

NICHOLAS TUCKER#, VICTORIA BROADWATER, DAN DILELLA, and RUTH CONLEY, Department of Chemistry, Shepherd University, Shepherdstown, WV, 25443, Department of Biology, Shepherd University, Shepherdstown, WV, 25443.

**Method development for the determination of biogenic amines in insect hemolymph by HPLC with electrochemical detection.**

An HPLC method was developed for the quantification of biogenic amines in insect hemolymph. The major challenge in the analysis is that a typical sample only contains a few hundred pictograms of the amine. A C18 column and electrochemical detection was used. The eluent was an aqueous 0.06 M phosphate buffer (pH- 6.7) mixed with 10-15% methanol and an ion-pairing reagent. With this method, the amines can be detected at picogram levels. However, there are hundreds of compounds in a typical biological fluid and, in some cases, another electro-active species can co-elute with the compounds of interest. To address this problem we have developed methods to selectively remove some potentially interfering compounds by pretreating the sample by solid phase extraction (SPE). Previously, we had used weak cation SPE columns to simplify the mixture but found that the method was too time consuming. We have now developed a method that uses weak anion SPE columns. The samples were collected in a syringe preloaded with an anticoagulant, either trichloroacetic acid or formic acid. The proteins were removed by centrifuging with a 10K Da filter. The solid phase extraction followed. This pretreatment was found to be effective for selective removal many interfering peaks. DHBA (3,4-Dihydroxybenzylamine hydrobromide) was used as an internal standard.