

Development of a 2D-HPLC/MS/MS analysis method for 3-nitrotyrosine, diesel exposure biomarker

A new analytical method has been developed for detection of 3-nitrotyrosine in biological samples using 2-dimensional (2-D) high pressure liquid chromatography (HPLC). Nitrotyrosine (NT), a biomarker of oxidative stress, can be formed in the body due to exposure to diesel exhaust particulate. Previous work in our laboratory identified that ion suppression was a significant problem during mass spectrometry (MS) detection of NT after reversed phase HPLC separation of solid phase extraction (SPE) extracts of biological samples. SPE was employed to remove background interferences found in the matrices of urine and blood plasma proteins. Blood plasma proteins were prepared by ultrafiltration and protein digestion prior to SPE. However, further sample preparation was necessary to remove the interfering compounds causing ion suppression, suggesting chromatographic separation with orthogonal characteristics to reversed phase. Therefore, the SPE extracts were first separated by strong cation exchange chromatography in order to isolate NT from the matrix interferences which may play a key role in ion suppression. The NT peak was then trapped onto a short reversed phase column and further eluted onto a longer analytical reversed phase column. The 2-dimensional chromatographic system (cation exchange/reversed phase) was optimized with ultraviolet detection and confirmed by tandem MS detection (MS/MS). This method will be applied to biological samples from a controlled exposure study where human volunteers were exposed to diesel exhaust particulate or filtered air. The aim is to determine if NT (protein-bound and free non-bound) levels become elevated in the body after diesel inhalation.