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as of 04-Jun-2018

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Begin Performance Period: 01-Aug-2016

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Major Goals: The purpose of this proposal was to seek a matching fund to acquire a liquid chromatography tandem mass spectrometry (LC-MSMS) instrument for John Jay College, a senior college within the City University of New York and a Minority-Serving Institution, to enhance its research capabilities and improve its educational program. Thanks to this award, the equipment ultra-fast liquid chromatograph Nexera, UFLC-XR coupled to a triple quadrupole mass spectrometer (LCMS-8050) from Shimadzu Corporation was installed in the Toxicology Laboratory in John Jay College in September 2016. The installation was completed on September 8th 2016, and the PI Marta Concheiro-Guisan and the co-PIs Shu-Yuan Cheng and Yi He attended the corresponding training on September 12th, 19th and 21st 2016. Since then, the equipment has been employed in undergraduate and graduate projects in "Human Performance and Biosystems" and "Environmental Chemistry" research areas. A total of 3 undergraduate students and 4 graduate students have been utilizing this instrument in their corresponding research projects.

The main problem studied with the equipment since its installation has been the waste-water analysis of tobacco and illicit drugs in New York City. Wastewater-based toxicology is an innovative and promising approach that provides information about exposure to external agents, such as drugs of abuse and medicines, in defined population groups by the analysis of human excretion products (biomarkers) in wastewater. Traces of almost everything we consume are excreted unchanged or as a mixture of metabolites in our urine and/or feces and ultimately end up in the sewer network (1). This approach is best described as large urine test, as the collective wastewater represents anonymous urine samples of thousands of individuals. By measuring the levels of licit and illicit drugs and their metabolites in wastewater, the quantity of drugs consumed in a community can be estimated (2).

The objective of this study is to develop and validate a wastewater analytical method for the simultaneous determination of tobacco and illicit drugs (marijuana, cocaine, heroin and amphetamines) and their metabolites in

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wastewater, and to apply this method to wastewater samples collected from wastewater treatment plants in New York City two different days of the week during four weeks throughout one year.

Another project that has been developed is the determination of alcohol exposure during pregnancy by maternal hair and umbilical cord analysis. Alcohol consumption during pregnancy puts the fetus at risk of developing conditions such as fetal alcohol syndrome and other alcohol related neurodevelopmental disorders, which manifest as cognitive impairments (3), as well as result in a range of birth defects and subsequent neurodevelopmental problems, including distinctive craniofacial dysmorphology, compromised growth, and cognitive and social impairments (4). Studies have shown that amounts such as 1 mg/g of ethanol ingested weekly during the early stages of fetal development can be harmful to a developing fetus (5). Objective analytical tools are critical to determine the timing and intensity of this exposure.

The objective of this study is to develop and validate sensitive and specific analytical methods for the determination of the ethanol minor metabolite EtG (ethyl glucuronide) in hair samples. The method will be applied to authentic samples collected from alcohol users to establish categorization of drinking habits using the cut off values proposed for hair by the Society of Hair Testing (SoHT), abstinent (EtG < 7 pg/mg), social drinker (7-30 pg/mg) or excessive drinker (EtG > 30 pg/mg). This study also wants to evaluate the utility of umbilical cord as alternative matrix to detect alcohol exposure during pregnancy; therefore, we develop and validate an analytical method for the determination of the ethanol minor metabolites EtG and EtS (ethyl sulfate) in umbilical cord. The method will be applied to authentic umbilical cord samples from newborns exposed to alcohol during pregnancy, which paired maternal hair, pubic hair and/or meconium samples tested positive for EtG.

We also have a project aiming at development of an advanced analytical method for determination of trace level explosives in environmental water (6). The investigated explosives include three major types of explosives commonly in use, i.e. nitroaromatic, nitramine, and nitrate ester explosives. The representative target analytes include nitrobenzene (NB); 1,3-dinitrobenzene (1,3-DNB), 1,3,5-trinitrobenzene (1,3,5-TNB), 2,4-dinitrotoluene (2,4-DNT); 2,4,6-trinitrotoluene (2,4,6-TNT), cyclotrimethylene trinitramine (RDX), cyclotetramethylene tetranitramine (HMX), and pentaerythritol tetranitrate (PETN). LC-MS/MS separation and detection parameters have been optimized. A poster presentation of this work entitled "Challenges and Accomplishments of the Routine LC/MS Analysis of Explosives in Water Matrices" has been submitted and accepted by Pittsburg Conference (Pittcon) 2018 in Orlando, FL. A manuscript of technique review of current analytical methods for explosive determination is in preparation.

