

Measurement of serum testosterone, androstenedione and dehydroepiandrosterone (DHEA) levels using ID-LC-MS/MS

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Background

The adrenal and gonadal androgens testosterone, androstenedione and dehydroepiandrosterone (DHEA) play an important role in sexual development and fertility as well as in several other processes. We developed a method to assess serum testosterone, androstenedione and DHEA levels in one run using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS).

Analytical method

Sample preparation consisted of addition of internal standards (¹³C₃-testosterone, ¹³C₃-androstenedione and ²H₆-DHEA) and a liquid-liquid extraction using hexane-ether. The samples were analyzed on an Acquity 2D UPLC system (Waters), equipped with a C4 column (Waters) and a Kinetex Fluorophenyl column (Phenomenex), and a Xevo TQ-S tandem mass spectrometer (Waters). Recovery was 101-107%, 99-106% and 92-104% for testosterone, androstenedione and DHEA, respectively.

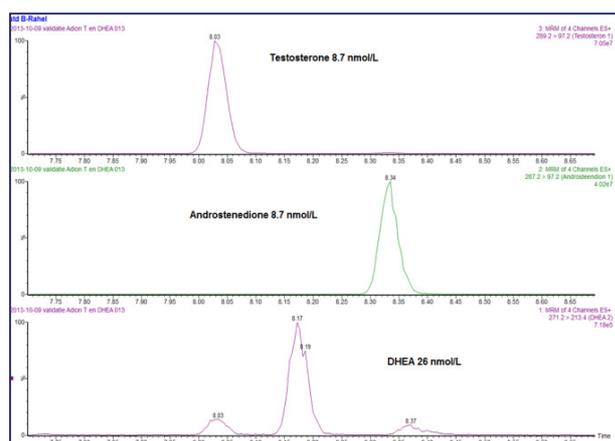


Fig.1: Chromatogram of testosterone, androstenedione and DHEA.

	Testosterone	Androstenedione	DHEA
Intra-assay CV	< 4,0%	<4,6%	<7,0%
Inter-assay CV	< 6,0%	<8.0%	<8.0%
Lower Limit of Quantitation	0,05 nM	Under investigation	1 nM

Table 1: Method characteristics

Linearity was shown in dilution series (mean R² was >0.999 for all analytes). This method tested negative for interference from several steroids and did not show ion suppression. The method was shown to be suitable for serum as well as EDTA and heparin plasma. The present testosterone method compared well ($y = 1.000x + 0.035$ nmol/L; $r = 0,9982$) to another ID-LC-MS/MS method for testosterone concordant with a published reference method (Bui et al. 2013) (Fig. 2). In the near future, the present method will also be compared to another LC-MS/MS method for androstenedione and DHEA.

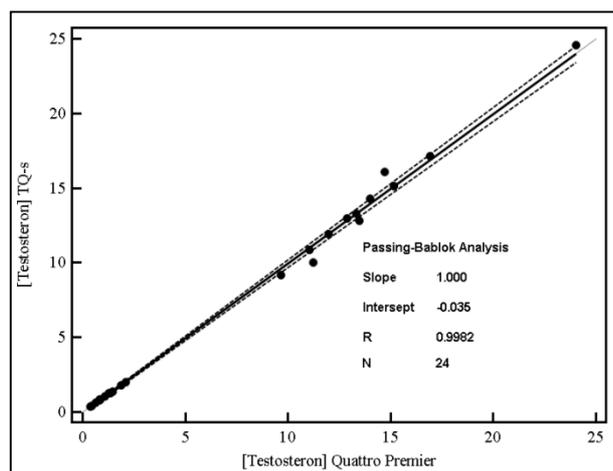


Fig. 2: Passing-Bablok analysis testosterone method comparison

Conclusion

We developed a sensitive and accurate method to measure serum testosterone, androstenedione and DHEA levels in one run.

