DEVELOPMENT OF A LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS) METHOD FOR THE ANALYSIS OF PEROXIDE EXPLOSIVE RESIDUES ON BUILDING MATERIALS

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NOMENCLATURE

°C	degrees Celsius
µg	microgram
µg/mL	microgram per milliliter
µL	microliter
AcCN	acetonitrile
ACS	Analytical Chemical Standard
APCI	atmospheric pressure chemical ionization
CIMS	chemical ionization mass spectrometry
CV	coefficient of variation
DC	direct current
EIMS	electron impact mass spectrometry
ESI	electrospray ionization
FDA	Food and Drug Administration
FEL	Forensic Explosives Laboratory
GC/EI/MS	gas chromatography with electron impact mass spectrometry
GC/MS	gas chromatography with mass spectrometry

GC/TEA	gas chromatography with thermal energy analysis
HMTD	hexamethylene triperoxide diamine
HPLC	high performance liquid chromatography
HPLC/MS	High-performance liquid chromatography, combined with mass spectrometry
ID1	identification ratio 1
ID2	identification ratio 2
IR	infrared spectrometry
kV	kilovolts
LC	liquid chromatography
LC	liquid chromatography
LC/MS	High-performance liquid chromatography, combined with mass spectrometry
LLOQ	lower limit of quantitation
LOD	limit of detection
LOQ	limit of quantitation
m/*z*	mass-charge ratio
mL	milliliter

mL/min	milliliter per minute
MRM	Multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NMR	nuclear magnetic resonance
NO	nitric oxide
NO_2	nitrogen dioxide
O_3	ozone
OK	Oklahoma, US
PMT	photomultiplier tube
psi	pound per square inch
Q1	first quadrupole
Q2	second quadrupole, collision cell
Q3	third quadrupole
R value	correlation coefficient (r^2 value)
RF	radiofrequency
SD	standard deviation

SIM	selected ion monitoring
SPME	solid phase microextraction
t-0:28	time zero through time 28, in days
TATP	triacetone triperoxide
TLC	thin layer chromatography
TNT	trinitrotoluene
ULOQ	upper limit of quantitation
Xg	times gravity

Chapter I Introduction

There is an urgent need for analytical methods capable of detecting trace quantities of peroxide explosives (Laine, Roske, & Cheng, 2007). Due to the ease of obtaining precursor chemicals and the simplicity of their manufacture, peroxide explosives are highly attractive for use in acts of terror and crimes involving homemade explosives (Xu, Craats, Kok, & Bruyn, 2004). With the increasing use of peroxide explosives, methods and techniques need to be developed to identify peroxide explosives where they are manufactured, in deactivated bombs, and in bomb postblast residues.

Hydrogen peroxide and a small number of other common chemicals can be used to make peroxide explosives. These well-known, common chemicals are inexpensive to purchase (Cotte-Rodrıguez, Hernandez-Soto, Chen, & Cooks, 2008) and can be found in most pharmacies, including materials like ammonia, acetone, formaldehyde and citric acid (Xu, et al., 2004). Directions for synthesis of many peroxide explosives, including the two most commonly encountered, namely triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD), can easily be found on the Internet (Xu, et al., 2004) and in publications (Davis, 1943).

The destructive power and relative ease of manufacture makes peroxide explosives appealing to terrorists (Widmer, Watson, Schlatter, & Crowson, 2002), as demonstrated in recent events. These events include the discovery of an explosives cache, dubbed a “bomb factory,” filled with the largest quantity of homemade explosives in a single location in the United States history. The cache including eight pounds of HMTD buried in the home’s yard and more HMTD inside the home (Wright & Schone, 2011). Another stockpile of precursors for manufacturing peroxide explosives, as well as the explosives themselves, was discovered belonging to Najibullah Zazi in 2009. Zazi pled guilty to being involved in a conspiracy to bomb the New York City subway system using the peroxide explosive TATP ("Zazi Admits Bomb Plot Against NYC Subways," 2010). A less fortunate example of peroxide explosives in the news is from July of 2005, when suicide bombers entered the London public transit system carrying peroxide-based explosives in homemade explosive devices. The detonation of these peroxide-based explosives resulted in 52 deaths and hundreds of injuries (*Report into the London Terrorist Attacks on 7 July 2005*, 2006).

Researchers have responded to the increasing prevalence of peroxide explosives and the subsequent need for analysis with the development of various techniques to analyze these chemicals. Unlike most other explosives, peroxide explosives do not contain nitro-groups making them un-amenable to some techniques that have been used to analyze other explosive compounds (Crowson & Beardah, 2001). Techniques suitable for analyzing and identifying peroxide explosives include thin layer chromatography (TLC), and high performance liquid chromatography (HPLC) coupled with fluorescence detection. Mass spectrometry under chemical ionization (CIMS) and electron impact

(EIMS) conditions can also be used to identify and analyze peroxide explosives, as well as infrared (IR) spectrometry and nuclear magnetic resonance (NMR) (Xu, et al., 2004).

High-performance liquid chromatography combined with mass spectrometry (LC/MS or HPLC/MS) is being used for the detection of explosives. Many explosive compounds are not amenable to analysis with GC/MS. HPLC is more appropriate for the study of compounds like peroxide explosives that are thermally labile, or not volatile enough for analysis with GC/MS. Currently, along with other methods of analysis, HMTD has been identified using HPLC/MS in atmospheric pressure chemical ionization (APCI) positive mode (Xu, et al., 2004).

The objective of this study was to develop an LC/MS method to detect HMTD at low levels and then use this method to determine the degradation times, and the possibility of recovery of HMTD from different common building materials using LC/MS/MS. Building materials were spiked with a known amount of HMTD standard and periodically sampled to determine decay rates. HMTD was then extracted from each of the different materials and analyzed using LC/MS/MS. The amount of remaining HMTD recovered from the building materials was then assessed to determine the degradation and recovery time of HMTD on each building material to then establish which materials would most likely have detectable residues of the explosive in the event of an investigation. By utilizing LC/MS, the identification of HMTD in building materials could provide information about the location of HMTD manufacture and possibly provide a link to the person or persons manufacturing the highly dangerous explosive.

Chapter II Review of Literature

2.1 Chemical Explosives

An explosive is defined as a material able to generate an explosion by liberating its own energy (Davis, 1943). Chemical explosives are mixtures of compounds, or a single compound, which after some form of initiation undergo an extremely rapid chemical reaction and build a huge amount of gaseous pressure and heat (Saferstein, 2007). Not all explosives generate heat, but almost all explosives produce gas when detonated, building gaseous pressure (Davis, 1943). The sudden buildup of pressure at the bombsite, or the origin of explosion, produces a disruption of the surrounding area. Once no longer contained, the gaseous products created by the explosion expand violently, creating what is known as the blast effect, moving out from the origin of the blast (Saferstein, 2007), and liberating energy (Davis, 1943).

Militaries and industries around the world use explosives for the blast power they provide. Explosives are also manufactured and/or assembled by individuals for the entertainment provided by an explosion. Also, the destructive power provided by

explosives makes them appealing weaponry for use in acts of terrorism (Saferstein, 2007).

Explosives often require a detonator, or a stimulus provided to the explosive, to provoke an explosion. Detonators can include a spark, a shock (Davis, 1943), friction, or heat (Xu, et al., 2004). This stimulus causes the liberation of the explosive's energy in an explosion, but the detonator does not impart energy to the explosion. The stimuli required for the detonation of an explosive, and the manner of the reaction of the explosive after the stimulus, are the basis for classifying explosive materials. Because behaviors of some explosives differ based on the environment in which they are used, and the stimuli used to initiate them, classes can overlap. Explosives are divided into three classifications: high explosives, low explosives, and primary explosives (Davis, 1943).

2.1.1 Classification of Explosives: Low Explosives

Also known as propellants, low explosives do not explode, they burn (Davis, 1943). The speed of deflagration, or the rate at which the explosive decomposes by burning, is relatively slow (Saferstein, 2007). Low explosives cause explosions by creating gas, which then produces the explosive power. Due to diverse rates of gas creation, and gas accumulation being the origin of the blast, rates with which low explosives deliver their energy vary greatly (Davis, 1943). The energy released may be as high as 1,000 meters per second (Saferstein, 2007). Low explosives contain all of the oxygen they require for combustion. Two examples of low explosives are smokeless powder and black powder (Davis, 1943). Like other low explosives, they produce a

propelling action, making them useful in the production of fireworks and ammunition (Saferstein, 2007).

2.1.2 Classification of Explosives: Primary Explosives

Primary explosives are composed of materials, which, under certain conditions explode without the need for an initiator. When subjected to shock or exposed to heat, primary explosives, or initiators, explode, not burn. Some primary explosives are unable to burn, due to their composition. Explosives that fall within this classification differ vastly in the amount of heat they produce as well as their sensitivity to initiate by heat. The amount of shock produced upon explosion, or brisance, is also variable. While some primary explosives have high brisance and can be used to initiate other explosives, other primary explosives are not suitable detonators due to low brisance. Examples of primary explosives include: lead azide, mercury fulminate, and nitrogen sulfide (Davis, 1943).

2.1.3 Classification of Explosives: High Explosives

Unlike primary explosives, high explosives are not detonated readily by shock or heat, but when a primary explosive provides the required shock to initiate the reaction, high explosives detonate (Davis, 1943). The energy released upon detonation is known as the speed of detonation. In high explosives, the speed of detonation is from 1,000 to 8,500 meters per second (Saferstein, 2007). Not all high explosives burn, and no explosive in the category functions through burning. When heated, through the explosives own combustion or by an external factor, high explosives are occasionally initiated and explode. Like primary explosives, high explosives explode if they are contained or uncontained. High explosives are generally more powerful and brisant than primary explosives and high explosives exert a higher mechanical effect on the area

surrounding the explosion than primary explosives. Examples of high explosives include: trinitrotoluene (TNT), dynamite, and nitroglycerin (Davis, 1943).

2.2 Peroxide Explosives

Explosives can be mixtures of compounds or a pure substance, and explosives that are a pure substance can be further divided into inorganic and organic compounds (Cooper & Kurowski, 1966). Within the group of organic compounds are peroxide explosives. Peroxide explosives are within the large chemical group of organic peroxides (Xu, et al., 2004).

Compounds classified as organic peroxides contain one or more of the peroxide functional group (R-O-O-R). This large group is divided into an alkyl and acyl peroxide class as well as a cyclic peroxide class (Widmer, et al., 2002). Alkyl/acyl peroxides have been well explored, but cyclic peroxides have not (Crowson & Beardah, 2001). Some organic peroxide compounds are explosive, and many of the different peroxide explosives fall into the class of cyclic peroxides (Xu, et al., 2004). The lack of information about cyclic peroxides is likely due to the inherent dangers of working with these compounds (Crowson & Beardah, 2001).

It is possible for larger ring sizes; however, cyclic peroxides typically are made up of 5-, 6-, or 9-membered rings. Other properties and the chemistry of cyclic peroxides are not well established. There is also a limited amount of experimental data due to the hazardous nature of the compound (Crowson & Beardah, 2001). Usually, peroxide explosives are unstable and easily detonated. They are very sensitive to friction, impact, heat, and shock (Xu, et al., 2004).

While some cyclic peroxides have a small number of limited uses in industry (Sigman et al., 2009), it is the explosive properties of peroxides that have attracted attention for decades (Urbanski, 1967). Many peroxide explosives initiate when burning, readily passing into detonation in a confined space (Urbanski, 1967). Several peroxide explosives are classified as primary explosives (Crowson & Beardah, 2001; Widmer, et al., 2002) and many are classified as high explosives (Xu, et al., 2004). In spite of their explosive power, virtually no practical application has been found for peroxide explosives because of the inherent dangers associated with working with them (Urbanski, 1967).

2.2.1 Manufacturing Peroxide Explosives

Hydrogen peroxide and a small number of other common chemicals can be used to make peroxide explosives. These well-known, common chemicals can be found in most pharmacies and include things like ammonia, acetone, formaldehyde and citric acid (Xu, et al., 2004). Starting materials are also inexpensive to purchase (Cotte-Rodrıguez, et al., 2008). Directions for synthesis of many peroxide explosives, including the two most commonly encountered, triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD), can easily be found on the Internet (Xu, et al., 2004) and in publications (Davis, 1943). Due to the availability of starting materials and the ease of production it is believed that the incidence of the criminal use of peroxide explosives will increase (Widmer, et al., 2002).

2.2.2 Recent Incidents Involving Peroxide Explosives

Recently, peroxide explosives are indicated in a number of crimes involving drugs, amateur chemist accidents, and acts of terror (Schulte-Ladbeck, Kolla, & Karst,

2003). Peroxide explosives like TATP and HMTD were first prepared in the late nineteenth century, however their use in acts of terror was not documented until the 1980s and 1990s (Xu, et al., 2004). Destructive power and relative ease of manufacture makes peroxide explosives appealing to terrorists, as demonstrated in recent events.

On November 18, 2010 a gardener stepped on something that exploded in the yard of a home, injuring the gardener's arm, chest and eye. The gardener's injuries led to the discovery of an explosives cache belonging to George Jakubec, a Serbian-born man living in Escondido, California. Jakubec's home was filled with the largest quantity of homemade explosives in a single location in United States history. This included eight pounds of HMTD buried in the yard and still more HMTD inside the house. Precursors for explosives, including those for peroxide explosives, were also discovered in the home, which was dubbed a "bomb factory." The house was burned to destroy the explosives safely. Jakubec faces eight federal crimes including possession and manufacturing of explosives (Wright & Schone, 2011).

In 2009 Najibullah Zazi also had a stockpile of precursors for building peroxide explosives, as well as the explosives themselves. Zazi was buying and storing beauty supply products to manufacture TATP. He then produced TATP in a Colorado hotel room and drove it to New York right before the anniversary of the September 11th terrorist attacks. Concerned he had been suspected of something, Zazi disposed of the TATP by flushing it down a toilet in New York. Zazi claims to have made approximately two pounds of TATP. In February of 2010 Zazi pled guilty to being involved in a conspiracy to bomb the New York City subway system using TATP ("Zazi Admits Bomb Plot Against NYC Subways," 2010).

Another large city public transit bombing plot was not thwarted prior to the attacks, and the consequences were devastating. In July of 2005, 52 people were killed and hundreds were injured in a terrorist attack on the London public transit systems. Suicide bombers entered the public transit system each carrying a rucksack with a homemade organic peroxide-based explosive device. Each bomber then detonated the explosive inside their rucksack at a different location in the extensive public transit system. Other homemade explosive devices containing peroxide-based explosives were also found in a car at the Luton railway station (*Report into the London Terrorist Attacks on 7 July 2005*, 2006).

