COMPOUNDER'S INTERNATIONAL
ANALYTICAL LABORATORY
Better Quality Through Quality Testing

HCG Formulations Facts

Human Chorionic Gonadotropin (HCG) is a protein containing a total of 237 amino acids and made up of 2 subunits, an alpha chain and a beta chain, which are held together by the weak forces of hydrogen bonding. Apparently, the protein activity requires the intact molecule which is lost or greatly reduced if these subunits break apart. In addition, because of its amino acid sequence and 3-dimensional orientation, the outer surface is highly charged ionically.

The official assay method which is spelled out in the USP, involves dosing laboratory rats, then sacrificing them and determining the weight of their uteruses, which can be correlated to the potency of HCG. Since this is a tedious, time consuming and distasteful process, it is not practical on a day to day basis. One of the alternate methods which has been used to assay the concentration of HCG, is by means of an immunoassay technique. This utilizes monoclonal antibodies that are specific to a specific sequence of amino acids found in the beta subunit of the protein. Such a technique works best for in-vivo testing because, in the body, the protein tends to maintain its structural integrity. Under in-vitro conditions however, such as a pharmaceutical preparation, this method can produce false high values because it will label both the intact protein as well as the disassociated beta subunit, and certain fragments of this subunit, as if they are the active intact form. Like all proteins, HCG is fragile and can be broken apart and denatured by processing, mechanical mixing, shaking, homogenizing, or ultrasonic dissolution. This can occur during the supplier's manufacturing processes as well as during compounding processes.

In our lab, we have developed and validated a chromatographic method which separates the intact protein from its subunits, as well as from various peptide pieces. This allows us to correctly assay only the intact protein concentration, which correlates well with its potency.

Compounding Suggestions:
HCG has a tendency to adhere to plastic more than glass, primarily when in solution. This becomes especially significant in concentrations below 1,000 units/mL. To help with this, we suggest not using plastic materials for anything that might come into contact with the HCG solution. (As a dry powder, this is not as critical). Also, for all glassware that will be exposed to solutions of HCG, pre-rinsing then wasting, with some of the HCG solution will help prevent losses. Like most proteins, some of it can adhere to filter materials. When sterile filtering, therefore, we suggest using a low-protein binding filter and wasting the first 1-2 mL that passes through the filter, then begin dispensing into sterile containers after that. HCG is sensitive to warm temperatures, so both in the solid form as well as in solution, it should be stored and shipped under refrigeration. Freezing, especially freezing then thawing and refreezing of any protein solution is not suggested because ice crystals, as they form, can damage the molecule. In our experience, we find that when HCG is dry mixed (often with mannitol powder) it is best accomplished by tumbling in a plastic or glass container. Like most proteins its exposure to metal should be limited. It is best not to mix in a mortar and pestle, which can cause fragmentation of the protein due to the inherent mechanical grinding action of this technique. And, finally, when making and mixing solutions of HCG, do not shake or sonicate. Instead, swirl or rock the container in a smooth action to avoid breaking the protein.