Quantitative Analysis of Organic Gunshot Residues by LC/MS/MS

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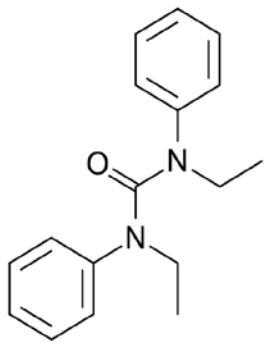
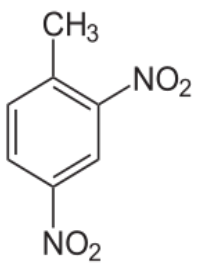
Introduction

The analysis of organic gunshot residues (GSR) on the hands of an individual that discharged a firearm has a long history in forensic science. The earliest tests for gunshot residue on a shooter's hands were chemical color tests for nitrates. Over the past fifty years, the analysis of gunshot residue has changed in response to advances in instrumentation capabilities and changes in the components associated with the manufacturing of ammunition. The primary technique that is used today for the detection of gunshot residue on a subject's hands is scanning electron microscopy with energy dispersive spectrometer (SEM/EDS). Even though this technique is automated, it still is time consuming requiring several hours and sometimes several days to search a single disk. In addition, some of the newer ammunition is lead free. Furthermore, for some of these the lead-free primer mixtures have elements that are not conducive to elemental analyses by automated SEM/EDS.

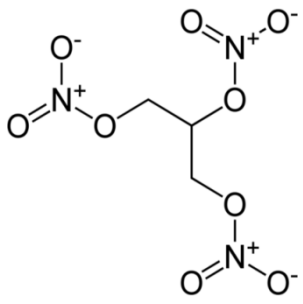
This research project would use liquid chromatography–triple quadruple mass spectrometry (LC/MS/MS) to evaluate its suitability in determining organic components of gunpowder and primer mixtures on a shooter hand. A robust, sensitive, fast and reliable method is presented for the quantitation of GSR using an LC triple quadrupole mass spectrometer. Significant variation exists between ammunition manufacturers in the formulation of both gunpowder and primer mixtures. In this study, three representative organic components of gunpowder, trinitroglycerin (TNG), dinitrotoluene (DNT) and ethyl centralite (CET), are selected to evaluate the feasibility of detecting organic components of gunshot residues from the hands of a shooter. The sampling techniques, brands of ammunition, the solutions used collecting gunshot residues from the surface of a hand and the reasonable length of time after shooting a gun are evaluated in this study.



Ethyl Centralite (CET)



Dinitrotoluene (DNT)



Trinitroglycerin (TNG)

Experimental

Sample Preparation:

Fired cases: Fired cases were sonicated in 4 mL acetonitrile for 10 min and subsequently dried under N_2 flow to a final volume of 0.8 mL. The concentrated solution (0.8 mL) was filtered via a 0.2 µm filter membrane.
Gun powder: About 20 mg of each gun powder was dissolved in 1 mL of acetonitrile. The solution was sonicated for 10 min and filtered via a 0.2 µm filter membrane.
Shooter hand swab tips: Four areas of hands are sampled using swabs with different solvents at 0, 2 and 4 hours. The polyester swab tip is cut off and sonicated in 0.75 mL of acetonitrile for 10 min and then filtered via a 0.2 µm filter membrane and subsequently dried under nitrogen flow. The residue is reconstituted into 50 µL of acetonitrile.