2.3 Hexamethylene triperoxide diamine (HMTD)

A cyclic organic peroxide explosive that has been partially described comparatively recently is hexamethylene triperoxide diamine (HMTD). HMTD was first synthesized in 1885 by Legler, but due to its instability its properties were not explored fully (Cotte-Rodrıguez, et al., 2008). HMTD is a white solid, and Baeyer and Villiger proposed its most plausible cyclic ring structure in 1900. Other structures have been proposed, up until recent use of structural characterization techniques like nuclear magnetic resonance spectroscopy and electron ionization mass spectrometry, the structure of HMTD was unclear (Crowson & Beardah, 2001). The structure of HMTD is shown in Figure 1.

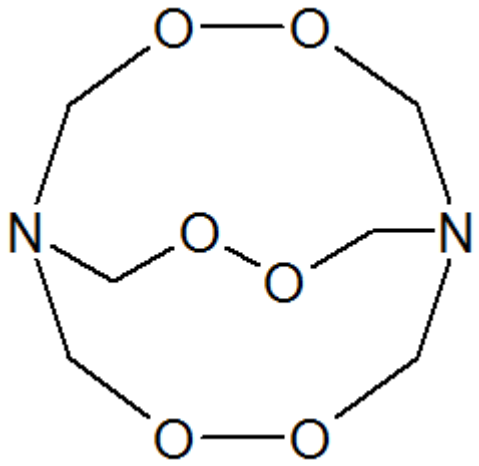

Figure 1. Chemical structure of HMTD

Instruction for the synthesis of many peroxide explosives, including HMTD, can easily be found on the Internet (Xu, et al., 2004), and they are published in books like The Chemistry of Power and Explosives by Tenney Davis (1943). Special equipment is unnecessary and the common chemicals required for production of HMTD are readily available (Widmer, et al., 2002) and inexpensive (Cotte-Rodrıguez, et al., 2008). HMTD can be prepared by combining hydrogen peroxide with hexamethylenetetramine in presence of citric acid. The presence of citric acid promotes the reaction by combining with the ammonia liberated by the combination of hydrogen peroxide with hexamethylenetetramine (Davis, 1943). This forces the reaction equilibration to the formation of HMTD, thereby increasing the yield.

Classified as a primary explosive, HMTD is especially susceptible to initiation by friction, impact, and electrical discharge (Crowson & Beardah, 2001). Due to the blast power and high brisance of HMTD it is effective as initiator for the detonation of other explosives. HMTD proves to be a powerful explosive, however it is unstable and chemically reactive, making it dangerous, unpredictable, and of little practical use (Davis,

1943). The ease of production, and availability of production materials make , HMTD readily manufactured by amateurs. Since no industrial or military uses have been identified, HMTD has been identified as an explosive used for unlawful circumstances (Crowson & Beardah, 2001).

2.4 Explosive Manufacture and Detection

Identification of post blast explosion residues, and of bulk explosive, can provide information about the type of explosive that was used in a bombing or manufactured for a bomb (Saferstein, 2007). These types of analysis done on samples that are collected directly from ambient surfaces (Cotte-Rodrıguez, et al., 2008). Explosives can be collected in airborne samples as well. Drugs of abuse, like methamphetamine, can also be found in air samples (Gordin & Amirav, 2000).

Methamphetamine use or production can also be analyzed through the collection of samples taken from ambient surfaces. When methamphetamine is manufactured or smoked, the drug is distributed into the surrounding area, onto and into building materials. Swabs collected from these materials show measurable amounts of methamphetamine. Remediation is required to decrease the possibility of exposure to methamphetamine in areas where methamphetamine has been manufactured, or cooked (*Voluntary Guidlines for Methamphetamine Laboratory Cleanup*, 2009).

Depending on the area where methamphetamine is produced, various materials are contaminated with methamphetamine. Methamphetamine manufactured in a building has the potential to contaminate all of the building materials in the vicinity. Different remediation procedures are required with different building materials. Carpets and materials that are porous, like unfinished wood, or absorbent, like drywall, are removed

and replaced when contaminated with methamphetamine. Removal is required because the methamphetamine has gotten down into these building materials and cleaning is not sufficient to remove the contaminant. Concrete contaminated with methamphetamine should be washed and all cleaning liquids removed and properly disposed of. If the concrete is sampled, even after cleaning, methamphetamine may still be detected. Concrete may then have to be removed, post-cleaning, because the methamphetamine gets into the concrete and cannot be removed (*Voluntary Guidlines for Methamphetamine Laboratory Cleanup*, 2009).

Due to the fact that methamphetamine and explosives can both become airborne (Gordin & Amirav, 2000), and that explosives can be recovered through swabbing ambient surfaces (Cotte-Rodrıguez, et al., 2008) like methamphetamine (*Voluntary Guidlines for Methamphetamine Laboratory Cleanup*, 2009), it is believed that peroxide explosives will distribute to nearby building materials during the manufacturing process. The explosives distributed to the building materials could also be collected and analyzed, as is the case with methamphetamine "cooks."

2.5 Current Analysis of Explosives Including Peroxide Explosives

In the field of forensic science, the development of analytical methods capable of detecting explosives in trace quantities has become increasingly important (Crowson & Beardah, 2001). This is particularly true of peroxide explosives, due to the ease of manufacture, and popularity for use in acts of terror and other criminal activity (Xu, et al., 2004).

There are many techniques that are particularly suitable for analysis and identification of trace amounts of explosives. For analyzing and identifying peroxide

explosives, techniques include thin layer chromatography (TLC), and high performance liquid chromatography (HPLC) coupled with fluorescence. Mass spectrometry under chemical ionization (CIMS) and electron impact (EIMS) conditions can also be used to identify and analyze peroxide explosives, as well as, infrared (IR) spectrometry, and nuclear magnetic resonance (NMR) (Xu, et al., 2004).

In general, for the analysis of small amounts of explosives the two most useful and sensitive techniques are gas chromatography with thermal energy analysis (GC/TEA), and gas chromatography with mass spectrometry (GC/MS). Both GC/TEA and GC/MS can detect trace amounts of explosives in the low nanogram range, though GC/TEA is not nearly as versatile as GC/MS. (Crowson & Beardah, 2001).

Thermal Energy Analysis (TEA) relies on a highly selective mechanism for the detection of specific nitro-containing (NO_2) compounds and many explosives contain nitro-groups. When nitro-containing explosives are burned nitric oxide (NO) is produced. The thermal energy analyzer takes the NO produced and reacts it with ozone (O_3) forming electronically excited nitrogen dioxide (NO_2). This excited product then relaxes, producing a red emission that can be detected by a photomultiplier tube (PMT) for detection. Because the mechanism of action requires nitro-containing compounds, GC/TEA cannot be used to detect organic peroxides, which do not normally contain nitro-groups (Crowson & Beardah, 2001).

For the analysis of explosives, GC/MS is limited to non-thermally labile, volatile explosives that can be eluted from a GC column. However, even with these limitations the number and types of explosives that can be successfully analyzed by GC/MS is extensive, including most organic peroxide explosives (Crowson & Beardah, 2001).

GC/MS analysis of HMTD was first done in 1981, using quantities of HMTD above trace amounts and using both chemical and electron ionization(Crowson & Beardah, 2001). A different group, in 1984, carried out this same study. Both studies produced relatively simple mass spectra with similar peaks. A GC/EI/MS study done in the Forensic Explosives Laboratory (FEL) showed similar results to the prior two studies. However, this time peaks were obtained using levels of HMTD in trace quantities. During repeated analysis of HMTD, in a number of different polar GC capillary columns, the solid phase became activated after an extremely short length of time. Activation of the solid phase within the columns resulted in the elution of asymmetrical chromatographic peaks that were very broad and thus complicated analysis (Crowson & Beardah, 2001).

High-performance liquid chromatography, combined with mass spectrometry (LC/MS or HPLC/MS) is being used for the detection of some explosives. Many explosive compounds are too thermally labile, or not volatile enough, for analysis with GC/MS. HPLC is more appropriate for the study of such compounds which include compounds like peroxide explosives. Currently, along with other methods of analysis, HMTD has been identified and quantified using HPLC/MS in atmospheric pressure chemical ionization (APCI) in positive mode (Xu, et al., 2004).

2.6 Liquid Chromatography with Tandem Mass Spectrometry

Liquid chromatography with tandem mass spectrometry (LC/MS/MS) uses the ability of LC to physically separate the compounds with the detection power of tandem MS to ionize and identify ions based on their mass-charge ratio (*m/z*). LC is a widely used separation technique due to its flexibility, sensitivity, ability to separate thermally

sensitive and nonvolatile compounds, and the ability to automate much of the process. Combining the LC with tandem MS provides "an ideal merger of separation and detection" (Skoog, Holler, & Crouch, 2007).

2.6.1 Liquid Chromatography

Liquid chromatography (LC) separates a mixture into its components based on their distribution between a moving liquid phase and the column filled with solid particles (Saferstein, 2007). The liquid mobile phase consists of organic solvent and water (Skoog, et al., 2007). Components with greater affinity for the liquid mobile phase travel through the solid particles in the column more quickly than the components with greater affinity for the column. Depending on the length of the interaction with the solid particles in the column, components that make up the sample are retarded to differing degrees, effecting separation of the mixture (Saferstein, 2007).

2.6.2 Mass Spectrometry

In a LC/MS/MS system, after the separation of sample components by LC, analysis of the analyte is accomplished using mass spectrometry (MS). The MS is directly connected to the LC system. From the LC, components enter a high-vacuum chamber where electrons collide with molecules, creating ions. These ions fragment and pass through an electrical or magnetic field where they are separated by mass (Saferstein, 2007). The separated ions are then sorted, detected, and identified based on their mass-charge ratio (*m/z*). They are then used to identify and quantify the analyte (Skoog, et al., 2007).

2.6.2.1 Ionization

Liquid is added to a sample during the LC process to push the sample through the

column, providing a mobile phase for chromatography. However, before a sample enters the MS to be analyzed, that large volume of liquid that must be removed through evaporation and ionization. Molecules are ionized outside of the MS, which helps to isolate and concentrate them as they are drawn into the MS. Inside the MS the ions can then be broken down and analyzed. Many techniques can be used for ionization outside of the MS, at atmospheric pressure. Two main types of ionization are used in LC-MS systems: atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) (Cody, 2006).

In ESI, effluent from the LC system is fed through a small capillary that has a voltage applied to it by the ESI source (Cody, 2006). A sheath gas surrounds the capillary tube. The sheath gas causes the effluent in the capillary tube to be nebulized, creating highly charged droplets in a fine spray that enter into mass spectrometer's region under vacuum. The applied voltage polarity determines whether the nebulized droplets will be negatively or positively charged (Politi, Groppi, & Polettini, 2006). By passing a gas through the chamber (Cody, 2006), with additional heat in the source the solvent evaporates, causing the droplets to shrink, subsequently increasing the charge concentration in the droplets (Politi, et al., 2006). The Rayleigh instability limit is ultimately reached in the small droplets (Cody, 2006) and the cohesive forces are exceeded by the repulsive forces among charges in the droplet (Politi, et al., 2006). The droplets then break apart and a charge is imparted to the molecules within. This ejects the ions into the gas phase. The ions then move, in the gas phase, into the mass analyzer (Politi, et al., 2006).

Atmospheric pressure chemical ionization (APCI) also removes the liquid from

the mobile phase of the liquid chromatography to allow for ionization and analysis by MS. With APCI, ionization does not occur in solution as in ESI, but rather ionization is done in the gas phase (Politi, et al., 2006). With APCI, effluent is forced through a capillary tube (Cody, 2006). Within the capillary tube the effluent is heated to between 400-500°C and a coaxial flow of nitrogen acts to nebulize the liquid (Politi, et al., 2006). Near to the end of the capillary tube is a needle with an applied high voltage called the corona discharge needle (2-5 kV) (Politi, et al., 2006). The corona discharge needle subjects the fine spray of liquid to a high voltage as it passes out of the capillary tube. The high voltage supplied by the corona discharge needle then creates ions within the sheath gas and the molecules in the solvent (Cody, 2006). Through the process of chemical ionization, the charged ions react with molecules in the analyte to form ions (Politi, et al., 2006). Analyte ions can then pass into the mass spectrometer to be analyzed (Cody, 2006).

2.6.2.2 Ion Separation

For analysis, ion separation must occur in the mass analyzer. There are many types of mass analyzers. Some mass analyzers include: ion trap mass spectrometer, linear ion trap mass spectrometer, time of flight mass spectrometer, and quadrupole mass spectrometer.

Most commonly, the quadrupole mass spectrometer is used for analysis. A quadrupole consists of four parallel poles, forming a square arrangement. Either radiofrequency (RF), or direct current (DC) is applied to each rod. Adjacent rods have an electrical current of the opposite charge, generating an electromagnetic field. This generated electromagnetic field acts almost like a sieve and based on a predetermined and

set mass to charge ratio (*m*/*z)*, the quadrupole determines which ions pass to the detector. Quadrupole mass analyzers can be operated in selected ion monitoring (SIM) mode, looking for a specified mass, or in scan mode, looking at the range ions present in the sample. Much more sensitive than scan mode, SIM mode focuses on specific ions (Politi, et al., 2006).

Quadrupole mass analyzers may also be linked together, called a triple quadrupole mass analyzer or tandem mass spectrometry (MS/MS) to analyze ions. In tandem mass spectrometry, generally, the first quadrupole (Q1) is used to remove ions that do not correspond to the *m*/*z* of the ion or ions of interest. The parent ion, or precursor ion or ion of interest, are allowed to pass into the second quadrupole (Q2) where an encounter with collision gas causes ion fragmentation. Fragmentation of precursor ions produces daughter ions, or product ions. These daughter ions then pass into the third quadrupole (Q3) where specific ions are separated out, which then pass through to the detector (Cody, 2006).

A triple quadrupole mass analyzer (MS/MS) can be operated different ways. By allowing either quadrupole (Q1 or Q3) to act as a filter, and let the other quadrupole be passive, a triple quadrupole mass analyzer can resemble a single quadrupole instrument. Settings on the instrument can also be changed to perform a product ion scan, with a precursor ion selected in Q1, product ions produced in Q2, and product ions scanned in Q3. This produces a product ion spectrum. Only two alterations are mentioned here, however many additional alterations and methods can also be performed with a triple quadrupole instrument (Politi, et al., 2006).

2.6.2.3 Ion Detection

Ions separated by the mass analyzer are converted into a measurable electronic signal by the detector of the mass spectrometer. Generally, detection of ions is done using an electron multiplier. Ions hit the surface of a dynode electrode and are converted to electrons inside the electron multiplier. The electrons emitted from the quadrupole create a current, the detector records this induced current. Using dynodes linked into a series, the signal of the electrons can be amplified. One dynode multiply the electrons produced from the previous dynode in the series, thus amplifying the signal. A continuous dynode, in a horn-shape, may also be used to amplify electrons. Amplification in the continuous dynode is due to the electrons colliding repeatedly with the internal surface of the horn-shaped detector (Politi, et al., 2006).

