Minimizing Collection Tube Interfering Substances in the Analysis of Testosterone using Atmospheric Pressure Chemical Ionization (APCI)

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Background

Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles, birds, and other vertebrates. It is secreted by the adrenal glands. The concentration of serum testosterone is lower in women and children and levels generally peak during puberty. Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles, birds, and other vertebrates. It is secreted by the adrenal glands. The concentration of serum testosterone is lower in women and children and levels generally peak during puberty.

Results

During the development of our assay, we observed significant interference that we attributed to gel separator tubes when ESI was used as the ionization source (see Figure 1A). In order to circumvent this issue, we chose to use APCI to attempt to resolve the interference (see Figure 1C). As a result of the development of this assay, we removed the gel separator tubes and removed the interfacing peaks, we felt this was also an opportune time to evaluate other possible collection sample tubes. In our study we used 43 sets of samples collected in Red, Gold, Green and Lavender top BD tubes; samples were collected from healthy volunteers 21 male and 22 female. All of the samples were processed and run using the APCI method over the course of 3 days. The data was entered into EP Evaluator: Multiple Instrument Correlation module. The Lavender top tubes were excluded from the study as an extremly pronounced negative bias, the other two tube types were evaluated against the preditected tube type. Red and BLue tubes were essentially equivalent. Further studies were performed to satisfy that we had eliminated commonly encountered endogenous interfering substances that maybe present in patient samples. We subjected our samples to high levels of hydrogen peroxide (5% v/v) and Propargyl alcohol (14.5% v/v) and analyzed the data using the UVP Evaluation protocol. No significant changes were observed in results due to ion suppression or interfering peaks.

Method and Design

Samples were evaluated from an assortment of the most commonly encountered blood collection tubes: BD Plus Serum/CAT with Gel Activator (Red), BD SST with Gel and C lot Activator (Gelad), BD K.EST (Lavender) and BD PST II Tubes with Gel and Lithium Heptane (Mint Green). In our study we used 43 sets of samples collected in Red, Gold, Green and Lavender top BD tubes; samples were collected from healthy volunteers 21 male and 22 female; samples were extracted, prepped and injected onto a Shimadzu 20AD, 20MI, 20AC, curtain gas 20 psi and a dwell time of 150 msec.

Conclusion

We have developed a highly sensitive and specific APCI LC-MS/MS method suitable for the analysis of testosterone that is largely free from collection tube and endogenous interfering substances. We have accurately and precisely measured Testosterone down to levels of 3.5 ng/dL. As a result of being relatively free of interferences and cross reactivity with structurally related compounds, the LC-MS/MS method offers better sensitivity than previously developed methods used for testosterone quantification, along with higher operational throughput and the smaller sample requirement are useful for pediatric testing.

References