Accomplishments: We developed a method to determine tobacco (cotinine), cocaine (benzoylecgonine, cocaethylene, and cocaine), amphetamines (methamphetamine, MDMA, MDA and amphetamine), opiates (6-monoacetilmorphine, morphine, codeine, oxycodone, hydromorphone, hydrocodone, fentanyl, norfentanyl, methadone, EDDP) and cannabis (delta-9-tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol) biomarkers in 50 mL of wastewater. Wastewater samples were filtered, extracted using mixed mode cation exchange solid phase extraction (SPE) cartridges, and analyzed by LC-MS/MS using positive electrospray ionization (ESI+). All compounds were analyzed on a reversed-phase C18 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases, using two different gradients (one for cannabinoids and another one for the rest of compounds) (Figure 1). Each compound was monitored by two multiple reaction monitoring (MRM) transitions (Table 1) in positive mode. Method validation included linearity (5-1,000 ng/L for all compounds, except 10-1,000 ng/L for cotinine), limit of detection (1-10 ng/L) and quantification (5-10 ng/L), imprecision (<20%), accuracy (80-120%), matrix effect (-48 to 11.6%) and extraction efficiency (7.7 to 112.3%), interferences (no interferences detected), and 24h auto-sampler stability (no analyte loss).

We applied this method to 48 wastewater samples collected from wastewater treatment plants in New York City (The Bronx, Brooklyn, Queens, and Manhattan) throughout one year. The specific wastewater plants were Hunts Point, North River, Tallman, Jamaica and Newtown Creek (Brooklyn-Queens and Manhattan pools). The collection time points were by 11am on May 27th, May 31st, July 1st, July 5th, September 2nd, September 6th and December 30th 2016 and January 3rd 2017. Tobacco biomarker cotinine was detected in all samples with concentrations ranging from 276 to 1467 ng/L. Most of opioids were also detected in all samples; morphine (145-1373.5 ng/L, n=48), codeine (20.8-219.6 ng/L, n=48), oxycodone (13-117.7 ng/L, n=48), hydromorphone (4-209.8 ng/L, n=48), oxycodone (13-80.5 ng/L, n=48), methadone (9.2-270.3 ng/L, n=48), EDDP (23.6-532.3 ng/L, n=48), hydrocodone (4.3-14.5 ng/L, n=33), 6-monoacetilmorphine (3.6-18.8 ng/L, n=8) and fentanyl (5.9 ng/L, n=1). In the case of cocaine and metabolites, cocaine was found in the 48 samples (76.8-1564 ng/L), as well as benzoylecgonine (266.4-2811 ng/L) and cocaethylene (3.1-46.3 ng/L). Amphetamine and methamphetamine were determined in the 48 samples with concentrations from 15 to 285.9 and from 21.6 to 372.2 ng/L, respectively. The amphetamine derivative MDA was detected in 22 samples (4.2-51.1 ng/L) and MDMA in 38 samples (5.6-187 ng/L). Cannabis markers were detected in 47 samples (THCCOOH, 68-1451.9 ng/L) and in 15 samples (THC, 4.7-33.3 ng/L).

We developed and validated an analytical method for the determination of EtG in hair samples. Hair samples were

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decontaminated with water, methanol and dichloromethane. After drying, they were cut in small pieces, and 25 mg were weighted and incubated in water in ultrasound bath for 2 h. The supernatant was extracted by graphite carbon-based solid phase extraction. The chromatographic separation was performed on porous graphitic column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases, and two MRM transitions were monitored in LC-MS/MS in electrospray negative mode (Figure 2). Method validation included linearity (5-100 pg/mg), limit of detection and quantification (5 pg/mg), imprecision (<7.2%), accuracy (80-120%), matrix effect (-32.3%), extraction efficiency (81.8%), interferences (no interferences detected), and 24h auto-sampler stability (no analyte loss).