The amplified electronic signal from either type of detector is conveyed to a controller, usually a computer, where the mass to charge ratio (*m*/*z)* of the ion detected can be determined. This determination is based on the quadrupole settings or time of flight at the time of detection (Politi, et al., 2006).

2.7 Method Development

Several steps are involved in successful development and optimization of a quantitative LC- MS/MS method. To begin the process of method development, first, a problem must be defined. Defining a problem involves; determining whether breakdown compounds will be examined with the progenitor compound, the composition of the matrix to be analyzed, the sample limitations, the lower limit of quantitation, linear range, and the many other factors associated with approaching the problem. After definition of a problem, a literature review for related materials and analyte information must be

executed. A literature search should also include the selection of a suitable internal standard to be used in the analysis. In LC-MS methods, there are three types of internal standards that can be used. A structural analogue of the analyte, the intended analyte labeled with several stable isotopes may be used, or any other chemical, as an internal standard. Radio-labeled internal standards are most often used in LC-MS method because labeled internal standards are chemically identical to the analyte (Taylor, 2006).

Following definition of a problem, a literature review, and the selection of a suitable internal standard, mass spectrometer conditions must be selected and optimized. To move the analyte of interest from the liquid mobile phase of chromatography to the gas phase required for analysis in the mass spectrometer, the proper ionization mode (ESI or APCI) must be selected (Taylor, 2006).

Fragment ions need to be chosen to examine and optimize the collision energy. Optimization of collision energies can be done by monitoring the mass transitions of the infused compound or compounds of interest (Taylor, 2006).

Following the optimization of mass spectrometer conditions for the analyte ions of interest, the source conditions are modified in an effort to increase sensitivity. Modifications intended to increase sensitivity include the optimization of temperature, the gas flows, the ionization parameters, and the ionization source voltage (Taylor, 2006).

Following the optimization of mass spectrometer conditions, the chromatography step must be examined. The most suitable type of liquid chromatography column must be selected to obtain the optimal selectivity and sensitivity for the analyte with the optimization of the flow rates and the mobile phase (Taylor, 2006).

Sample preparation is often the next step. In order to get the sample into a form

that can be analyzed by the instrument, sample preparation is usually required. Sample preparation strategies should be developed with the intent of retaining the largest amount of the intended analyte as possible. Sometimes, in order to get a sample into a form that can be analyzed, a specific extraction procedure is required. Though there are many extraction methods, common extraction methods include solid phase extraction, liquid-liquid extraction, and sample dilution with protein precipitation (Taylor, 2006).

After method development, the method must go through a validation process to confirm that it is sensitive, precise, selective, reproducible, and accurate.

2.8. Method Validation

After a method has been developed for the LC/MS/MS, is established a method validation is required. Method validation represents a collection of tests that must be performed to determine if the newly developed method can be applied in practice to the intended target to produce and collect the intended data (Zhou, Song, Tang, & Naidong, 2005). The main parameters evaluated in method validation are accuracy, precision, and selectivity. Stability, sensitivity, and reproducibility are also examined during method validation in order to determine the reliability of the method (*Guidance for Industry: Bioanalytical Method Validation* 2001).

2.8.1. Accuracy of a Method

Accuracy is the most critical aspect of method validation. Accuracy, or trueness, is typically the first parameter evaluated during method validation. The difference between a sample with a known value and the value of an experimental sample is the accuracy.

The accuracy of a method can be determined in different ways. One approach is

comparing results from an existing validated method to the results obtained using the newly developed method. Another way to determine accuracy is analyzing both a reference sample with a known concentration and an experimental sample, and then comparing the calculated value of the reference sample to the calculated value of the experimental sample (Shabir, 2003). The Food and Drug Administration (FDA) states that at the lowest concentration of a sample can be quantified above background noise, or the lower limit of quantitation (LLOQ), for an unknown should be within 20% of the known value for the reference sample (*Guidance for Industry: Bioanalytical Method Validation* 2001). At concentrations above the LLOQ, the FDA states the calculated value of the experimental sample should be within 15% of the calculated value of the references sample (*Guidance for Industry: Bioanalytical Method Validation* 2001).

2.8.2. Precision of a Method

The second parameter in method validation is precision. By repeatedly analyzing the same sample, at the same concentration, and determining the clustering of the each of the quantitative values, one can determine precision. Generally, at minimum, three concentrations should be run in triplicate to determine precision. The samples tested should represent a low concentration, a medium concentration, and a high concentration or analyte (Araujo, 2009). While only three concentrations of analyte are required, to determine precision, a standard curve developed using samples with five different concentrations, is recommended. A standard curve developed using five different sample concentrations with five points on the curve, yields a measure of precision commonly known as the five-point standard curve (Stöckl, D'Hondt, & Thienpont, 2009). The FDA states that at the LLOQ, precision should be within 20% of the coefficient of variation

(CV) of the reference sample, and at concentrations above the LLOQ, the FDA maintains that precision should be within 15% of the coefficient of variation (CV) (*Guidance for Industry: Bioanalytical Method Validation* 2001). To validate reproducibility for a method, the determination of precision is vital. Precision can be determined for both the tests that have been conducted on different days, using the interday differences, or the tests that have been conducted on the same day, using intraday differences (Peters, 2006).

2.8.3. Selectivity of a Method

The third parameter that is part of method validation is selectivity. The ability to detect an intended analyte without interference from other components present in a matrix is selectivity. Selectivity can be investigated by analyzing samples with none of the intended analyte and determining if there is interference, or matrix effects. Specificity for the intended analyte, without interference from the matrix compounds represents maximal selectivity.

When a method said to be specific, it has 0% interference from matrix effects, or 100% selectivity. Specificity is an exact term. Selectivity, however, is not an exact expression; it can be expressed in qualifying modifiers, using terms like low, high, good, poor, etc. (Araujo, 2009).

2.8.3.1. Matrix Effects within a Sample

The presence of co-eluting substances in a sample causes alterations in the ionization efficiency, producing matrix effects. The exact mechanisms underlying matrix effects are unknown; however, they are thought to be caused by competition between an undetected co-eluting component from the matrix and the intended analyte in the matrix. Matrix effects must be evaluated during any LC/MS/MS method validation because they

may interfere with or complicate quantitation of analyte in a given sample. Matrix effects could severely alter the accuracy, precision, selectivity, and sensitivity of the data being collected. An increase in the analyte ion formation is known as ion enhancement where as a decrease in formation of the analyte ions in known as ion suppression. Enhancement and/or suppression by matrix effects cause inaccurate quantitation of the intended analyte. (Taylor, 2005).

Matrix effects can be detected using postcolumn infusion or postextraction addition techniques, with postcolumn infusion being the more robust technique. Using the postcolumn infusion technique, a syringe pump and HPLC system are both coupled to the mass spectrometer for the same run. Using the syringe pump, analyte is infused into the flow of eluent from the LC, with the analyte being added before the mass spectrometer ionization source, but after the chromatographic column. This permits the response of the analyte to be examined, allowing for the determination of matrix effects over the entirety of the run (Taylor, 2005).

With the postextraction addition technique, samples with postextraction addition of the analyte are compared to pure samples that have been prepared in the mobile phase. Taking the difference between the response of the postextraction sample and the pure sample, then dividing this sum by the response of the pure sample can test for possible matrix effect. Unlike the postcolumn infusion technique, the postextraction addition technique evaluates matrix effects only at the intended analyte's point of elution. (Taylor, 2005).

Elimination or minimization of matrix effects can be achieved through or improved chromatographic separation or modifications of extraction technique used,.

Matrix effects are most frequently observed within a run's solvent front. By modifying the chromatographic separation, and retaining the analyte on the column, the longer period of time reduces matrix effect. Also, several reports have been published showing electrospray ionization (ESI) has a greater chance of contributing to matrix effects than does atmospheric pressure chemical ionization (APCI). This indicates that switching the source may also reduce matrix effects (Taylor, 2005).

Extraction techniques like liquid-liquid extraction or solid phase extraction produce fewer matrix effects compared to an extraction like protein precipitation or a "dilute and shoot" method of sample preparation. This is due to some extraction methods producing a sample that contains fewer components that may contribute to matrix effects. The interfering compound determines what matrix effects are observed. For example, polar compounds have greater ion suppression than less polar or nonpolar compounds, therefore less polar and nonpolar compounds produce fewer matrix effects (Taylor, 2005).

2.8.4. Sensitivity of a Method

Sensitivity refers to the modification of the response of a measuring device over the corresponding alteration in the stimulus, or the standard curve slope. Sensitivity describes the lowest concentration of an analyte that a method can detect (limit of detection (LOD)) or quantitate (limit of quantitation, LOQ). LOQ is sometimes written as the lower LOQ, or LLOQ. A method is determined to be sensitive if the analyte can undergo a minor alteration in its concentration and this concentration change then results in the instrumentation displaying a detectable adjustment in the measured signal produced (Taverniers, Loose, & Bockstaele, 2004).

2.8.5. Reproducibility and Stability of a Method

The fifth parameter in method validation, reproducibility, is the capability of replicating comparable results over time. By definition, reproducibility is the precision of a method after varying factors over some defined period of time or number of tests (Araujo, 2009). Reproducibility is not only applied to a method with a single user on a single instrument, the precision of results obtained between different laboratories using the same method is also connected to reproducibility (Peters, 2006).

The last parameter for method validation is stability. Knowledge of appropriate storage conditions for a sample to prevent degradation is important for analysis. If there is sample degradation, breakdown products in the sample may produce results that differ from those seen with an un-degraded sample. Stability is the capability of maintaining the intended analyte, stored in the matrix, over a set interval of time (Peters, 2006).

2.8.6. Linearity of the Data

Linearity is the straight-line relationship between the analytical concentration and the experimental response value. When validating a method, linearity should be assessed as well (Araujo, 2009). Based on the standard curve, which ideally is a straight-line relationship between the analytical concentration and the experimental response value, a correlation coefficient (r^2 value) can be determined. With perfect linearity, the r^2 value will be equal to 1. The standard curve, and the corresponding calculated r^2 value, should be reproducible among runs; both within runs from a single day and among runs that are produced from day to day (Peters, 2006).

2.8.7. Carryover from Previous Analysis of an Analyte

When a sample is analyzed, a small amount may sometimes be retained on the column or elsewhere within the LC system, this is known as carryover. Carryover can cause contamination of new samples that are subsequently analyzed. For any given sample, the highest concentration of analyte that can be determined quantitatively, with accuracy and precision, is the upper limit of quantitation (ULOQ). Following an injection of a sample with a concentration equal to the ULOQ, a sample that contains no analyte, a blank sample, is injected into the LC/MS/MS to test for carryover (Clouser-Roche, Johnson, Fast, & Tang, 2008). A peak seen after injection of a blank sample indicates that an analyte from a prior injection has not been fully eluted from the instrument and carryover is occurring. Carryover can occur from the sample just prior to the blank run, or from any of the previously run samples. If carryover is seen, in order for the run not to be counted as a failure, the peak produced by the analyte eluted with a blank sample must have an area that is less than 20% of the determined lower limit of quantitation (LLOQ) for that analyte (Clouser-Roche, et al., 2008). It is important to note that the limit of detection (LOD) is the lowest detectable concentration of an analyte in a sample, whereas the upper and lower limit of quantitation (ULOQ and LLOQ) are the upper and lower limit values at which the concentration of the sample can be quantitated accurately (Shabir, 2003).

It is critical to ensure the reliability of results by validating all new methods that are to be used in any laboratory. High quality sample analysis and data can be obtained reliably using a new method after proper validation of the newly created method (Peters, 2006).

Chapter III Methods

Identification of HMTD in a building or on building materials can provide information about where HMTD was manufactured for use in an explosive. The objective of this study was to determine the degradation time of HMTD from different common building materials using developed extraction methods in conjunction with analysis using a method developed for LC/MS/MS. Building materials were spiked with a known amount of HMTD standard and allowed to rest, exposed to a room environment for differing amounts of time. HMTD was then extracted from each of the different building materials and analyzed using LC/MS/MS. Remaining HMTD extracted from each of the building materials was then calculated to determine the degradation of HMTD on different common building materials.

3.1 Instrumentation

All samples were analyzed with a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan). This system consists of the following components: a system controller, (CBM-20A); a solvent delivery unit, (LC-20AD); an auto-sampler, (SIL-20AC;) and a

column oven, (CTO-20AC). For LC separation, a Restek biphenyl 50 mm x 2.1 mm column was used (Restek, Bellefonte, PA).

An Applied Biosystems 4000 Q-Trap LC/MS/MS System (Applied Biosystems, Foster City, CA) was coupled to the Shimadzu HPLC system. The mass spectrometer was equipped with a Turbo V™ electrospray ionization source and a Harvard Apparatus syringe pump (Holliston, MA). The source of instrument gases was a NitroGen N300DR nitrogen generator (Peak Scientific Instruments Ltd, Paisley, United Kingdom). Analyst® 1.5 Software was used for data analysis and to control the instrument.

3.2 Materials

Methanol (VWR International, West Chester, PA) and 98% formic acid (EMD Chemicals, Gibbstown, NJ) were both Analytical Chemical Standard (ACS) grade; and acetonitrile (OmniSolv, EM Science, Gibbstown, NJ) was HPLC grade. Ammonium formate, 99%, was obtained from Alfa Aesar (Alfa Aesar, Ward Hill, MA). HMTD analytical standard (100 µg/mL) was purchased from AccuStandard (AccuStandard Corporation, New Haven, CT). Filters-used: costar® 8170, Spin-X® Centrifuge Tube Filter, 0.45 µm Nylon filter with a 2.0 mL Polypropylene Tube.

3.2.1 Building Material Specifications

- Carpet: Shaw® carpeting, Style/Color: Full Throttle Suede, Description: 100% Polyester, Product number: 710HD00720. Acquired from The Home Depot (in Tulsa, OK). Cut into .5 inch X .5 inch squares.
- Wood: woodgrain millwork®, product name and number: Lattice 267, Pine. Purchased from The Home Depot (in Tulsa, OK). Cut into .5 inch X .5 inch squares.

- Concrete: DTS Pavestone Company, 12” Square Stepping Stone – Gray, Drycast Concrete, Product number: 71200. Purchased from The Home Depot (in Tulsa, OK). Cut into .5 inch X .5 inch squares by a marble and granite fabricating company.
- Drywall: SHEETROCK Brand Gypsum Patching Panel Drywall, 2 ft. x 2 ft. x .5 in., Model number: 1441133. Purchased from The Home Depot (in Tulsa, OK). Cut into .5 inch X .5 inch squares.

3.3 Preparation of Standards

HMTD analytical standard (100 µg/mL) was used neat during the duration of the study.

3.4 Method Development

The method presented here is the final optimized LC and MS/MS parameters created through method development. Many adjustments had to be made to produce the final method used in the study. The following are examples of only two types of modifications made during the long, arduous, method development process.

