1 pg/mL, 10 pg/mL, 100 pg/mL, 100 pg/mL, 500 pg/mL and 50 pg/mL, respectively. The system used to achieve this simultaneous quantitation is a UPLC-MS/MS (Qtrap 6500). With this method, the sample preparation is the combination of liquid-liquid extraction and a simple derivatization. It is simple and practically eliminates potential contamination. A full validation has been performed for the seven compounds in compliance with GLP and FDA Guidelines for bioanalytical method development and validation. The linearity and accuracy are within the bias range of 15% for all seven compounds. All testing results of other parameters also meet the acceptance criteria of EndoCeutics SOPs and FDA guidelines.