With regard with the explosives project, we developed mobile phase gradient for UPLC separation, investigated various MS ionization modes (Table 2), and obtained precursor and MRM transition ions information. It is demonstrated the feasibility of LC-MS/MS for determination of trace level explosives that might be found in environmental water.

Training Opportunities: A total of 3 undergraduate students and 4 graduate students have been utilizing the LC-MS/MS instrument LCMS-8050 from Shimadzu in their corresponding research projects, since its installation in the Toxicology Laboratory of John Jay College about a year ago.

Also two presentations will be performed in scientific meetings thanks to the work developed with the LCMS-8050. These presentations are:

- "A wastewater analysis for tobacco and drug detection in New York City", Alethea Jacox, Nicole Centazzo, Bonnie-Marie Frederick, Jasmine Gayle, Marta Concheiro-Guisan. American Academy of Forensic Sciences, Seattle, Washington, February 19-24, 2018.

- "Challenges and Accomplishments of the Routine LC/MS Analysis of Explosives in Water Matrices", Cristina Veresmortean, Yi He. Pittcon, Orlando, FL, February 26-March 1, 2018.

Results Dissemination: Nothing to Report

Honors and Awards: - Cycle 47 PSC-CUNY Research Award

Effective date: July 1st 2016 –July 1st 2017

Name of PI: Marta Concheiro-Guisan

Project title: "Wastewater-based epidemiology of tobacco and illicit drugs in New York City".

Funding Agency: The City University of New York.

Total costs: \$3,484

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI

Participant: Marta CONCHEIRO-GUISAN

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Co PD/PI

Participant: Shu-Yuan Cheng

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

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as of 04-Jun-2018

National Academy Member: N
Other Collaborators:

Participant Type: Co PD/PI

Participant: Yi He

Person Months Worked: 12.00

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Publication Status: 3-Accepted

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Date Received: 08-Dec-2017

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Conference Location: Seattle, Washington

Paper Title: A wastewater analysis for tobacco and drug detection in New York City

Authors: Alethea Jacox, Nicole Centazzo, Bonnie-Marie Frederick, Jasmine Gayle, Marta Concheiro-Guisan

Acknowledged Federal Support: **N**

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Conference Location: Orlando, FL

Paper Title: Challenges and Accomplishments of the Routine LC/MS Analysis of Explosives in Water Matrices

Authors: Cristina Veresmortean, Yi He

Acknowledged Federal Support: **N**

Contract Number: W911NF-16-1-0430

Proposal No. 68834-RT-REP: “Liquid chromatography tandem mass spectrometry in toxicology and environmental sciences research and education programs”

PI: Marta Concheiro-Guisan; **Co-PI:** Shu-Yuan Cheng; Yi He

Institution: John Jay College of Criminal Justice, City University of New York

Final Progress Report

1. Foreword

The purpose of this proposal was to seek a matching fund to acquire a liquid chromatography tandem mass spectrometry (LC-MSMS) instrument for John Jay College, a senior college within the City University of New York and a Minority-Serving Institution, to enhance its research capabilities and improve its educational program. Thanks to this award, the equipment ultra-fast liquid chromatograph Nexera, UFLC-XR coupled to a triple quadrupole mass spectrometer (LCMS-8050) from Shimadzu Corporation was installed in the Toxicology Laboratory in John Jay College in September 2016. The installation was completed on September 8th 2016, and the PI Marta Concheiro-Guisan and the co-PIs Shu-Yuan Cheng and Yi He attended the corresponding training on September 12th, 19th and 21st 2016. Since then, the equipment has been employed in undergraduate and graduate projects in “Human Performance and Biosystems” and “Environmental Chemistry” research areas. A total of 3 undergraduate students and 4 graduate students have been utilizing this instrument in their corresponding research projects.

2. List of Appendixes

Table 1. Mass spectrometry parameters of the analytes and internal standards of the wastewater project.

Table 2. Investigation of different ionization modes for target analytes in the explosives project.

Figure 1. A, Total Ion Chromatogram (TIC) of cotinine (1), morphine (2), oxymorphone (3), hydromorphone (4), codeine (5), amphetamine (6), MDA (7), methamphetamine (8), oxycodone (9), 6-monoacetilmorphine (10), hydrocodone (11), MDMA (12), norfentanyl (13), benzoylecgonine (14), cocaine (15), cocaethylene (16), fentanyl (17), EDDP (18) and methadone (19) in water sample fortified at 20 ng/L. B, Total Ion Chromatogram (TIC) of delta-9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy- tetrahydrocannabinol (THCCOOH) in water sample fortified at 20 ng/L.