The final method culminates in the use of an aqueous mobile phase (comprised of Eluent A: 0.2% Formic acid, 0.2 % Ammonium formate in water) as well as an organic mobile phase (comprised of Eluent B: 0.2% Formic acid, 0.2 % Ammonium formate in methanol) to move the analyte through the LC system. This set of eluents represents only the final eluents determined most fitting for use in this method. Many other sets of eluents were attempted in this study including a pure water aqueous mobile phase and a pure methanol organic mobile phase. Another technique using the eluents, called postcolumn infusion, was also attempted. Peroxide explosives have volatile oxygen -

oxygen bonds and addition of acids into a solution with peroxide explosives would break this bond, forming a compound other than the intended analyte, HMTD. Acids, like very weak solutions of formic acid, are used to help create ions prior to the MS/MS. In this study, postcolumn infusion of Eluent A (0.2% Formic acid, 0.2 % Ammonium formate in water) was attempted in an effort to achieve better ionization of the HMTD when using eluents that provided no ionization. The postcolumn infusion, while it did work, was slow, could not be fully automated, and was found to be less effective than the final eluents eventually used.

Not only the were the sets of eluents used in the method modified many times through method development, the ratio in which they were used was modified as well. Through method development it was shown that HMTD requires a high volume of the aqueous mobile phase to elute from the column fully or within a within a reasonable run time. The volume of aqueous eluent pushing the analyte through the LC system must be balanced by the appropriate ratio of organic eluent, however, or the HMTD elutes in the solvent front. Many eluent ratios were attempted in this method development, ranging from 20% aqueous to 100% aqueous before determining that 94% aqueous eluent provided separation of the solution, keeping HMTD out of the solvent front, and eluted the HMTD solution relatively quickly while provide complete elution of HMTD from the LC system.

3.4.1 Method

A 50 µg/mL HMTD solution was used for optimization of the mass spectrometer. At a flow rate of 60 µL/min, the dilution of HMTD standard was infused using a syringe

pump, directly into the mass spectrometer through a Turbo V™ source in electrospray configuration.

In the first quadrupole mass analyzer, a single quadrupole scan was performed to determine the presence of HMTD at a mass-charge ratio (*m*/*z)* of 209.12. After determining the HMTD precursor ion of 209.12 *m/z* a product ion scan was then performed to analyze all products of the HMTD precursor ion. The first quadrupole was fixed at 209.12 *m*/*z* while the third quadrupole was set to scan for products created in the collision cell (Q2) over a defined mass range. Multiple product ions were seen, however three product ions (145.10, 120.00, and 179.15) were selected for inclusion based on selectivity and sensitivity.

Progression of each of the monitored ions representing HMTD (209.12, 145.10, and 179.15), and the parameters that affect the progression of these ions, through the mass spectrometer were then optimized to increase sensitivity.

3.4.2 LC Parameters

An aqueous mobile phase (Eluent A: 0.2% Formic acid, 0.2 % Ammonium formate in water) as well as an organic mobile phase (Eluent B: 0.2% Formic acid, 0.2 % Ammonium formate in methanol) was used to carry the sample through the HPLC column. The total flow rate of 0.5 ml/min was comprised of 94% aqueous mobile phase (Eluent A) for the duration of the 10 minute run. The sample was injected in a volume of 10 µl.

3.4.3 Source Parameters

The source parameters were as follows:

- Curtain Gas: 20.0 psi
- Gas 1: 40.0 psi
- Gas 2: 40.0 psi
- Temperature: 350.0°C
- Entrance Potential: 10 volts
- Ionspray Voltage: 4000 volts

3.4.4 MS Parameters

The mass spectrometer was run in positive mode. Multiple reaction monitoring (MRM) was used to monitor multiple user defined ion fragments. MRM parameters are shown in Table 1.

Table 1. HMTD MS Parameters

Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)	CXP (volts)
209.12	145.10	36.00	9.00	6.00
209.12	120.00	36.00	13.00	6.00
209.12	179.15	36.00	7.00	8.00

Analyst® software and Excel software were used to generate best fit lines of the data for each of the different building materials and determine quantitative values of the HMTD concentrations in the extractions from the samples of carpet, wood, drywall and concrete extractions.

3.5 Preparation of Samples

3.5.1 Multiple Low Level Extractions for Determination of LOD and LOQ

A spike of 0.2, 0.3, or 0.4 µg of HMTD analytical standard (100 µg/mL) from AccuStandard was applied to each building material (carpet, wood, drywall, and concrete), and allowed to dry. HMTD was then extracted using the method developed for the individual type of building material (described in Section 3.6) and analyzed using the LC/MS/MS. For example, using carpet; three individual pieces of carpet were each spiked with an aliquot of 2, 3, or 4 µL of HMTD analytical standard (100 µg/mL). The five spiked carpet samples were then allowed to dry, were extracted for HMTD, and analyzed.

Each building material (carpet, wood, drywall, and concrete) was spiked as follows:

- Carpet: aliquot on the pile of the carpet, the side of the carpet normally facing up into a carpeted room, on the carpet fiber
- Wood: aliquot on the side of the wood that is finished and factory sealed (with pencil, mark the side of the wood without the spike to differentiate it from the spiked side)
- Drywall: place the aliquot on the side of the drywall that is finished to be facing into a room with drywall, the side with the thinner paper backing (with pencil mark, the side without the spike to differentiate it from the spiked side if needed)
- Concrete: aliquot onto the side of the concrete that is factory finished, not cut (with pencil, mark the side without the spike to differentiate the non-

spiked end from the spiked end)

3.5.2 Blank Samples for the Determination of LOD and LOQ

A spike of 50 µL of acetonitrile was applied to 12 individual pieces of each building material (carpet, wood, drywall, and concrete), on the unmarked side (as described in Section 3.5.1.) and allowed to dry. The acetonitrile spiked building materials were then extracted using the method developed for the individual type of building material (described in Section 3.6) and analyzed using the LC/MS/MS. For example, using carpet; 12 individual pieces of carpet were each spiked with an aliquot of 50 µL of acetonitrile. The 12 spiked carpet samples were then allowed to dry, were extracted, and were analyzed.

3.5.3 Recovery Study of HMTD Extractions From Building Materials

Three solutions of 25 µL of HMTD analytical standard (100 µg/mL) from AccuStandard and 225 µL of methanol were created and analyzed using the LC/MS/MS.

Building material extractions were made as follows: three individual pieces of each building material were spiked with 25 µL of HMTD analytical standard (100 µg/mL) and allowed to dry. The spiked building materials were then extracted using the method developed for the individual type of building material (described in Section 3.6) and analyzed using the LC/MS/MS.

3.5.4 Degradation Study of HMTD

Twelve pieces of each building material (carpet, wood, drywall, and concrete) were laid out, not touching anything. A spike of 50 µL of HMTD analytical standard (100 µg/mL) from AccuStandard was then applied to each building material on the

unmarked side as above in Section 3.5.1.

Six pieces of each building material (carpet, wood, drywall, and concrete) were laid out, not touching anything. A spike of 50 µL of acetonitrile was applied to these building materials as a blank.

Two samples of each building material (carpet, wood, drywall, and concrete), that were spiked with 50 µL of HMTD, as well as one sample of each building material spiked with acetonitrile, were extracted and analyzed after a 1 hour dry period (t=o), as well as after three days (t=3), seven days (t=7), fourteen days (t=14), twenty-one days (t=21), and twenty-eight days (t=28). This creates 12 samples, three of each building material, two spiked with HMTD and a blank spiked with acetonitrile, for each of the six different times. Table 2, below, shows the degradation study set up.

Table 2. HMTD Degradation Study (value represents number of replicates at each timepoint)

Sample Type	t=0	t=3	t=7	t=14	t=21	t=28
Carpet w/ HMTD	2	2	2	2	2	2
Carpet w/ AcCN	1	1	1	1	1	1
Wood w/ HMTD	2	2	2	2	2	2
Wood w/ AcCN	1	1	1	1	1	1
Drywall w/ HMTD	2	2	2	2	2	2
Drywall w/ AcCN	1	1	1	1	1	1
Concrete w/ HMTD	2	2	2	2	2	2
Concrete w/ AcCN	1	1	1	1	1	1

3.6 HMTD Extraction from Building Materials

Extraction was attempted using solid phase microextraction (SPME). The extraction failed and a direct rinse extraction method had to be developed for each building material. Do to the inherent differences in carpet, wood, drywall, and concrete, HMTD must be extracted from each of the different building materials with a slightly different method.

3.6.1 Extraction of HMTD from Carpet

Spiked carpet samples were folded in half (fiber side touching inside of the fold), and pushed into the bottom of the filter cartridge insert of the polypropylene centrifuge tube with the carpet-weave backing facing the filter cartridge with the carpet fibers toward the middle. 500 µL of methanol was added to the filter cartridge insert inside the outer polypropylene centrifuge tube, the tubes capped, and centrifuged for one minute at 10,000 Xg. The filter cartridge insert was then removed from the polypropylene centrifuge tube and the remaining solution was evaporated down to 250 µL, according to the 250 µL mark on the polypropylene centrifuge tube. The solution was then pipetted out of each test tube and transferred to an individual injection vial for LC/MS/MS analysis.

3.6.2 Extraction of HMTD from Wood

Spiked wood samples were placed spiked side down into a 10 mL disposable plastic cup. 1000 µL of methanol was added down the side of the plastic cup and the wood in the methanol allowed to sit for 10 minutes. After 10 minutes the solution was then pipetted out of the plastic cup and into the filter cartridge insert inside the outer

polypropylene centrifuge tube, the tubes were capped and centrifuged for one minute at 10,000 Xg. The filter cartridge insert was then removed from the tube and the remaining solution evaporated down to 250 µL, according to the 250 µL mark on the polypropylene centrifuge tube. The solution was then pipetted out of each test tube and transferred to an individual injection vial for LC/MS/MS analysis.

3.6.3 Extraction of HMTD from Drywall

Spiked drywall samples were placed on a clean piece of paper. The drywall cubes were then cut, about one third of the way into the drywall from the side with the spiked paper backing. The drywall on the spiked paper backing was then scraped and the drywall broken into powder using a scoopula. The drywall powder and paper backing were poured from the paper into a 10 mL disposable plastic cup. 2000 µL of methanol was added to the plastic cup and allowed to sit and evaporate down to about 1000 µL. The solution was then pipetted out of the plastic cup and into the filter cartridge insert inside the outer polypropylene centrifuge tube, the tubes were capped and centrifuged for one minute at 10,000 Xg. The filter cartridge insert was then removed from the tube and the remaining solution evaporated down to 250 µL, according to the 250 µL mark on the polypropylene centrifuge tube. The solution was then pipetted out of each test tube and transferred to an individual injection vial for LC/MS/MS analysis.

3.6.4 Extraction of HMTD from Concrete

Spiked concrete samples were placed on a clean piece of paper. The spiked end of the concrete sample was broken off, about one third of the way into the concrete from the manufacturer-finished spiked end. The end piece that was broken off of the concrete

was then wrapped in plastic pulverized using a hammer with a mortar and pestle. The pulverized concrete was poured from the paper into a 10 mL disposable plastic cup. 3000 µL of methanol was added to the plastic cup and allowed to sit and evaporate down to about 1000 µL. The solution was then pipetted out of the plastic cup and into the filter cartridge insert inside the outer polypropylene centrifuge tube, the tubes were capped and centrifuged for one minute at 10,000 Xg. The filter cartridge insert was then removed from the tube and the remaining solution evaporated down to 250 µL, according to the 250 µL mark on the polypropylene centrifuge tube. The solution was then pipetted out of each test tube and transferred to an individual injection vial for LC/MS/MS analysis.

3.7 Method Validation

3.7.1 Precision

By examining the variability of calibrators run on the same day, and the variability of calibrators run on different days, precision was determined. The variability of calibrators run on the same day is known as intraday variability and the variability of calibrators run on different days is the interday variability. Intraday variability was calculated for each lower end calibrator concentration by taking the standard error of the mean of each day when multiple samples are run at the same level, then dividing by the mean of the analyte peak area for that level, and multiplying that number by the number of samples run that day. Daily intraday variabilities were then added together and divided by the total number of samples. For one calibrator level this process can be represented by the formula:

IntradayVariability =

((nday1*(SEMday1/Meanday1))+((nday2*(SEMday2/Meanday2))

Interday variability was calculated for each calibrator concentration by using the mean, standard error of the mean, and number of calibrators for each different day, then determining the variability in the daily numbers using column statistics in GraphPad Prism®.

3.7.2 Accuracy

The accuracy of the different building material extractions was calculated by determining the percent error of the extracted analyte peak area in the recovery study through comparison of the calculated value from extrapolation of the best fit line of the pooled data with known concentrations. This was calculated by:

((calculated value – true value)/ true value)*100

3.7.3 Selectivity

An HMTD solution was made by taking 30 µl of HMTD standard with 1470 µl of methanol. A syringe was filled with the HMTD solution and connected to a capillary tube attached to the MS/MS using a 3-way connector to allow for direct infusion of the HMTD solution. As a background comparison, a blank extraction of each building material was placed in the LC autosampler. For each of the building materials, the blank extraction sample was run with the same HMTD MRM acquisition method that was used to run all other sample samples with the HMTD solution infused as well at an injection flow rate of 10 µl/min. The chromatograms were reviewed for ionization enhancements and suppressions.

3.7.4 Sensitivity

3.7.4.1 Sensitivity: Multiple Low Level Extractions for Determination of LOD and LOQ

The LOD and LOQ of each building material were calculated by using three runs of HMTD extractions from each of the building materials. The analyte peak area values for the low level calibrators, 0.2-0.4 µg from each run were plotted in a graph to obtain the y-intercept and slope for each run. The y-intercept and slope values were then be used to calculate the LOQ and LOD. The standard deviation of the three y-intercepts will be calculated along with the mean of the three slopes. The LOQ = $(10*SD_{Yint})/mean_S$ and the LOD equals $(3.3*SD_{Yint})/mean_S$ where SD means standard deviation (Peters, 2006).

The sets of solutions were analyzed in the following order: a blank, extracted solution from the building material spiked with 2 µL of HMTD, extracted solution from the building material spiked with 3 µL of HMTD, extracted solution from the building material spiked with 4 µL of HMTD. All peaks were reviewed for correct integration.

3.7.4.2 Sensitivity: Estimation of LOD and LOQ using Blank Extractions of Building Materials

The LOD and LOQ using blank extractions of each of the building materials were calculated by using 12 runs of acetonitrile extractions from each of the building materials. The average and standard deviation of the 12 analyte peak areas from each of the building material blanks was calculated. The LOQ = (10*SD)+mean and the LOD equals (3.3*SD)+mean where SD means standard deviation (Peters, 2006).

The sets of solutions were analyzed in the following order: extracted solutions from carpet spiked with AcCN (1-12), a methanol blank, extracted solution from wood

spiked with AcCN (1-12), a methanol blank, extracted solution from concrete spiked with AcCN (1-12), a methanol blank, extracted solution from drywall spiked with AcCN (1-12), a methanol blank. Samples were run in this order to enable the identification of the presence of carryover. All peaks were reviewed for correct integration.