LC Method:
Agilent 1200 SL series binary pump, well plate sampler, thermostatted column compartment
Column: Eclipse Plus C18, 2.1x50mm 1.8 um, 600 bar
Column temperature: 40 ºC
Injection volume: 1 mL
Autosampler temp: 4 ºC
Needle wash: flushport (MeOH:water 75:25), 10 seconds
Mobile phase: A(CET) = 0.1 % formic acid in water
B(CET) = 0.1 % formic acid in acetonitrile
A(DNT) = 5 mM ammonium acetate in water
B(DNT) = methanol
A(TNG) = 25 µM ammonium chloride in water
B(TNG) = methanol
Flow rate: 0.4 mL/min
Gradient: 50% B up to 100% B in 1 minute and 100% B for 2 min

MS Method:
Agilent 6430 triple quadrupole mass spectrometer

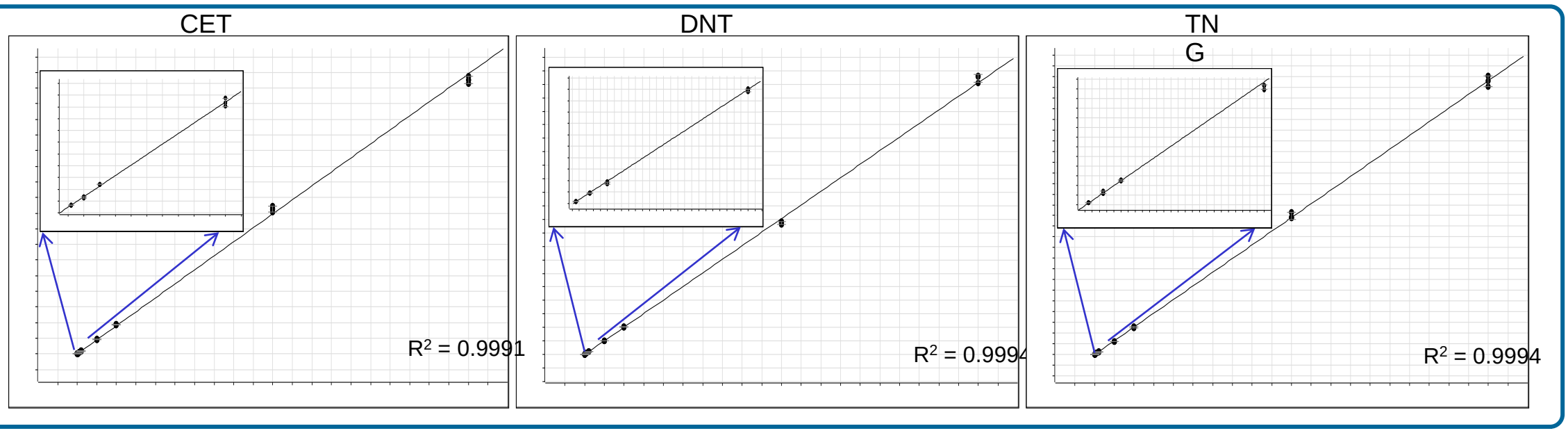
Ion mode:	ESI+	APCI-	APCI-
Gas temperature:	350 ºC	325 ºC	200 ºC
Drying gas (nitrogen):	10 L/min	4 L/min	4 L/min
Nebulizer gas (nitrogen):	35 psi	20 psi	20 psi
Vaporizer temperature:		350 ºC	200 ºC
Capillary voltage:	2500V	3500V	1000V
Corona current:		4 µA	4 µA

MRM acquisition:
(Q1 and Q2 peak width = 0.7 unit, Q1 peak width is 2.5 units for TNG)