Figure 2. Quantifier (220.8>84.9) and qualifier (220.8>75.2) MRM transitions of ethylglucuronide (EtG) in hair at the limit of quantification (5 pg/mg).

3. Statement of the problem studied

The main problem studied with the equipment since its installation has been the wastewater analysis of tobacco and illicit drugs in New York City. Wastewater-based toxicology is an innovative and promising approach that provides information about exposure to external agents, such as drugs of abuse and medicines, in defined population groups by the analysis of human excretion products (biomarkers) in wastewater. Traces of almost everything we consume are excreted unchanged or as a mixture of metabolites in our urine and/or feces and ultimately end up in the sewer network (1). This approach is best described as large urine test, as the collective wastewater represents anonymous urine samples of thousands of individuals. By measuring the levels of licit and illicit drugs and their metabolites in wastewater, the quantity of drugs consumed in a community can be estimated (2).

The objective of this study is to develop and validate a wastewater analytical method for the simultaneous determination of tobacco and illicit drugs (marijuana, cocaine, heroin and amphetamines) and their metabolites in wastewater, and to apply this method to wastewater samples collected from wastewater treatment plants in New York City two different days of the week during four weeks throughout one year.

Another project that has been developed is the determination of alcohol exposure during pregnancy by maternal hair and umbilical cord analysis. Alcohol consumption during pregnancy puts the fetus at risk of developing conditions such as fetal alcohol syndrome and other alcohol related neurodevelopmental disorders, which manifest as cognitive impairments (3), as well as result in a range of birth defects and subsequent neurodevelopmental problems, including distinctive craniofacial dysmorphism, compromised growth, and cognitive and social impairments (4). Studies have shown that amounts such as 1 mg/g of ethanol ingested weekly during the early stages of fetal development can be harmful to a developing fetus (5). Objective analytical tools are critical to determine the timing and intensity of this exposure.

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dinitrobenzene (1,3-DNB), 1,3,5-trinitrobenzene (1,3,5-TNB), 2,4-dinitrotoluene (2,4-DNT); 2,4,6-trinitrotoluene (2,4,6-TNT), cyclotrimethylene trinitramine (RDX), cyclotetramethylene tetranitramine (HMX), and pentaerythritol tetranitrate (PETN). LC-MS/MS separation and detection parameters have been optimized. A poster presentation of this work entitled “Challenges and Accomplishments of the Routine LC/MS Analysis of Explosives in Water Matrices” has been submitted and accepted by Pittsburg Conference (Pittcon) 2018 in Orlando, FL. A manuscript of technique review of current analytical methods for explosive determination is in preparation.

4. Summary of the most important results

We developed a method to determine tobacco (cotinine), cocaine (benzoylecgonine, cocaethylene, and cocaine), amphetamines (methamphetamine, MDMA, MDA and amphetamine), opiates (6-monoacetylmorphine, morphine, codeine, oxycodone, oxycodone, hydromorphone, hydrocodone, fentanyl, norfentanyl, methadone, EDDP) and cannabis (delta-9-tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol) biomarkers in 50 mL of wastewater. Wastewater samples were filtered, extracted using mixed mode cation exchange solid phase extraction (SPE) cartridges, and analyzed by LC-MSMS using positive electrospray ionization (ESI+). All compounds were analyzed on a reversed-phase C18 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases, using two different gradients (one for cannabinoids and another one for the rest of compounds) (Figure 1). Each compound was monitored by two multiple reaction monitoring (MRM) transitions (Table 1) in positive mode. Method validation included linearity (5-1,000 ng/L for all compounds, except 10-1,000 ng/L for cotinine), limit of detection (1-10 ng/L) and quantification (5-10 ng/L), imprecision (<20%), accuracy (80-120%), matrix effect (-48 to 11.6%) and extraction efficiency (7.7 to 112.3%), interferences (no interferences detected), and 24h auto-sampler stability (no analyte loss).