The average analyte peak area values for the lower level (0.2-0.4 µg) HMTD extractions from three runs of each of the building materials were plotted in a graph to obtain the y-intercept and slope of the best fit line, which were then used to extrapolate the LOQ and LOD concentrations from the acetonitrile blanks in µg (Peters, 2006).

3.7.4.3 Sensitivity: Recovery Study of HMTD by Extraction

Calibrators were prepared by adding 25 µL of HMTD analytical standard to 225 µL of methanol. These calibrators were analyzed and the analyte peak area of the calibrators was compared to the analyte peak areas of the extractions from the building materials that were spiked with 25 µL of HMTD analytical standard and percent recovery was determined.

The sets of solutions were ordered in the following way: a methanol blank, a solution of 25 µL of HMTD analytical standard with 225 µL of methanol, a methanol blank, a solution of 25 µL of HMTD analytical standard with 225 µL of methanol, a methanol blank, a solution of 25 µL of HMTD analytical standard with 225 µL of methanol. Samples were run in this order to enable the identification of the presence of carryover. All peaks were reviewed for correct integration.

3.8 HMTD Degradation on Building Materials

The sets of solutions were ordered in the following way: a methanol blank, extracted solution from carpet (1) spiked with HMTD, extracted solution from carpet

spiked with AcCN, extracted solution from carpet (2) spiked with HMTD, a methanol blank, extracted solution from wood (1) spiked with HMTD, extracted solution from wood spiked with AcCN, extracted solution from wood (2) spiked with HMTD, a methanol blank, extracted solution from concrete (1) spiked with HMTD, extracted solution from concrete spiked with AcCN, extracted solution from concrete (2) spiked with HMTD, a methanol blank, extracted solution from drywall (1) spiked with HMTD, extracted solution from drywall spiked with AcCN, extracted solution from drywall (2) spiked with HMTD, a methanol blank. Samples were run in this order to enable the identification of the presence of carryover. All peaks were reviewed for correct integration.

Separate ID ratios (designated as ID1 and ID2) of the calibrators will be used to confirm the calculated concentrations of HMTD. ID1 was calculated taking the analyte peak area of the second largest Q3 product ion and dividing it by the analyte peak area of the largest Q3 product ion. ID2 was calculated taking the analyte peak area of the third largest Q3 product ion and dividing it by the analyte peak area of the largest Q3 product ion. The average ID1 and ID2 ratio using all of the samples was calculated and a 30% upper and lower range was determined from that number. For the calculated concentration of HMTD to be confirmed, the ID1 and ID2 ratio must be within the 30% upper and lower calculated range.

3.9 Statistical Analysis

All statistical analyses will be performed using Microsoft Excel® 2007 (Microsoft Corporation, Redmond, WA) and GraphPad Prism® Version 5.0 (GraphPad Software, San Diego, CA).

Chapter IV Results

4.1 Precision

Table 3 shows interday and intraday variability for the lower end calibrators (0.2-0.4 µg HMTD) for each building material.

Table 3. Interday and Intraday Variability of Carpet, Wood, Concrete, and Drywall Extractions

Building Material	Type of Variability	0.2 µg	0.3 µg	0.4 µg
Carpet	Interday	13%	28%	21%
	Intraday	2%	22%	18%
Wood	Interday	14%	20%	4%
	Intraday	22%	14%	36%
Concrete	Interday	29%	32%	26%
	Intraday	14%	28%	7%
Drywall	Interday	25%	2%	20%
	Intraday	7%	30%	14%

4.2 Accuracy

Accuracy of the building material extractions from the recovery study are shown in Table 4.

Table 4. Accuracy of the Building Material Extractions in the Recovery Study

Material	Known HMTD (µg)	Mean amount HMTD (µg)	% Error
Carpet	2.00	1.86	-7.04
	1.50	1.73	15.62
	1.00	0.95	-4.64
	0.50	0.45	-9.43
Wood	2.00	2.05	2.54
	1.50	1.43	-4.82
	1.00	0.99	-0.78
	0.50	0.53	5.85
Concrete	2.00	2.15	7.60
	1.50	1.45	-3.41
	1.00	0.65	-35.37
	0.50	0.82	64.88
Drywall	2.00	3.19	59.70
	1.50	2.37	57.83
	1.00	1.96	96.47
	0.50	0.85	69.38

4.3 Selectivity

Using the postcolumn infusion method matrix effects were examined. Extraction blanks from each building material were examined. Chromatograms were

then examined for indications of suppressions or enhancements of the HMTD signal. A lack of overlap between the analyte retention time of HMTD and enhancement or suppression of the signal indicates that the quantitation was unaffected by matrix effects. An example of the chromatogram examined in shown in Figure 2.

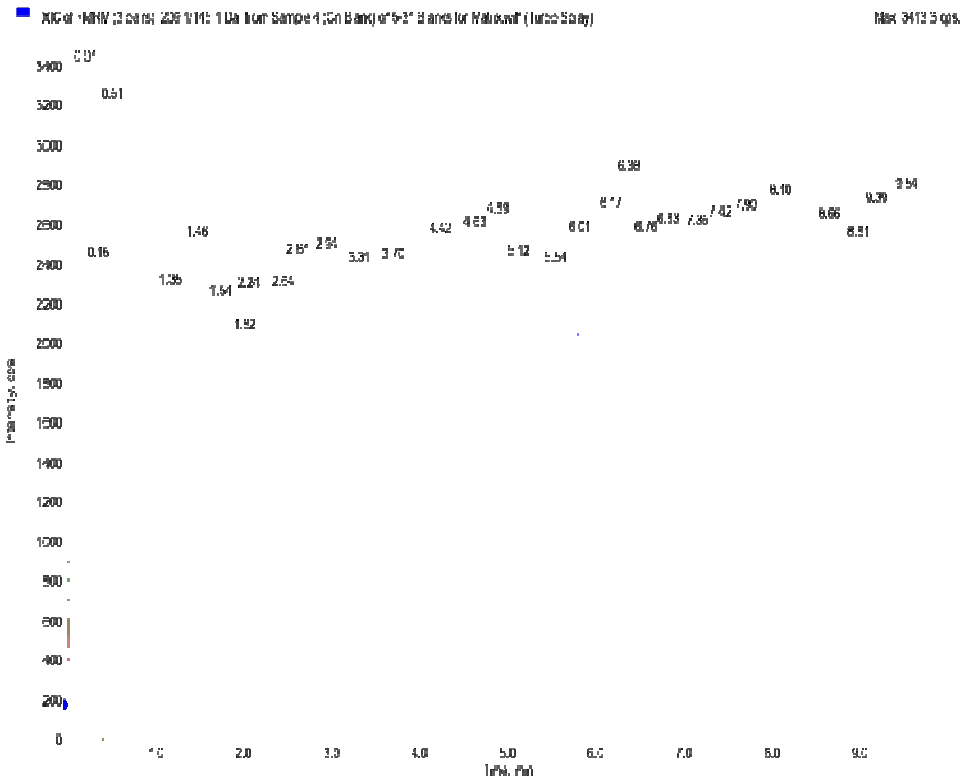

Figure 2. Chromatogram of Matrix Effects from Concrete Extractions
The chromatogram shows the matrix effects from concrete using the postcolumn infusion method. The red, blue, and green lines represent the HMTD ions selected for. There is no suppression or enhancements of those ions between 2.5 and 4 minutes where the HMTD would elute in a normal run.

4.4 Sensitivity

4.4.1 Multiple Low Level Extractions for Determination of LOD and LOQ

4.4.1.1 Low Level Carpet Extractions

Table 5 shows the analyte peak areas from 0.2-0.4 µg HMTD on carpet extractions. These analyte peak areas were used to determine the sensitivity of the method for the carpet extraction.

Table 5. Analyte Peak Area (counts) Used to Calculate LOD/LOQ for Low Level Carpet Extraction Method

HMTD (µg)	Analyte Peak Area (counts)			Average (counts)
0.2	2.38E+04	1.87E+04	1.80E+04	2.02E+04
0.3	6.27E+04	2.74E+04	4.31E+04	4.44E+04
0.4	7.13E+04	3.80E+04	5.42E+04	5.45E+04

The analyte peak areas (counts) were plotted on a graph (Figure 3) and the mean and standard deviation of the Y-intercepts shown in the graph (Table 6) were calculated to determine LOD and LOQ.

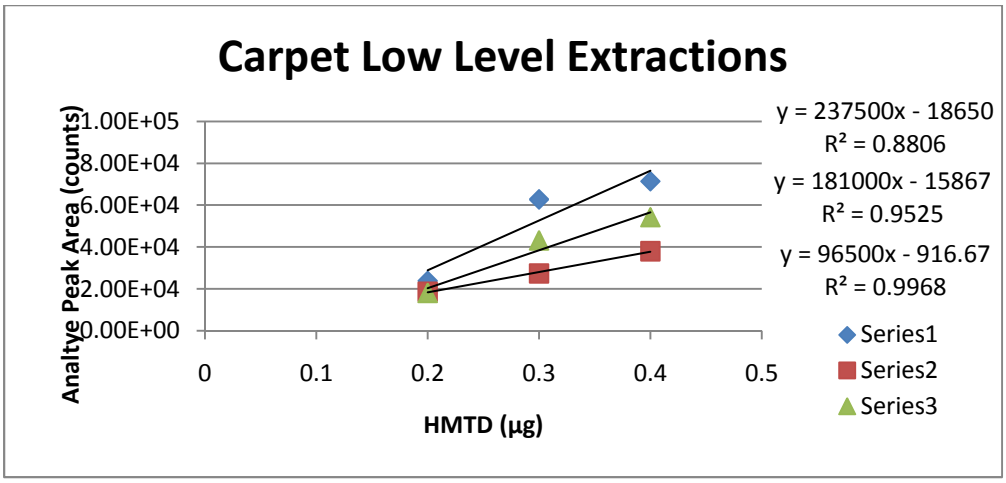

Figure 3. Carpet Extraction LOD/LOQ Graph
Analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ calculations.

Table 6. Y-Intercepts and Slopes Used to Calculate LOD/LOQ for Low Level Carpet Extraction Method

Run	Y-intercept	Slope
1	-18650	237500
2	-916.67	96500
3	-15866.67	181000
Standard Deviation	9536.96	
Mean	171667	
LOD (µg)	0.18	
LOQ (µg)	0.56	

4.4.1.2 Low Level Wood Extractions

Table 7 shows the analyte peak areas from 0.2-0.4 µg HMTD on wood extractions. These analyte peak areas were used to determine the sensitivity of the method for the wood extraction.

Table 7. Analyte Peak Area (counts) Used to Calculate LOD/LOQ for Low Level Wood Extraction Method

HMTD (µg)	Analyte Peak Area (counts)			Average (counts)
0.2	6.85E+03	6.33E+03	4.07E+03	5.75E+03
0.3	9.59E+03	7.24E+03	5.48E+03	7.44E+03
0.4	8.93E+03	1.32E+04	6.24E+03	9.46E+03

The analyte peak areas (counts) were plotted on a graph (Figure 4) and the mean and standard deviation of the Y-intercepts shown in the graph (Table 8) were calculated to determine LOD and LOQ.

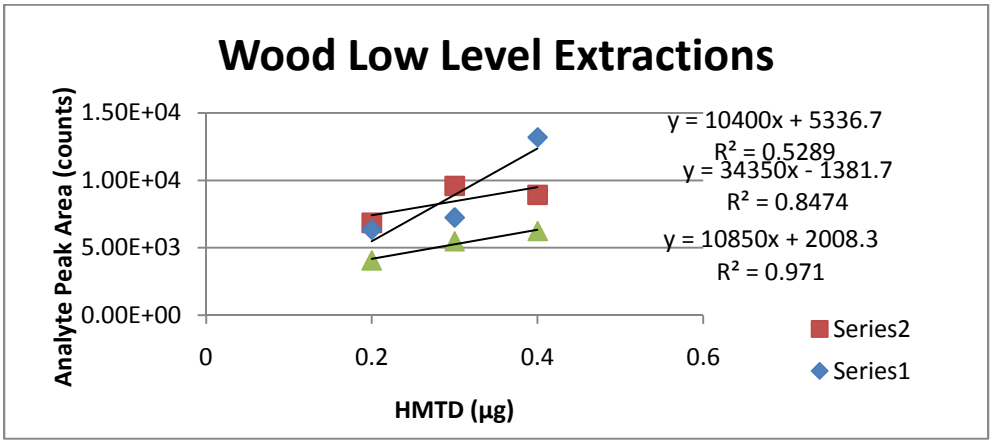

Figure 4. Wood Extraction LOD/LOQ Graph
Analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ calculations.

Table 8. Y-Intercepts and Slopes Used to Calculate LOD/LOQ for Low Level Wood Extraction Method

Run	Y-intercept	Slope
1	5336.67	10400
2	-1381.67	34350
3	2008.33	10850
Standard Deviation	3359.21	
Mean	18533	
LOD (µg)	0.60	
LOQ (µg)	1.81	

4.4.1.3 Low Level Concrete Extractions

Table 9 shows the analyte peak areas from 0.2-0.4 µg HMTD on concrete extractions. These analyte peak areas were used to determine the sensitivity of the method for the concrete extraction.

Table 9. Analyte Peak Area (counts) Used to Calculate LOD/LOQ for Low Level Concrete Extraction Method

HMTD (µg)	Analyte Peak Area (counts)			Average (counts)
0.2	1.69E+03	4.59E+03	6.14E+03	4.14E+03
0.3	2.91E+03	6.41E+03	1.14E+04	6.91E+03
0.4	4.10E+03	1.26E+04	1.44E+04	1.04E+04

The analyte peak areas (counts) were plotted on a graph (Figure 5) and the mean and standard deviation of the Y-intercepts shown in the graph (Table 10) were calculated to determine LOD and LOQ.

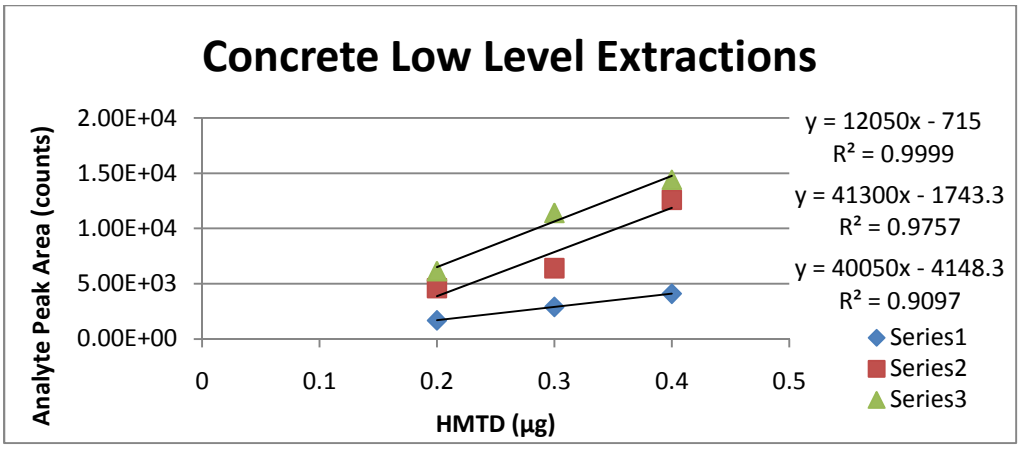

Figure 5. Concrete Extraction LOD/LOQ Graph
Analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ calculations.