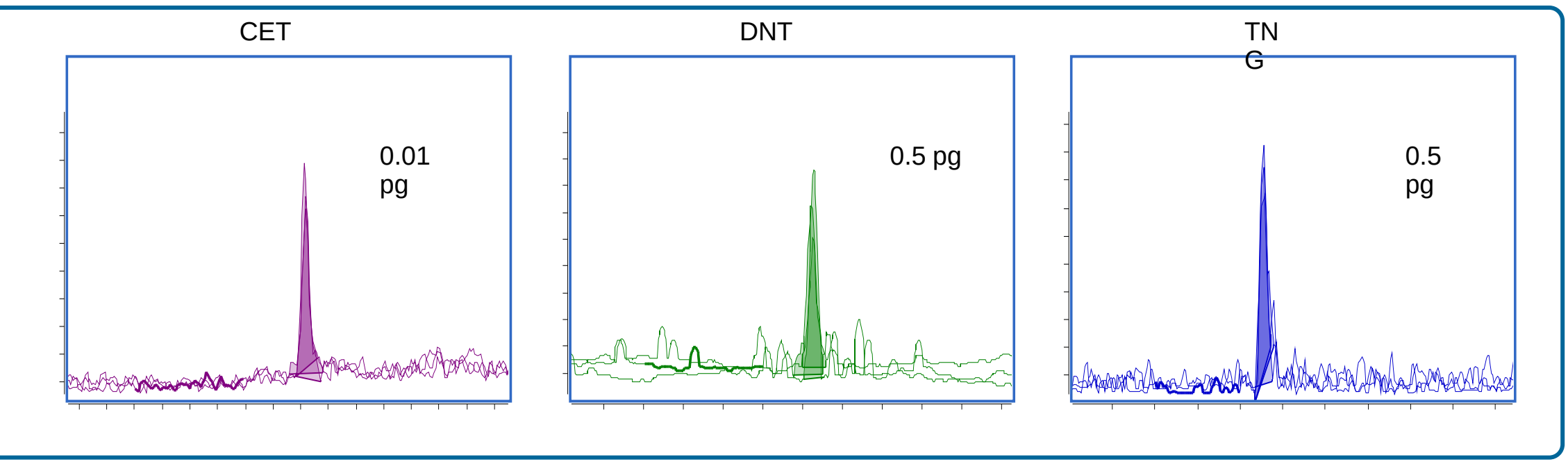
	Mode	MRM	Fragmentor (V)	Dwell (msec)	Collision Energy (V)
CET	ESI+	269.2>119.9	100	200	20
		269.2>147.8	100	200	8
DNT	APC-	181.0>45.9	100	150	30
		181.0>134.9	100	150	20
TNG	APCI-	262.9>61.9	60	200	10
		262.9>46.0	60	200	8

Results and Discussion

Dynamic Range: Calibration Curves



Sensitivity: Limits of Detection



Method Validation: Accuracy, Reproducibility and Precision

Compound	LOD (pg)	LOQ (pg)	S/N	Range (ng/mL)	R^2	Reproducibility (%, n=3)	Accuracy (%)	Precision (%)
CET	0.01	0.01	> 15:1	0.01 – 100	0.9991	0.83 – 4.64	86.3 – 108.5	4.18
DNT	0.5	1	> 10:1	1 – 10000	0.9994	0.39 – 5.93	88.3 – 118.6	6.27
TNG	0.5	1	>10:1	1 – 10000	0.9994	0.94 – 5.41	85.4 – 112.8	6.51

Measurement of CET, DNT and TNG in fired cases and gunpowder of three brands of ammunition

Fired Cases	CET		DNT		TNG	
	Conc. (µg)	%RSD	Conc. (ng)	%RSD	Conc. (µg)	%RSD
Winchester Fired Case (WFC)	1.62	4.18	< LOQ	N/A	16.5	3.93
Remington Fired Case (RFC)	2.86	2.28	< LOQ	N/A	44.6	1.83
Federal Fired Case (FFC)	0.0685	2.80	2.50	13.1	44.2	N/A
Gunpowder	**Conc. (µg/mg)**	**%RSD**	**Conc. (ng/mg)**	**%RSD**	**Conc. (µg/mg)**	**%RSD**
Federal American Eagle (FAE)	0.210	2.45	1.01	12.2	255	0.22
Remington Golden Saber (RGS)	9.47	0.85	< LOQ	N/A	264	1.00
Winchester Super X (WSX)	19.1	0.45	6.95	2.67	223	5.05

