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MEETING ABSTRACTS

DETERMINATION OF BChE ACTIVITY BY MASS SPECTROMETRIC ANALYSIS OF ITS ADDUCT WITH RUSSIAN Vx

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Phosphonylated butyrylcholiesterase (BChE) is a marker of exposure to organophosphorus compounds, including nerve agents and pesticides. In cases of poisoning with nerve agents, it is important not only to establish the fact of poisoning, but also to give a quantitative estimate. The most common quantitative characteristic is BChE inhibition. We developed a highly sensitive method for the quantification of BChE inhibition by Russian Vx (VR) by mass spectrometry. For model experiments we used donor human blood plasma exposed to VR at concentrations of 1–100 ng/ml.

Butyrylcholinesterase was selectively isolated from plasma by immunoprecipitation and then subjected to enzymatic hydrolysis with pepsin. The hydrolysate was analyzed by HPLC-MS/MS using MRM mode, which allowed determination of the VR-modified nonapeptide FGESAGAAS (m/z 930 Da) at a very low level of VR (1 ng/mL). To measure the inhibition of BChE, an excess of VR is added to one sample, and the nonapeptide peak area is considered to correspond to 100% inhibition. The inhibition of BChE in samples containing different concentrations of VR are determined by ratio of the nonapeptide peak area in each specific sample to that at 100% inhibition.

BChE inhibition,% = (S_{930}/S_{930}100%)*100

It was found that the VR-modified nonapeptide peak area is linearly related to VR concentration. The BChE inhibition measured by mass spectrometry was consistent with the results of Ellman's assay ($R^2 \geq 0.98$). The advantages of the proposed approach over Ellman's assay include the possibility of quantification of inhibition at low doses of nerve agent and lack of necessity to construct calibration plots.

Keywords: butyrylcholiesterase; nerve agents; VR; adduct; inhibition