Table 10. Y-Intercepts and Slopes Used to Calculate LOD/LOQ for Low Level Concrete Extraction Method

Run	Y-intercept	Slope
1	-715	12050
2	-4148.33	40050
3	-1743.33	41300
Standard Deviation	1762.07	
Mean	31133	
LOD (µg)	0.19	
LOQ (µg)	0.57	

4.4.1.4 Low Level Drywall Extractions

Table 11 shows the analyte peak areas from 0.2-0.4 µg HMTD on drywall extractions. These analyte peak areas were used to determine the sensitivity of the method for the drywall extraction.

Table 11. Analyte Peak Area (counts) Used to Calculate LOD/LOQ for Low Level Drywall Extraction Method

HMTD (µg)	Analyte Peak Area (counts)			Average (counts)
0.2	9.84E+03	5.44E+03	6.31E+03	7.20E+03
0.3	1.66E+04	2.10E+04	1.12E+04	1.63E+04
0.4	2.99E+04	2.29E+04	1.73E+04	2.34E+04

The analyte peak areas (counts) were plotted on a graph (Figure 6) and the mean and standard deviation of the Y-intercepts shown in the graph (Table 12) were calculated to determine LOD and LOQ.

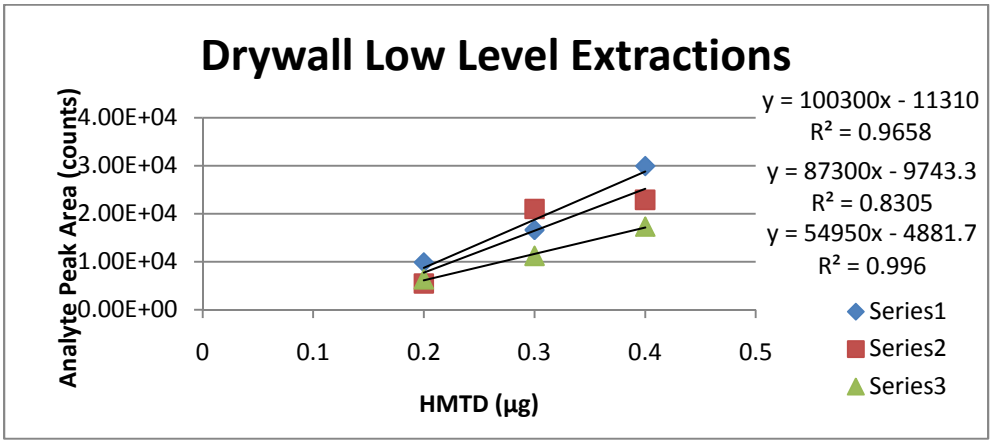

Figure 6. Drywall Extraction LOD/LOQ Graph
Analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ calculations.

Table 12. Y-Intercepts and Slopes Used to Calculate LOD/LOQ for Low Level Wood Extraction Method

Run	Y-intercept	Slope
1	-11310	100300
2	-9743.33	87300
3	-4881.67	54950
Standard Deviation	3351.96	
Mean	80850	
LOD (μg)	0.14	
LOQ (μg)	0.42	

4.4.2 LOD and LOQ using Blank Extractions From Building Materials and the HMTD Building Material Extractions

4.4.2.1 Blank Carpet Extractions: LOD and LOQ

The area of baseline normally in the region of the analyte peak was manually integrated and average analyte peak area (counts) from the HMTD carpet extractions were plotted on a graph (Figure 7). Then the mean and standard deviation of analyte peak area of blank extractions (Table 13) were calculated to determine LOD and LOQ.

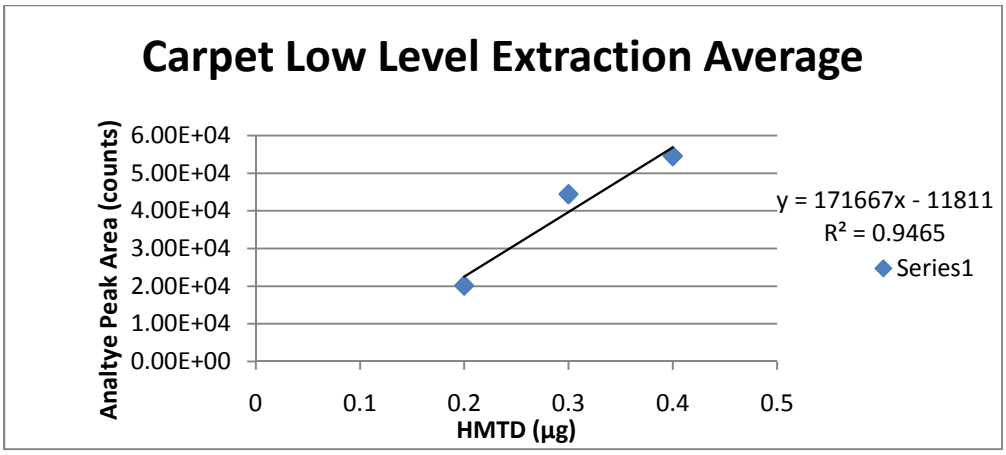

Figure 7. Low Level Carpet Extraction Mean LOD/LOQ Graph
Average analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ concentration calculations.

Table 13. Average and Standard Deviation Used to Calculate LOD/LOQ from the Low Level Carpet Blank Extractions

Average Analyte Peak Area	SD	LOD	LOQ
1078.50	635.87	3176.86	7437.15
Calculated concentration:		LOD	LOQ
(in µg)		0.09	0.11

4.4.2.2 Blank Wood Extractions: LOD and LOQ

The average analyte peak area (counts) from the HMTD carpet extractions were plotted on a graph (Figure 8) and the mean and standard deviation of analyte peak area of blank extractions (Table 14) were calculated to determine LOD and LOQ.

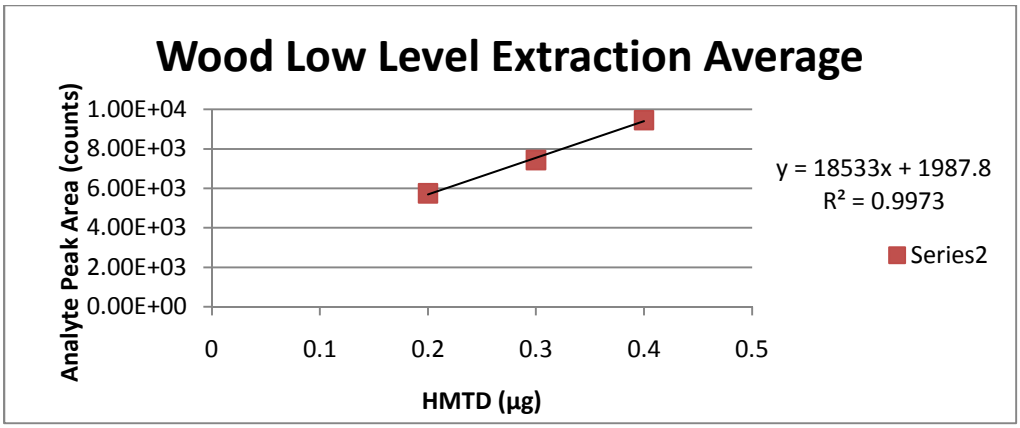

Figure 8. Low Level Wood Extraction Mean LOD/LOQ Graph
Average analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ concentration calculations.

Table 14. Average and Standard Deviation Used to Calculate LOD/LOQ from the Low Level Wood Blank Extractions

Average Analyte Peak Area	SD	LOD	LOQ
898.92	281.51	1827.89	3713.98
Calculated concentration:		LOD	LOQ
(in µg)		-0.01	0.09

4.4.2.3 Blank Concrete Extractions: LOD and LOQ

The average analyte peak area (counts) from the HMTD carpet extractions were plotted on a graph (Figure 9) and the mean and standard deviation of analyte peak area of blank extractions (Table 15) were calculated to determine LOD and LOQ.

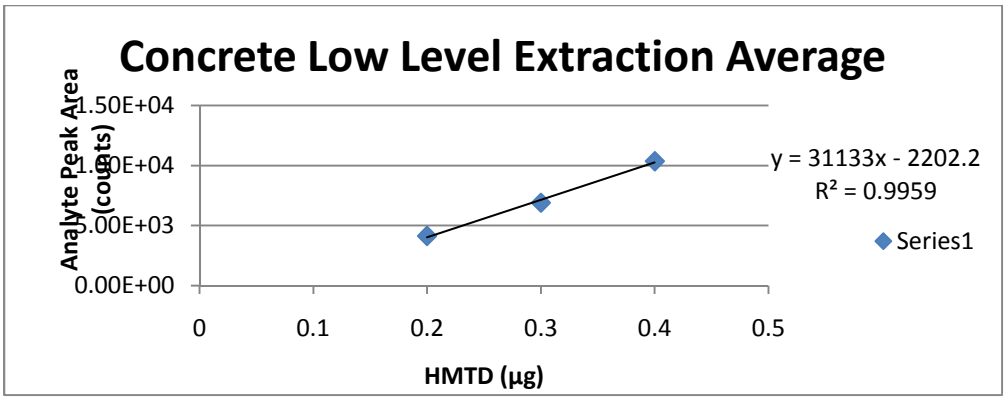

Figure 9. Low Level Concrete Extraction Mean LOD/LOQ Graph
Average analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ concentration calculations.

Table 15. Average and Standard Deviation Used to Calculate LOD/LOQ from the Low Level Concrete Blank Extractions

Average Analyte Peak Area	SD	LOD	LOQ
827.58	480.22	2412.32	5629.82
Calculated concentration:		LOD	LOQ
(in µg)		0.15	0.25

4.4.2.4 Blank Drywall Extractions: LOD and LOQ

The average analyte peak area (counts) from the HMTD carpet extractions were plotted on a graph (Figure 10) and the mean and standard deviation of analyte peak area of blank extractions (Table 16) were calculated to determine LOD and LOQ.

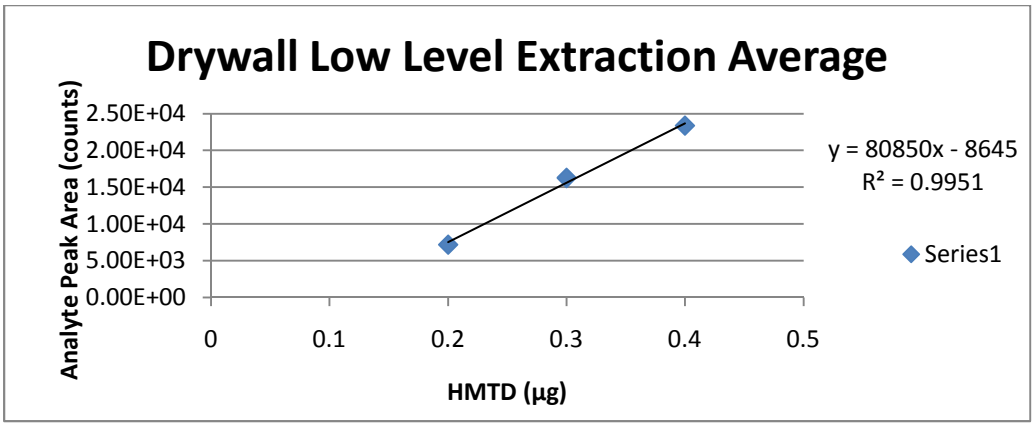

Figure 10. Low Level Drywall Extraction Mean LOD/LOQ Graph
Average analyte peak area (counts) of the low level extractions (0.2-0.4 µg) of three runs were plotted to obtain y-intercepts and slopes for LOD/LOQ concentration calculations.

Table 16. Average and Standard Deviation Used to Calculate LOD/LOQ from the Low Level Drywall Blank Extractions

Average Analyte Peak Area	SD	LOD	LOQ
1035.33	435.95	2473.98	5394.85
Calculated concentration:		LOD	LOQ
(in µg)		0.14	0.17

4.4.2.5 Summary of LOD and LOQ from Building Material HMTD Extractions and Blank Extractions From Building Materials

Table 17 shows the LOD and LOQ values calculated from both the building material low level extractions (0.2-0.4 µg), as well as the blank extraction method with the LOD and LOQ value in µg from the low level extraction curve.

Table 17. Calculated LOD and LOQ from Building material HMTD Extractions as well as Blank Extractions

Building Material	Method	LOD (µg)	LOQ (µg)
Carpet	Low Level Calibration Curve estimate	0.18	0.56
	Blank Repetition estimate	0.09	0.11
Wood	Low Level Calibration Curve estimate	0.60	1.81
	Blank Repetition estimate	-0.01	0.09
Concrete	Low Level Calibration Curve estimate	0.19	0.57
	Blank Repetition estimate	0.15	0.25
Drywall	Low Level Calibration Curve estimate	0.14	0.41
	Blank Repetition estimate	0.14	0.17

4.5 Recovery of HMTD in Extractions From Building Materials

4.5.1 Carpet Extraction: HMTD Recovery

Table 18 shows the analyte peak area for extractions of 2.5 µg of HMTD from carpet and the percent of recovered HMTD when compared to a prepared solution with 2.5 µg of HMTD.

Table 18. Percent of HMTD Recovered During Carpet Extraction

	HMTD Solution	Carpet Analyte Peak Area	% Recovery
	1.42E+06	7.83E+05	84.28%
	6.72E+05	7.81E+05	84.07%
	6.95E+05	6.87E+05	73.95%
Average	9.29E+05	7.50E+05	80.77%
Standard Deviation	4.25E+05	5.49E+04	5.90%

4.5.2 Wood Extractions: HMTD Recovery

Table 19 shows the analyte peak area for extractions of 2.5 µg of HMTD from wood and the percent of recovered HMTD when compared to a prepared solution with 2.5 µg of HMTD.

Table 19. Percent of HMTD Recovered During Wood Extraction

	HMTD Solution	Wood Analyte Peak Area	% Recovery
	1.42E+06	1.99E+05	21.42%
	6.72E+05	1.98E+05	21.31%
	6.95E+05	1.27E+05	13.67%
Average	9.29E+05	1.75E+05	18.80%
Standard Deviation	4.25E+05	4.13E+04	4.44%

4.5.3 Concrete Extractions: HMTD Recovery

Table 20 shows the analyte peak area for extractions of 2.5 µg of HMTD from concrete and the percent of recovered HMTD when compared to a prepared solution with 2.5 µg of HMTD.

