K26 Simultaneous Determination of the Nerve Gases GB (Sarin) and VX and the Vesicant HD (Sulfur Mustard)

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The goal of this presentation is to present methodology for rapidly detecting exposure or contamination from the chemical warfare or potential terrorist agents, GB (sarin), VX, and HD (sulfur mustard).

The U.S. as a signatory to the Chemical Weapons Convention plans upon destroying the domestic stockpile of chemical warfare agents stored at six different sites by 2007. Some of these storage sites, such as the Pine Bluff (Arkansas) Arsenal, have substantial populations living near the demilitarization facility. In the unlikely event that an accidental release occurs, monitoring of persons potentially exposed and environmental contamination will be necessary for assessing effects on public health. The nerve gases GB (sarin) and VX are two of the chemical warfare agents currently scheduled for destruction. These toxic gases are relatively easy to synthesize and have previously been used for terrorist activities in Japan. Additionally, it is known that several rouge nations supporting terrorist activities also possess these nerve agents. The vesicant (blistering agent) HD in addition to being in U.S. stockpiles slated for destruction is also known to be in the possession of some rouge states. Therefore, having an assay for detection of these chemical agents becomes important in any forensic investigation following an incident.

GB and VX hydrolyze in the environment and are metabolized in humans by essentially identical pathways. These organophosphates form a common end product, methylphosphonic acid (MPA) which if identified would indicate that either of these agents was utilized. More specific identification was ascribed by determination of the immediate precursors to MPA, either isopropylmethylphosphonic acid (IMPA) or ethylmethylphosphonic acid (EMPA) derived from GB and VX, respectively. HD also undergoes environmental hydrolysis and human metabolism in identical manners and forms thiodiglycol (TDG) and thiodiglycol sulfoxide (TDGS). Therefore, an analysis method that can detect MPA, IMPA, EMPA, TDG, and TDGS can be utilized for multiple matrices by modifying the pre-analytical work-up.

A GC-MS method was developed that simultaneously detects MPA, IMPA, EMPA, TDG, and TDGS as their respective silylated derivatives in a 10-minute analysis. For urine analysis, 100 IL of a 1,000 ng/mL aqueous solution of d_7-IMPA and d_8-TDG was added as internal standards to 3 mL of urine. Calibrators containing 3.1, 6.3, 12.5, 25, 50, and 100 ng/mL of MPA, IMPA, EMPA, TDG, and TDGS in laboratory workers' urine were used to determine replicate urine specimens to which 0, 10, and 80 ng/mL of these hydrolysis compounds were added. Following addition of the internal standards, 1 mL of 5% HCl was added followed by extraction with 3 mL 9:1 $CHCl_3$:Isopropyl alcohol and centrifugation to separate the organic layer that was evaporated to dryness under nitrogen at 50°C. To the resultant residue was added 30 IL BSTFA and 70 IL ethyl acetate followed by heating at 75°C for 15 min. GC-MS conditions were as follows: injection volume 1 IL; injector port 180°C; interface 280°C; column, HP-1 (12m x 0.2 mm i.d.); oven program 50°C for 4 min, 40°C/min, 280°C for 0.25 min; helium flow 0.5 mL/min; SIM mode with 50 ms dwell; and EM 400 volts above daily tune. Retention times and ions (where *q* is the quantitative ion) were: EMPA, 6.02 min, *m/z* 153 (*q*), 154, 137; d_7-IMPA 6.15 min, *m/z* 154 (*q*), 171, 155; IMPA, 6.17 min, *m/z* 153 (*q*), 195, 169; MPA 6.39 min, *m/z* 225 (*q*), 226, 227; d_8-TDG 7.81 min, *m/z* 119 (*q*), 183, 168; TDG 7.83 min, *m/z* 116 (*q*), 176, 130; TDGS 8.64 min, *m/z* 166 (*q*), 117, 267.

The LOQ of the developed method was 3.1 ng/mL for all the analytes of interest and the LOD was 1.5 ng/mL. Because exposure of humans to the nerve gases and vesicant constitute unethical experimental paradigms, validation of the method will require the determination of a baseline levels of the compounds in a substantial number of non-exposed humans. TDG is known to occur at low levels in human urine as a by-product of dietary habits. Once a background-level for all the analytes is determined, a level of 2SD above the mean could be used for indicating exposure.

Nerve Gases, Vesicant, Urine Analysis

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