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5. Bibliography

1. Castiglioni S., Thomas K.V., Kasprzyk-Hordern B., Vandam L., Griffiths P. (2014), "Testing wastewater to detect illicit drugs: State of the art, potential and research needs", *Science of the Total Environment*, 487:613-620.
2. Zuccato E., Chiabrando C., Castiglioni S., Bagnati R. Fanelli R.(2008),"Estimating community drug abuse by wastewater analysis", *Environmental Health Perspectives*, 116(8):1027–1032.
3. Denkins Y. M., Woods J., Whitty J. E., Hannigan J. H., Martier S. S., Sokol R. J., & Salem N. (2000). Effects of gestational alcohol exposure on the fatty acid composition of umbilical cord serum in humans. *The American Journal of Clinical Nutrition*, 300s-306s.
4. Vaiano F., Favretto D., Palumbo D., Cooper G., Mactier H., Busardò, F. P., . . . Bertol E. (2016). A novel, simultaneous extraction of FAEE and EtG from meconium and analysis by LC-MS/MS. *Analytical and Bioanalytical Chemistry*, 408(10), 2587-2594.
5. Morini L., Groppi A., Marchei E., Vagnarelli F., Algar O. G., Zuccaro P., & Pichini S. (2010). Population baseline of meconium ethyl glucuronide and ethyl sulfate concentrations in newborns of nondrinking women in 2 Mediterranean cohorts. *Therapeutic Drug Monitoring*, 32(3), 359-363.
6. Mu R., Shi H., Yuan Y., Karnjanapiboonwong A., Burken J. G., Ma, Y. (2012), Fast Separation and Quantification Method for Nitroguanidine and 2,4-Dinitroanisole by Ultrafast Liquid Chromatography–Tandem Mass Spectrometry, *Anal. Chem*, 84, 3427-3432.

7. Appendixes

Table 1. Mass spectrometry parameters of the analytes and internal standards of the wastewater project.

Compound	Precursor (M+H)+	Product 1	Q1	CE	Q3	Product 2	Q1	CE	Q3
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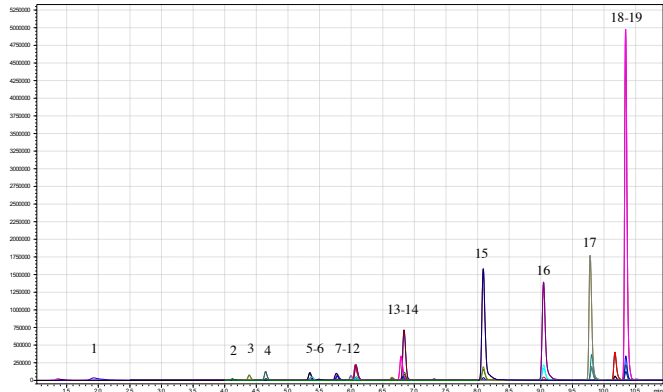
11-nor-9-Carboxy- Δ^9 -THC	345.05	299.3	-13	-21	-14	193.2	-13	-27	-20
11-nor-9-Carboxy- Δ^9 -THC-D ₅	348.05	302.2	-13	-21	-14				
6-Acetylmorphine	328	165.1	-12	-40	-29	211.15	-13	-28	-22
6-Acetylmorphine-D ₅	331	165.2	-13	-39	-16				
Amphetamine	136	91.15	-22	-22	-16	119.2	-23	-14	-12
Amphetamine-D ₅	140.95	93.15	-24	-17	-16				
Benzoylcegonine	290.05	168.25	-10	-20	-18	105.15	-11	-28	-10
Benzoylcegonine-D ₅	293.05	171.2	-11	-21	-18				
Cocaethylene	318	196.2	-15	-20	-20	82.15	-10	-29	-30
Cocaethylene-D ₅	321.1	199.2	-10	-20	-21				
Cocaine	304.05	182.2	-12	-21	-19	82.15	-12	-30	-14
Cocaine-D ₅	306.9	185.3	-11	-22	-19				
Codeine	300	165.2	-21	-39	-29	215.15	-15	-28	-23
Codeine-D ₅	303.05	165.15	-22	-43	-27				
Cotinine	177.15	80.25	-12	-24	-29	53.2	-29	-45	-19
Cotinine-D ₅	180.15	80.15	-12	-25	-29				
EDDP perchlorate	279.1	235.25	-10	-32	-26	220.2	-18	-45	-24
EDDP-D ₅ perchlorate	282.05	235.25	-11	-32	-25				
Fentanyl	337.1	188.3	-10	-24	-19	105.2	-10	-39	-10
Fentanyl-D ₅	342.1	188.3	-13	-24	-19				
Hydrocodone	300.05	199.2	-11	-30	-20	171.2	-21	-39	-18
Hydrocodone-D ₅	303	199.2	-12	-31	-21				
Hydromorphone	286.05	185.15	-11	-32	-18	157.2	-10	-44	-16
Hydromorphone-D ₅	289.05	185.2	-11	-32	-20				
MDA	180.15	163.2	-12	-15	-18	105.25	-12	-25	-17
MDMA	194.15	163.15	-19	-15	-16	105.15	-20	-24	-10
MDMA-D ₅	199.2	165.25	-13	-15	-17				
Methadone	310.05	105.2	-12	-26	-10	77.2	-12	-53	-13
Methadone-D ₅	312.95	268.3	-15	-16	-29				
Methamphetamine	150.15	119.2	-16	-15	-12	91.2	-16	-21	-16
Methamphetamine-D ₅	155.25	121.25	-15	-16	-22				
Morphine	286.05	165.2	-11	-41	-16	201.2	-11	-28	-21
Morphine-D ₅	289.05	181.15	-11	-35	-19				
Norfentanyl oxalate	232.95	84.2	-16	-19	-30	55.2	-15	-35	-15
Norfentanyl-D ₅ oxalate	238.15	84.25	-15	-20	-29				
Oxycodone	316.05	241.2	-12	-30	-11	212.15	-10	-43	-22
Oxycodone-D ₅	319.1	244.25	-12	-30	-25				
Oxymorphone	302.05	227.2	-10	-28	-23	198.15	-11	-48	-20
Oxymorphone-D ₅	305.05	230.25	-11	-30	-28				