Table 20. Percent of HMTD Recovered During Concrete Extraction

	HMTD Solution	Concrete Analyte Peak Area	% Recovery
	1.42E+06	5.72E+04	6.16%
	6.72E+05	7.55E+04	8.13%
	6.95E+05	9.30E+04	10.01%
Average	9.29E+05	7.52E+04	8.10%
Standard Deviation	4.25E+05	1.79E+04	1.93%

4.5.4 Drywall Extractions: HMTD Recovery

Table 21 shows the analyte peak area for extractions of 2.5 µg of HMTD from drywall and the percent of recovered HMTD when compared to a prepared solution with 2.5 µg of HMTD.

Table 21. Percent of HMTD Recovered During Drywall Extraction

	HMTD Solution	Drywall Analyte Peak Area	% Recovery
	1.42E+06	3.53E+05	38.00%
	6.72E+05	2.13E+05	22.93%
	6.95E+05	3.34E+05	35.95%
Average	9.29E+05	3.00E+05	32.29%
Standard Deviation	4.25E+05	7.59E+04	8.17%

4.5.5 Summary of HMTD Recovery from Building Materials

Table 22 shows the shows the average percent of analyte peak area of HMTD and standard deviation recovered from each of the building materials.

Table 22. Average Percent of HMTD Recovered from Each Building Material

Material	Percent of Average Analyte Peak Area Recovered	Standard Deviation
Carpet	80.77%	5.90%
Wood	18.80%	4.44%
Concrete	8.10%	1.93%
Drywall	32.29%	8.17%

4.6 Degradation of HMTD from Building Materials

4.6.1 Degradation of HMTD from Carpet

Table 23 shows the analyte peak areas at different times during the degradation study that were used to determine the remaining detected HMTD on carpet.

Table 23. Analyte Peak Area from HMTD Degradation Study in Carpet Extractions

Peak Area (counts)	t0	t3	t7	t14	t21	t28
Carpet Sample						
1	1.27E+05	1.56E+05	5.88E+04	7.66E+04	3.30E+04	4.53E+05*
2	3.56E+05	1.79E+05	4.88E+04	4.57E+04	2.63E+04	6.75E+05*
Average	2.42E+05	1.68E+05	5.38E+04	6.12E+04	2.97E+04	5.64E+05*
Standard Deviation	1.62E+05	1.63E+04	7.07E+03	2.18E+04	4.74E+03	1.57E+05*

***28 day timepoint excluded**

4.6.2 Degradation of HMTD from Wood

Table 24 shows the analyte peak areas at different times during the degradation

study that were used to determine the remaining detected HMTD on wood. If the ID1 and ID2 ratios were not within the 30% upper and lower calculated range then the samples were marked as not detected (ND).

Table 24. Analyte Peak Area from HMTD Degradation Study in Wood Extractions

Peak Area (counts)	t0	t3	t7	t14	t21	t28
Wood Sample						
1	9.12E+03	1.58E+04	1.06E+04	4.10E+03	1.12E+03	ND
2	4.50E+03	2.32E+04	6.51E+03	ND	4.07E+03	ND
Average	6.81E+03	1.95E+04	8.56E+03	4.10E+03	2.60E+03	
Standard Deviation	3.27E+03	5.23E+03	2.89E+03		2.09E+03	

4.6.3 Degradation of HMTD from Concrete

Table 25 shows the analyte peak areas at different times during the degradation study that were used to determine the remaining detected HMTD on concrete. If the ID1 and ID2 ratios were not within the 30% upper and lower calculated range then the samples were marked as not detected (ND).

Table 25. Analyte Peak Area from HMTD Degradation Study in Concrete Extractions

Peak Area (counts)	t0	t3	t7	t14	t21	t28
Concrete Sample						
1	1.10E+04	1.06E+04	1.50E+03	ND	ND	ND
2	5.70E+03	1.35E+04	ND	1.33E+03	ND	ND
Average	8.35E+03	1.21E+04	1.50E+03	1.33E+03		
Standard Deviation	3.75E+03	2.05E+03				

4.6.4 Degradation of HMTD from Drywall

Table 26 shows the analyte peak areas at different times during the degradation study that were used to determine the remaining detected HMTD on drywall. If the ID1 and ID2 ratios were not within the 30% upper and lower calculated range then the samples were marked as not detected (ND).

Table 26. Analyte Peak Area from HMTD Degradation Study in Drywall Extractions

Peak Area (counts)	t0	t3	t7	t14	t21	t28
Drywall Sample						
1	9.77E+03	8.72E+04	1.06E+04	1.94E+04	ND	ND
2	9.19E+03	1.80E+04	6.78E+03	ND	ND	ND
Average	9.48E+03	5.26E+04	8.69E+03	1.94E+04		
Standard Deviation	4.10E+02	4.89E+04	2.70E+03			

4.6.5 Degradation of HMTD from Building Materials Summary

Figure 11 represents the average analyte peak areas representative of the remaining HMTD in carpet, wood, concrete, and drywall extractions (Tables 27, 29, 31, and 33) for time 0-21 days. Data from the timepoint of 28 days (extracted from carpet) was not included here because the data was collected after the instrument had been cleaned, altering sensitivity, discussed further in the Discussion section.

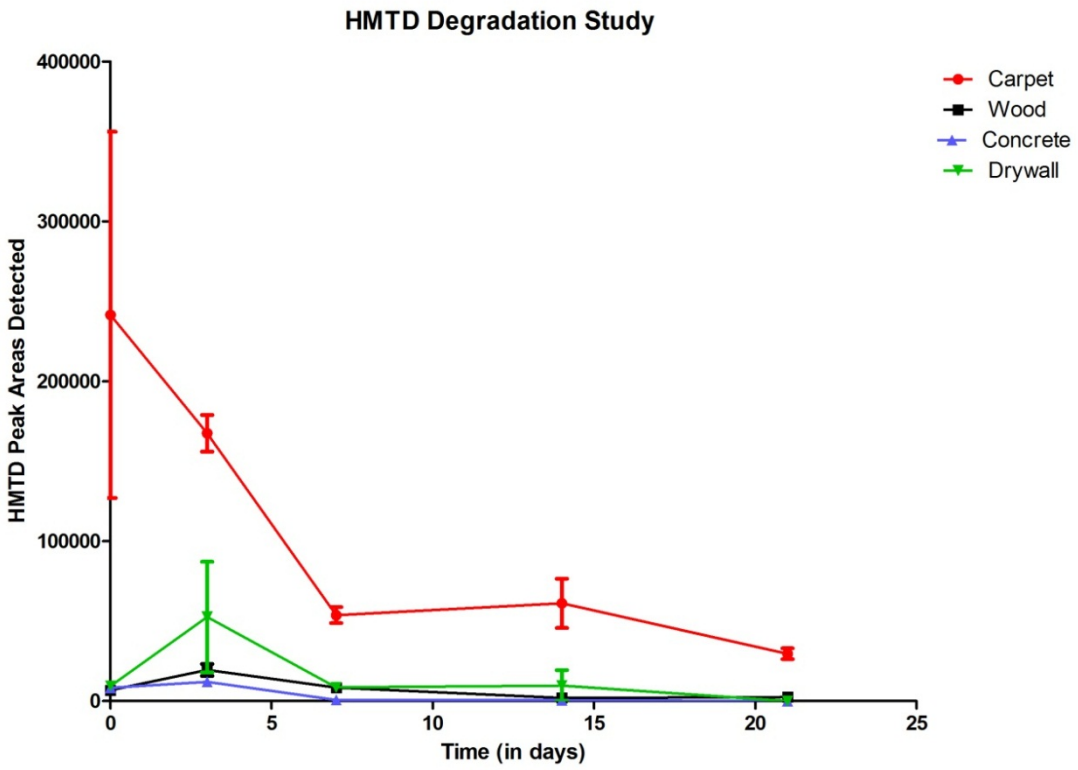

Figure 11. HMTD Extracted from Each Building Material During Degradation Study

Average HMTD analyte peak areas extracted from each building material plotted against time during the degradation study.

Chapter V Discussion

The goal of the study was to develop a LC/MS/MS method to analyze HMTD extracted from building materials after given time periods to determine which building materials retained HMTD for the longest amount of time in the highest quantity. Sensitivity was explored, providing calculated LOD and LOQ values for each of the building materials. Also, the percentage of HMTD recovered from each building material during the extraction process was calculated.

5.1 Precision

Interday and intraday variability was examined for the lower end calibrators (0.2-0.4 µg HMTD) for each building material to determine the precision of each building material method. Interday variability ranges from 4 to 29% for the four building materials, while intraday variability ranges from as low as 2% to as high as 36%. Commonly acceptable parameters for analytical methods were outlined in the literature review and the precision falls outside of guidelines routinely established. While the precision is not sufficient for a bioanalytical quantitation, it is sufficient for the semi-quantitative estimation of explosive residues in building materials.

5.2 Accuracy

Accuracy was calculated using the high-end calibrators for each of the building materials. Of the four building materials, the calibrators for the drywall HMTD extractions showed the lowest accuracy. Other building materials showed better accuracy, particularly those of carpet and wood. Concrete was challenging due to the rigor of the pulverization and extraction procedure, where it is difficult to generate a fine powder for uniform extraction.

5.3 Selectivity

Using the postcolumn infusion method, matrix effects were examined. Extraction blanks from each building material were examined. Chromatograms were then examined for indications of suppressions or enhancements of the HMTD signal. A lack of overlap between the analyte retention time of HMTD and enhancement or suppression of the signal indicates that the peaks in extracted samples were representative of the analyte HMTD and were not simply due to matrix effects. This also allows us to conclude variations in sensitivity were due to analyte presence or absence in extracts.

5.4 Sensitivity

Two accepted statistical methods were used to calculate two different values for both the LOD and LOQ on each of the four different building materials. Typically, one statistical method is used to determine LOD and LOQ. In this study, two statistical methods were used to calculate these values in an effort to best establish the true LOD and LOQ of the method for each of the four building materials.

Originally, one set of LOD and LOQ calculations was to be performed by taking HMTD spiked building materials (0.5-2.5 µg), extracting and analyzing them, and creating a best fit line to calculate the LOD and LOQ values. This approach, however, gave values that looked falsely inflated based on what could be seen in the data. Clearly peaks could be identified below the LOD calculated value for each of the different building material. Because peaks could be identified, with acceptable ID ratios for HMTD, this calibration curve was determined to be too high to be representative of the actual LOD and LOQ for each of the building materials.

To correct for the calibration curve being too high multiple injections of HMTD extracted from each of the building materials were done at the lower HMTD levels. The amount of HMTD spiked on each set of the building materials ranged from .2 to .4 µg. The goal was to go below the calculated limit of detection for each of the building materials and determine when one would not be able to detect HMTD. This was done in an effort to offer confirmation or further grounds for rejection of the calculated LOD and LOQ calculations or a method of statistical assessment of LOD and LOQ values. Despite the very low volume of the HMTD aliquot onto each set of building materials, all of the building materials showed peaks and the ID1 and ID2 ratios within the typical limits of identification. HMTD was being detected below the calculated LOD value based on either statistical method, calculation from blanks or standard curves.

The blank extractions consisted of acetonitrile on each of the building materials. The area where an HMTD peak would normally be seen was integrated and this integrated analyte peak area was used in conjunction with the calibration curve produced from the extractions above to calculate the LOD and LOQ values in µg. The lower level

LOD and LOQ calculations were performed by taking HMTD spiked building materials (0.2-0.4 µg), extracting and analyzing them, and creating a best fit line to calculate the LOD and LOQ values. This approach gave values that looked more realistic when compared to the HMTD building material extractions with the higher concentrations of HMTD and values that are lower than the calculated value using blank extractions. These low level extractions are representative of the actual LOD and LOQ values for each of the building materials.

The calculated LOD and LOQ values using the blank method are lower than any of the values calculated from the extraction of HMTD, but they are consistent with the other calculated values for LOD and LOQ. These calculated values for LOD and LOQ could be representative of the actual LOD and LOQ values for each of the building materials. This is another accepted mathematic method for the determination of LOD and LOQ.

The calculated values for LOD and LOQ using the blank extractions gives a negative value for amount in µg when using the low level extraction curve for wood (-0.0086 µg). This is due to the mathematical method used to calculate a solutions concentration using the information in the equation of a line. When the average analyte peak area (in counts) is plotted, a linear line of best fit is added to the data with an R value and equation. The R value and the equation are generated based on the slope of the line. Because of the generated equation of a line based on the on the HMTD building material extractions, and the average analyte peak areas from the acetonitrile blanks, when the LOD and LOQ are calculated a negative number is sometimes produced. If the line had been forced through zero, the concentrations would not be negative. However, if

the line is forced through zero, the equation is altered and the R value moves further from one, indicating that the newly created line is less representative of the data points. The data points represent the amount of HMTD in the analyzed extraction. To accurately calculate a value in µg from analyte peak area, a line with an R-value closest to one, or most representative of the data, is imperative. It would also be beneficial to bracket the unknowns with calibrators that were run in conjunction with them. While no suitable internal standard was found in this study, an internal standard would provide normalization for the complicated extractions and provide for a more representative best fit line and unknown quantitation from extrapolation.

5.5 Recovery Study

This study was conducted without the use of an internal standard. LC-MS methods usually incorporate a suitable internal standard to be used in analysis. An internal standard is a compound that is added to a solution in constant, known, amounts. The internal standard can then be used to correct for the loss of analyte during extraction or sample preparation. Because an internal standard was not used in this study there is no way to correct for HMTD loss during the extraction process. A recovery study was performed in effort to better understand how much of the aliquot of HMTD was being recovered through the extraction processes from each of the building materials. By taking analyte peak areas of HMTD from each building material extractions and comparing them to a standard solution of a theoretical same concentration, a percentage value for recovery was determined. Only carpet extractions, with an average recovery of 81%, were above a 50% recovery rate. The other three building materials showed less than 50% of HMTD recovered through the extraction process, with drywall being the

highest of the three at 32%, followed by wood (19%) and then concrete at only 8%.

These percent recovery rates indicate that through refined extraction processes the amount of HMTD recovered could be higher than what is seen in this study. With refined extraction processes, and recovery of larger quantities of HMTD, it is possible that the amount of time that HMTD is detectable in the different building materials would be prolonged.

5.6 Degradation Study

A degradation study was conducted in an effort to determine how long HMTD could be extracted from different building materials and detected. The study was set up with six different time points (t-0, 3, 7, 14, 21, and 28 days) and each building material was evaluated for the remaining HMTD in the extraction of the building material. Building materials used were common building materials; carpet, wood, concrete, and drywall.

