SIERRA BADLEY, Josue Montenegro, Dan DiLella, Dept. of Chemistry, and Ruth Conley, Dept. of Biology, Shepherd University, Shepherdstown, WV, 25443. Method Development for the Determination of Octopamine in Insect Hemolymph by HPLC with Electrochemical Detection.

An HPLC method has been developed for the quantification of octopamine in insect hemolymph. The samples were collected in a syringe preloaded with formic acid as an anticoagulant and with DHBA (3,4-Dihydroxybenzylamine hydrobromide) as an internal standard. Proteins were removed by centrifuging with a 10K Da filter. The eluent was an aqueous 0.05 M citrate buffer (pH= 5.7) mixed that included an ion pairing reagent and mixed with 5-10% methanol. A C18 column and electrochemical detection was used. With this method, the amines can be detected at picogram levels. The major challenge in the analysis is that there are hundreds of compounds in a typical biological fluid and, in some cases, another electro-active species will co-elute with the amine of interest. Adjusting the pH, the percentage methanol, and the amount and type of ion pairing reagent had a very significant effect on the retention times. The measured octopamine level varied from 30 to 600 pg. of octopamine per mg. of hemolymph.