Δ^9 -THC	315.05	193.2	-12	-22	-21	123.15	-11	-33	-12
Δ^9 -THC-D ₉	318.1	196.2	-12	-24	-21				

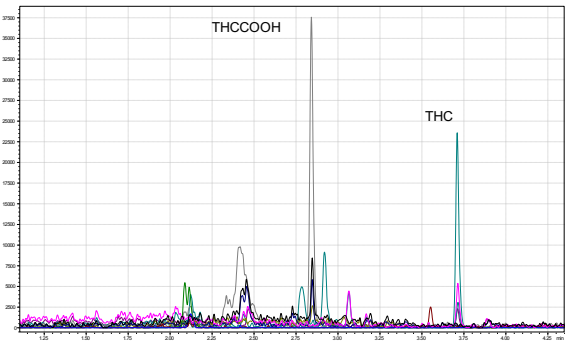
Table 2. Investigation of different ionization modes for target analytes in the explosives project.

Compound	MW	ESI(+)	ESI(-)	DUIS(+)	DUIS(-)
NB	123.06		✓	✓	
1,3 DNB	168.11		✓	✓	
1,3,5 TNB	213.10		✓	✓	
1,3,5 TNT	227.13	✓	✓	✓	✓
2,4 DNT	182.13	✓	✓		
RDX	222.12		✓	✓	✓
HMX	296.15	✓	✓		✓
PETN	316.14	✓	✓		✓

Figure 1. A, Total Ion Chromatogram (TIC) of cotinine (1), morphine (2), oxymorphone (3), hydromorphone (4), codeine (5), amphetamine (6), MDA (7), methamphetamine (8), oxycodone (9), 6-monoacetilmorphine (10), hydrocodone (11), MDMA (12), norfentanyl (13), benzoylecgonine (14), cocaine (15), cocaethylene (16), fentanyl (17), EDDP (18) and methadone (19) in water sample fortified at 20 ng/L. B, Total Ion Chromatogram (TIC) of delta-9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy- tetrahydrocannabinol (THCCOOH) in water sample fortified at 20 ng/L.



A



B

Figure 2. Quantifier (220.8>84.9) and qualifier (220.8>75.2) MRM transitions of ethylglucuronide (EtG) in hair at the limit of quantification (5 pg/mg).

