

Background

- Organization for the Prohibition of Chemical Weapons (OPCW) ensures compliance with the Chemical Weapons Convention that bans the use of chemical weapons.
- OPCW maintains a network of designated labs that can be used to analyze authentic environmental samples (i.e. samples collected in Syria in 2013).
- ECBC Forensic Analytical Center (FAC) has been designated for the analysis of environmental samples since 1998.
- Designated labs were proficient in analysis of environmental samples (e.g. soil, water) for chemical warfare agents (CWAs), but the capability to confirm human exposure to CWAs was uncertain.
- In 2009, OPCW implemented the first confidence building exercise for analysis of biomedical samples.

Biomedical Sample Analysis

- The FAC has successfully participated in all five of the confidence building exercises that OPCW has executed.
- Human plasma, urine, and synthetic urine have all been used as matrices.
- Challenges:
 - Detection of biomarkers at low ppb (ng/mL) level in biological matrices.
 - Limited sample volume.
 - Legally defensible report.
- Official biomedical sample proficiency tests to begin in 2016.

GC-MS/MS Sample Preparation

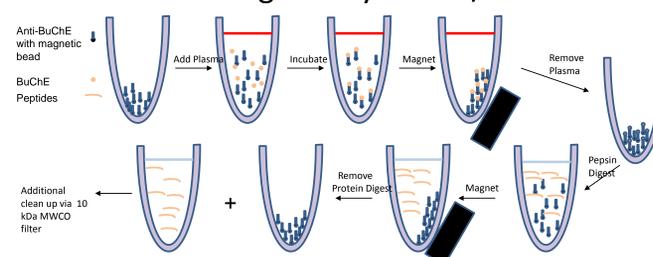
- Urine preparation generally involved solid phase extraction (SPE), followed by derivatization (i.e. PFB-Br, HFBI)
- Plasma preparation:
 - Nerve agents: fluoride activation then SPE
 - HD: Protein precipitation, NaOH cleavage, derivatization with PFB-Cl, then SPE

| CW class | Test Year | Matrix | Biomarker/Analyte | Analytical Technique |
|-----------------------------|-----------|-----------------|---|---|
| Nerve Agents (G-agents, VX) | 2010 | Synthetic Urine | EMPA, IMPA | LC-MS/MS (-ESI) GC-MS/MS (-CI) |
| | 2012 | Urine | PMPA, EMPA, IMPA | LC-MS/MS (-ESI) GC-MS/MS (-CI) |
| | 2013 | Plasma Urine | IMPA IMPA, PMPA, EMPA | GC-MS/MS (+CI) LC-MS/MS (-ESI) GC-MS/MS (-CI) |
| | 2014 | Plasma | BuChE nonapeptide w/GD or VX adduct GD, VX | LC-MS/MS (+ESI) GC-MS/MS (+CI) |
| Blister Agents (HD) | 2010 | Synthetic Urine | TDG B-lyase metabolite, SBMSE | GC-MS/MS (+CI), GC-MS (EI) LC-MS/MS (+ESI) |
| | 2012 | Urine | TDG B-lyase metabolite, SBMTE B-lyase metabolite, SBMSE | GC-MS/MS (+CI), GC-MS (EI) GC-MS/MS (+CI) LC-MS/MS (+ESI) |
| | 2015 | Plasma | HSA tripeptide w/HD adduct HD | LC-MS/MS (+ESI) GC-MS/MS (-CI) |

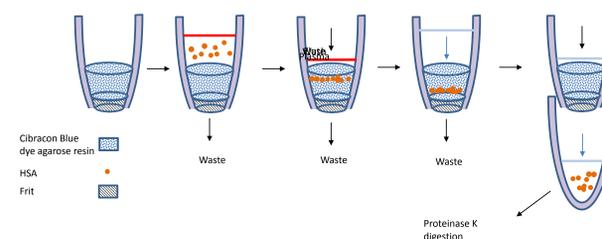
LC-MS/MS Sample Preparation

LC-MS/MS detection of nerve agents and sulfur mustard in plasma required two different preparations for detecting the agent adducts on plasma proteins.

Plasma Sample Preparation for Detection of Nerve Agents by LC-MS/MS

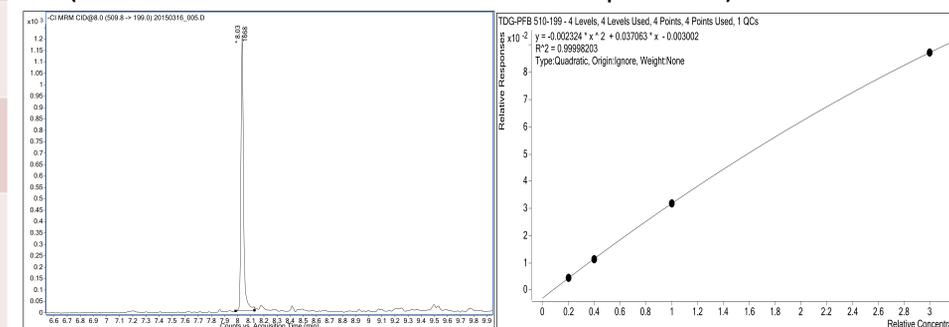


Plasma Sample Preparation for Detection of Sulfur Mustard by LC-MS/MS



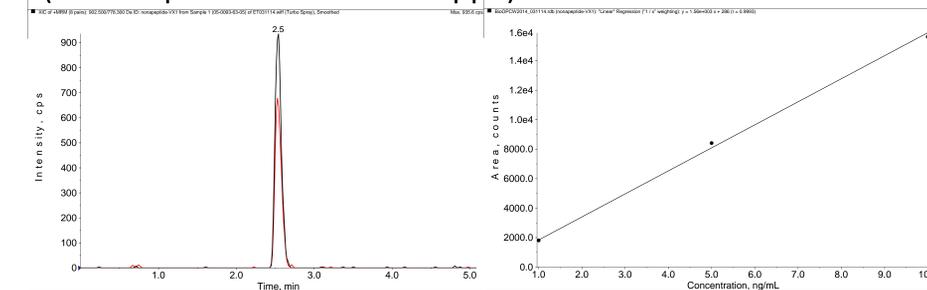
GC/MS/MS Analysis

- Analysis on an Agilent 7890 GC/7000 MS/MS (NCI, methane)
- Pentafluorobenzoyl derivative of TDG (cleaved from protein adduct).
- Quantitation using TDG-d8 as internal standard (spiked as HD-d8).
- Four point curve (10-150 ppb) using 509.8→199 transition (509.8→211 and 509.8→167 used as qualifiers)



LC-MS/MS Analysis

- Analysis on an ABSciex 4000Qtrap with Waters Acquity UPLC.
- Detection of Butylcholinesterase nonapeptide digestion product with ethyl methyl phosphonate adduct.
- Two transitions were used for identification, 902.5→778.3 and 902.5→673.4.
- Transition 902.5→778.3 was used for quantitation using a 3-point calibration curve, 1ppb-10 ppb.
- The concentration detected via our analysis was 3.3 ppb (OPCW spiked amount was 5 ppb).



Acknowledgements

Analytical Toxicology Branch (ECBC) - Dr. Mike Jakubowski, Jeff McGuire, and Ron Evans.
MRICD - Dr. Rick Smith and Dr. Ben Capacio.