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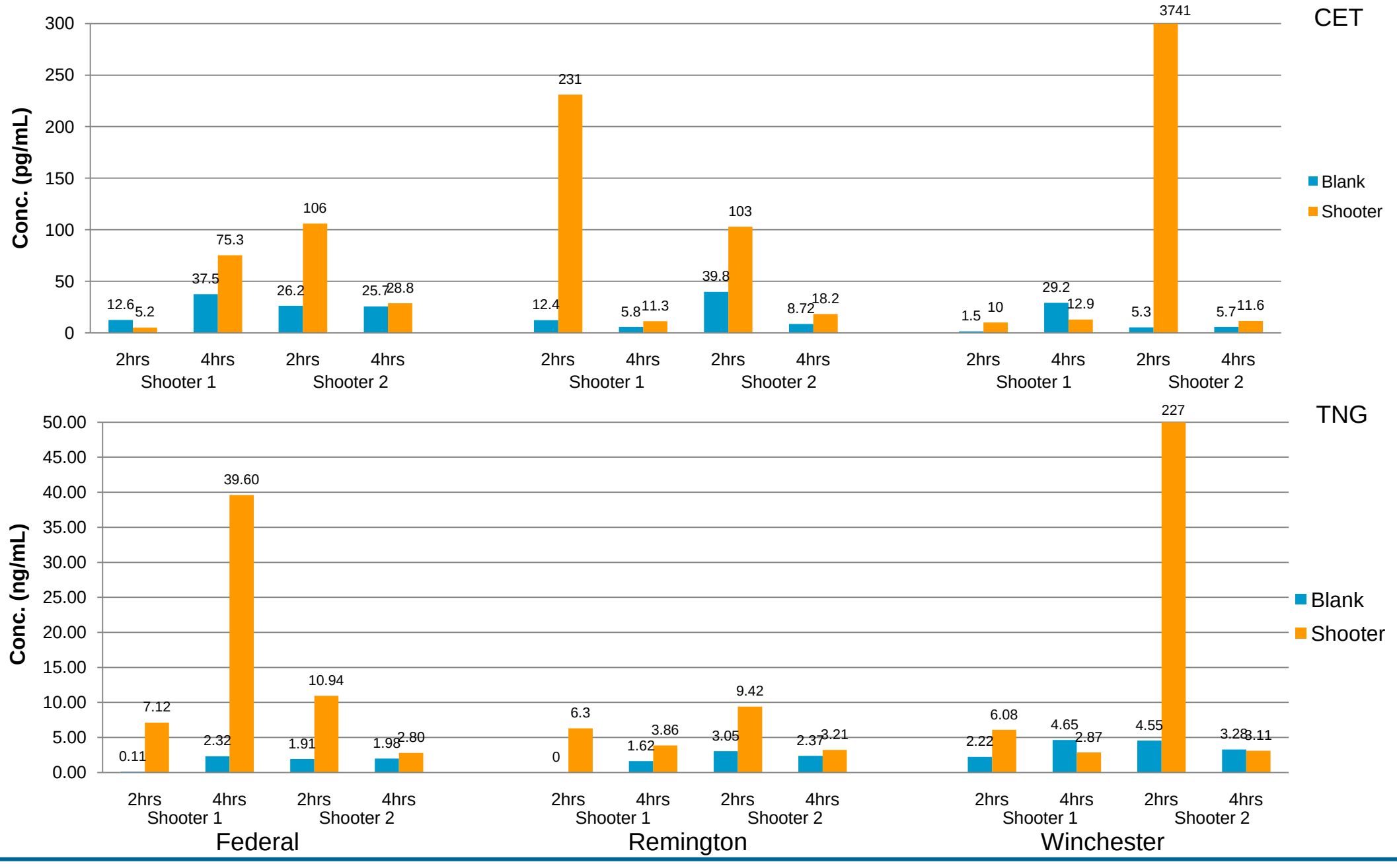
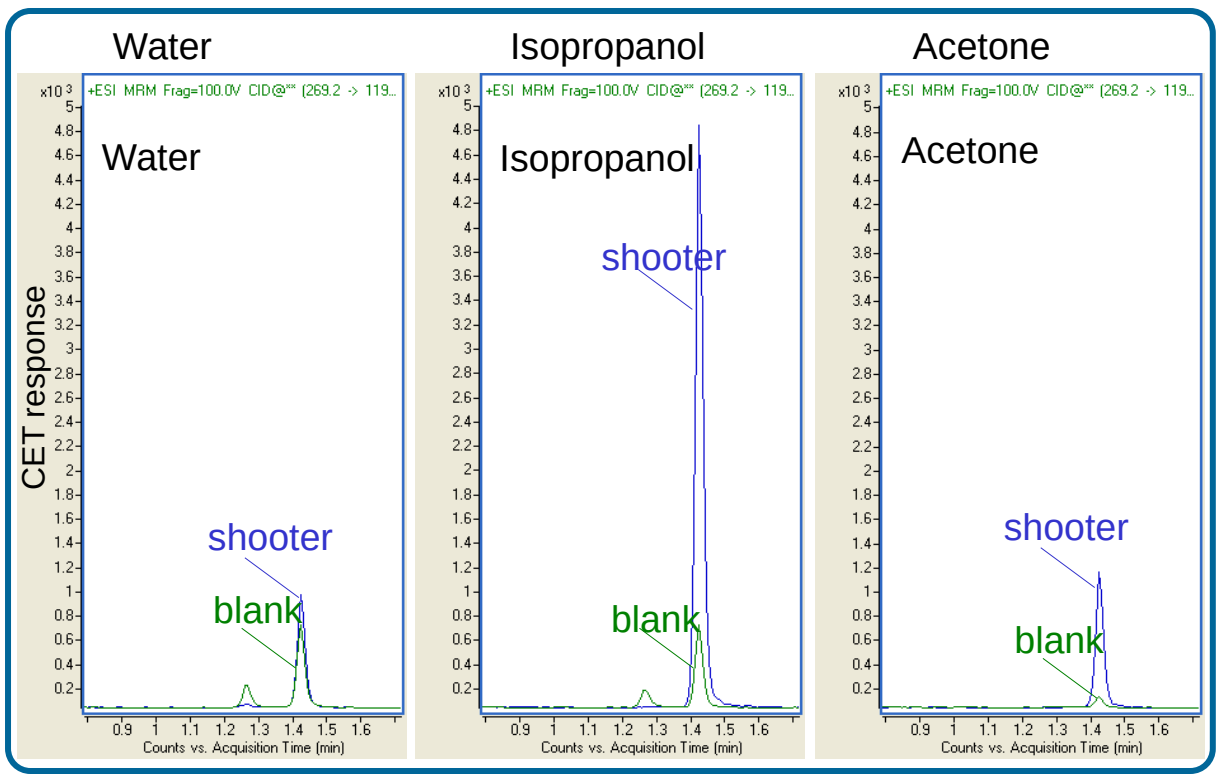
Analysis of Samples Collected from the Hands of a Shooter



Test subjects fired three brands of ammunition, Federal, Remington and Winchester. Four areas of the hands were sampled using a polyester swab at 2 and 4 hours. CET and TNG are detected in the samples instead of DNT, which is not an abundant ingredient in gunpowder. In most cases, the 2 hour level is higher than the 4 hour sample and blank. To provide significant evidence, more time-course points and statistic study on samplings need to be evaluated in the further study.

Shooter Hand Sampling Solvent Comparison



Conclusions

- *Agilent 6430 triple quadrupole demonstrates excellent sensitivity for detecting organic gunshot residues. The limit of detection (LOD) is 0.01, 0.5, and 0.5 pg on-column for CET, DNT and TNG, respectively.*
- *The calibration curves show excellent linearity with four orders of dynamic range (e.g. 0.01-100 ng/mL for CET). Great accuracy, precision and reproducibility of LC/MS/MS (QQQ) quantitative analyses were observed for all analytes.*
- *These organic gunshot residue compounds were successfully screened in gunpowder and fired cases from different brands of ammunition.*
- *This LC/MS/MS method is technically feasible for routine analysis of organic gunshot residues from the shooter's hands at a reasonable length of time after shooting a gun. More statistical evaluation needs to be done in future studies.*