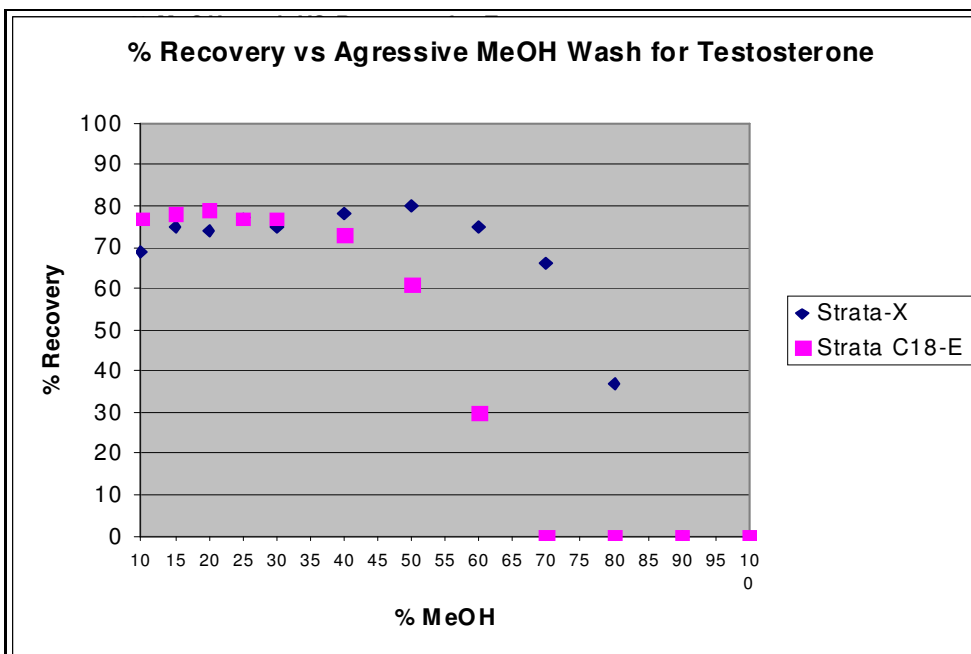




Optimizing Wash and Elution Protocols in SPE Method Development

One key step in the development of any SPE method is the wash step. Ideally, SPE methods should be optimized to allow the most aggressive organic wash possible to remove matrix components that can otherwise interfere with the subsequent sample analysis (i.e. via LC/UV or LC/MS). However, overly-aggressive washing can also result in a loss of target analyte(s). Therefore, an effective SPE method development process has to strike the ideal compromise sample recovery and cleanliness. In this tech tip, we will use the development of an SPE method for testosterone as a model for optimizing wash steps in the SPE method development process.

Testosterone is a very hydrophobic compound, sometimes subject to abuse by athletes for its performance enhancing properties. Because of the neutral, non-polar nature of the compound, it is logical that the SPE would be performed using a silica-based SPE sorbent, such as Strata C18-E, or a hydrophobic, polymeric sorbent such as Strata-X. The 96-wp format of 30mg Strata-X and 50 mg C18-E were used for this demonstration, with the following the protocol. The sorbent was first conditioned with 1 mL of methanol, followed by 1 mL of water. 500 μ L of plasma spiked with testosterone (0.5 μ g/mL) was diluted with 1mL of water and then loaded into multiple wells of the well-plates. To determine the ideal washing conditions, we applied a series of washes varying from 10 to 100% methanol at 10% increments (each well had a different wash). After the wash step, 1 ml of methanol was applied to elute the retained components. A curve relating % recovery as a function of % of methanol in the wash step was then reconstructed based upon LC-MS-MS analysis of the elutents. As is clear from the curve, when the % of methanol in the wash step is increased beyond ~40%, we begin to see a decrease in the recovery of testosterone from the Strata C18-E media. However, when using the polymeric Strata-X, we were able to increase the concentration of methanol in the wash to ~50-60%, which should result in a slightly cleaner final eluent. Based upon these results, we would conclude that the use of Strata-X with a 50-60% methanol wash step would be optimal for this application.



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