Carpet extractions provided detectable amounts of HMTD through the entire time of the degradation study. Amounts extracted from the carpet do not display a linear degradation pattern. There is, however, a rough pattern of decreasing HMTD concentration from time zero to time 21. The amount of HMTD in time 28 is significantly higher than any other time point. This time point was collected after the LC/MS/MS system was cleaned during preventive maintenance, and is not comparable to the other time points (explained further in Section 5.8). There is variance in the amount of HMTD detected in the extraction samples, both in the two extractions samples from each time point and in the extraction samples over the six time points. In all likelihood,

the variation in the two extraction samples at each time point is mostly due to the difference in the amount of HMTD extracted from the carpet sample (explained further in Section 5.7). Carpet was the only building material tested that showed HMTD throughout the time of the degradation study.

Extractions from wood provided detectable amounts of HMTD through the degradation study to t-21. Detectable peaks were visible after t-21 in t-28 but the ID1 and ID2 ratios were not consistent with HMTD identification. Amounts of HMTD extracted from the wood do not display a linear degradation pattern, and no real pattern in the data is obvious. Like the carpet samples, there is variance in the amount of HMTD detected in the extraction samples, both in the two extractions samples from each time point and in the extraction samples over the five time points in which HMTD was identified. In all likelihood, the variation in the two extraction samples at each time point is mostly due to the difference in the amount of HMTD extracted from the wood sample (explained further in Section 5.7).

Concrete extractions provided detectable amounts of HMTD through the degradation study to t-14. Detectable peaks were visible after t-14 but the ID1 and ID2 ratios were not consistent with HMTD identification. Like wood, amounts of HMTD extracted from the concrete do not display a linear degradation pattern, and no real pattern in the data is obvious. Like the carpet and wood samples, there is variance in the amount of HMTD detected in the extraction samples, both in the two extractions samples from each time point and in the extraction samples over the four time points in which HMTD was identified.

Extractions from drywall provided detectable amounts of HMTD through the

degradation study to t-14. Detectable peaks were visible after t-14 but the ID1 and ID2 ratios were not consistent with HMTD identification. Like wood and concrete, amounts of HMTD extracted from the drywall do not display a linear degradation pattern, and no real pattern in the data is obvious. Like the carpet, wood, and concrete samples, there is variance in the amount of HMTD detected in the drywall extraction samples, both in the two extractions samples from each time point and in the extraction samples over the four time points in which HMTD was identified.

This data collected in the degradation study implies that carpet would be best for the recovery of HMTD, when compared to wood, concrete, and drywall. After carpet samples, drywall has the highest amount of HMTD recovered, however wood samples yield detectable HMDT for a longer period of time. Concrete samples yield the lowest amount of HMTD throughout the study and showed a slightly shorter timeframe for detection when compared to drywall, making it the worst of the four samples in yield and timeframe for recovery. Extractions from carpet showed the highest amount of HMTD recovered on average. HMTD was also recoverable for a longer period of time from carpet samples, when compared with the other three building materials.

5.7 Building Material Differences and Extraction Methods

Building materials are diverse; they do not necessarily provide a homogenous matrix and they do not lend well to extraction. The differences within the building materials lead to variations in the amount of HMTD that is recovered during extractions on different samples of the same building material. Also, different types of building materials like Berber carpet, thicker paper backed drywall, sealed lumber, and concrete made with larger filler materials (defined as the screed materials) are not necessarily

going to act in a similar manner to the building materials used in this study.

The variations in carpet sample extractions are partially due to the carpet fibers being different from the carpet backing (the woven material that the carpet fibers are attached to). The carpet fibers are flexible and liquid (like the methanol used to rinse the carpet) flows freely around the fibers. The woven backing does not allow methanol to rinse through as freely. An aliquot of HMTD may deposit in the backing of the carpet or amongst the fibers of the carpet. The different types of areas within the carpet could extract differently, creating variations in the amount recovered from a given carpet sample, causing aberrations in the data. For example, in the recovery study between 73 and 84% of HMTD were extracted from comparable carpet samples.

Wood extractions show great variation in the amount of HMTD recovered (between 13 and 21%). Wood soaks up the methanol being used to extract the HMTD, not allowing the methanol to act as a rinse to remove the aliquot of HMTD. Some wood squares soak up much more methanol then others. The amount of rough edge as well as variations in the face and edge of the wood contributes to the variation in the amount of methanol absorbed by the wood samples. The wood density and the presence of knots would certainly be expected to affect these results also. Basically, the methanol is absorbed and HMTD is not eluted from the wood square samples very well (only an average recovery of 18.80%) or in comparable amounts between samples.

In drywall samples, it is likely that the paper-backing and the pressed gypsum extract differently due their composition. Drywall samples show a large deviation in the amount of HMTD extracted (16% in the recovery study) with an average recovery less than 50% (32%). It has not been determined if the aliquot of HMTD deposits in the

paper backing or travels through the paper backing and into the gypsum. Where the HMTD is deposited in the drywall may cause deviations in the data. The paper-backing portion of the drywall would probably be more difficult to extract, whereas the gypsum portion of the drywall could be powdered and rinsed more effectively. It is likely that a portion of the HMTD is deposited into both the gypsum part of the drywall as well as the paper-backing portion. The portion deposited into each section of the drywall is variable. These variations in where the HMTD is deposited will cause the differences in the amount of HMTD extracted in comparable extractions of different samples.

Concrete is composed of sand and rock materials (as well as some other ingredients) and the final product, though solid, is far from void of holes. These crevasses may hold HMTD, or provide a gateway for the HMTD to be absorbed or imbedded far inside the concrete. It would be unreasonable to assume that HMTD stayed on the surface of the concrete, or that each of these gaps could be rinsed completely or evenly to extract HMDT. Also, each area of poured concrete is different, based on a number of factors. Any given sample will extract differently based on the irregularities within the concrete sample itself. The amount of HMTD extracted from comparable samples is widely variable (between 6 and 10% in the recovery study). The amount of HMTD recovered from concrete is the lowest of the four building materials (an average of 8.10%). This low recovery is probably due to the crevasses within the concrete where HMTD may be deposited as well as the ability of the acetonitrile and HMTD solution to distribute throughout the concrete sample, making extraction more difficult.

The extraction methods developed to remove the HMTD from each building material may also contribute to variations in the data. The extraction methods (all four)

were completed with an evaporation step. In an effort to more fully extract the HMTD from the building materials, large amounts of methanol, 3 mL in the case of concrete, were used to submerge and rinse the building materials. This amount of methanol had to be decreased for analysis, so evaporation was used. The samples were evaporated down to a 250 µL mark placed on a polypropylene centrifuge tube. The samples were run on different days and not all sample levels could be compared to one another. Not being able to compare the sample liquid amounts visually, as well as the shape of the tubes and human error in the interpretation of the level liquid in the tubes, leads to different amounts of solution being analyzed. The evaporation method lends to inaccuracy in the amount of solution and therefore the concentration of HMTD in the samples being analyzed and the final determination of HMTD present in the sample.

5.8 Proliferation of Buildup Within the Liquid Chromatography - Tandem Mass (LC/MS/MS) Spectrometry System

Prior to the final time period of the degradation study and prior to the samples being run for the low level building extractions and the recovery study, a problem with the LC/MS/MS had to be addressed. The sample did not appear to be being analyzed by the mass spectrometer. The LC/MS/MS had a buildup of a coating on the face of the mass spectrometer and around the inlet in the ionization source.

The buildup could be from the HMTD extractions being analyzed, a caution for use of this method. The buildup could also have been from a myriad of other substances analyzed on the LC/MS/MS or a combination of the samples that have been analyzed. It is recommended that if HMTD is being analyzed using the LC/MS/MS that data are examined throughout the study and compared for anomalies, namely suppression of the

analyte peak areas. A cleaning of the faceplate with a mild acid solution, rinsed gently with methanol, and wiped thoroughly will prevent this buildup and anyone attempting follow on use of these methods would be wise in cleaning the source regularly.

The buildup on the faceplate could have affected my results by lowering the analyte peak areas in samples that were run approaching the time of the LC/MS/MS problem. Also, the analyte peak areas produced by samples prior to cleaning and the samples run after the cleaning cannot be compared based on the analyte peak areas alone. Looking at the recovery study, extractions from carpet as an example, the average analyte peak area for comparable samples prior to cleaning was 3.98E+4 counts, while after cleaning it is 7.50E+5 counts. If the recovery extractions are carried out using the analyte peak areas from samples run prior to cleaning the LC/MS/MS compared to samples analyzed after the cleaning of the LC/MS/MS the recoveries are 4.29% compared to 80.77%, respectively.

5.9 Significance of a Method for the Analysis of HMTD and its Degradation Rate

The destructive power and relative ease of manufacture makes peroxide explosives appealing (Widmer, et al., 2002). Peroxide explosives are easy to manufacture (Xu, et al., 2004) with common chemicals that are inexpensive to purchase (Cotte-Rodrıguez, et al., 2008) with recipes easily referenced in publications (Davis, 1943) and on the Internet (Xu, et al., 2004).

Events like the “bomb factory,” filled with the largest quantity of homemade explosives in a single location in the United States history (Wright & Schone, 2011), point to the need for analytical methods capable of detecting trace quantities of peroxide

explosives. With the increasing use of peroxide explosives (Laine, et al., 2007)., methods and techniques need to be developed to identify peroxide explosives where they are manufactured, in deactivated bombs, and in bomb postblast residues.

If trace amounts of HMTD can be collected and analyzed where HMTD is being manufactured or being integrated into an explosive device it could provide a link between the person manufacturing the HMTD or building an explosive device. By extracting HMTD from building materials HMTD may be recoverable in a place of explosive manufacture or assembly even after a cleaning of the area, similar to methamphetamine detection after methamphetamine production. After the manufacture of explosives and the assembly of explosives, it is important to understand how long there is viable evidence tying the perpetrator to the transgression, as with any other criminal offense. This is where the degradation study comes in. By having an idea how long HMTD can be recovered, and from which building materials, resources can be best applied to the collection and analysis of the building material most likely to provide the best results.

5.10 Comparing Other HMTD LC/MS/MS Methods with This Method

Probably due to the inherent dangers presented with peroxide explosives, there is a limited amount of information on peroxide explosives. A method was not found in the literature comparable to the method developed here. HPLC is more appropriate for the study of peroxide explosives like HMTD, which are thermally labile, or not volatile enough for analysis with GC/MS. Currently, along with other methods of analysis, HMTD has been identified using HPLC/MS in atmospheric pressure chemical ionization (APCI) positive mode. These methods analyze solutions of HMTD in methanol or acetonitrile and calculations based on those solutions (Xu, et al., 2004) (Crowson &

Beardah, 2001). The method developed in this study has LOD and LOQ values calculated based on extractions from building materials. This method also uses a different ionization source then the other methods found.

5.11 Future Work

In the literature, there is a limited amount of information on peroxide explosives, probably due to the inherent dangers presented with peroxide explosives. With such a limited amount of information, there is so much work that could be done with peroxide explosives.

Further work could be done on more building materials, or materials commonly found in homes that may be contaminated with HMTD. Other types of the building materials could also be looked into. Carpet, for example, comes in many types of materials, weaves, and backing materials. All of those variations could cause HMTD to extract differently, giving different data. Wood also has many variations; finish, cut (based on grain), smoothness, and hardness of the wood may affect the extraction of HMTD.

To remove the HMTD from the building materials multiple extraction processes were employed. The extraction processes themselves could be refined in future work in hopes of reducing the variable in amount of HMTD extracted from samples and increasing the amount of HMTD extracted from samples. In the case of concrete, only 6-10% of HMTD was recovered with the extraction method used, with an 8% standard deviation. If a method could be developed to extract more HMTD, maybe through a better method to pulverize the concrete and thereby free the HMTD, then concrete may become a more viable building material for the recovery of HMTD.

Future work could also be done to refine or eliminate the evaporation step in the extraction process. All four building material extraction methods depend on an evaporation step. This evaporation step lends to the variability in the amount of solution, and concentration of HMTD in the solution, being analyzed. By finding a way to either remove the evaporation step, or refine the process to be more precise or at the least comparable among samples, the variation in the data would be lessened.

A buildup on the face of the mass spectrometer was encountered in this study. This build up prevents the data gathered before cleaning to the data after cleaning from being accurately compared. It is unclear what the buildup was composed of, or even what it was from. This method could be repeated in future work, in an effort to see if the buildup is produced. If the buildup is produced, information on the rate of buildup may be useful. Also, the LC/MS/MS method as well as the extraction methods could be altered in an effort to reduce the amount of build up or eliminate it entirely. Without the buildup the method would be much more user friendly and practical for further application.

It is believed that manufacturing HMTD broadcasts the HMTD throughout the place of manufacturing like during a methamphetamine cook. This theory has not been proven. Experiments need to be done to prove that HMTD is dispersed throughout the place of manufacture. Experiments could also be done to determine the amount of HMTD deposited onto the building materials.

5.12 Conclusions

The work presented here demonstrates a sensitive LC/MS/MS method for the detection of HMTD; it has been applied to construction materials to determine likely

evidential sources. The degradation and recovery time of HMTD on each building material was used to then establish which materials would most likely have detectable residues of the explosive in the event of an investigation. In an area where HMTD may have been produced the best samples to be collected for the recovery of HMTD are those of carpet. Carpet will yield the highest amount of HMTD for the longest amount of time when compared to wood, concrete, or drywall. If carpet is unavailable for collection, other building materials should be collected. After carpet samples, drywall has the highest amount of HMTD recovered, however wood samples yield detectable HMDT for a longer period of time. Concrete samples yield the lowest amount of HMTD throughout the study and showed a slightly shorter timeframe for detection when compared to drywall, making it the worst of the four samples in yield and timeframe for recovery.

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VITA

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Master of Science

Thesis: DEVELOPMENT OF A LIQUID CHROMATOGARPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS) METHOD FOR THE ANALYSIS OF PEROXIDE EXPLOSIVES ON BUILDING MATERIALS

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Title of Study: DEVELOPMENT OF A LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS) METHOD FOR THE ANALYSIS OF PEROXIDE EXPLOSIVE RESIDUES ON BUILDING MATERIALS

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Scope and Method of Study: The purpose of this research was to develop and validate a method for the detection of low levels of HMTD extracted from building materials using liquid chromatography with tandem mass spectrometry (LC/MS/MS). Method validation was performed to determine precision, accuracy, sensitivity, and selectivity. For each building material an extraction method was developed, and the percent of HMTD recovered through extraction was determined. Carpet, wood, concrete, and drywall samples were spiked with a known amount of HMTD analytical standard and the HMTD was then periodically extracted from each of the different materials and analyzed using LC/MS/MS. The amount of HMTD recovered from the building materials was then assessed to determine the degradation and recovery time of HMTD and establish which materials would most likely have detectable residues of the explosive in the event of an investigation.

Findings and Conclusions: The LC/MS/MS method was successfully developed to detect low amounts of HMTD that was extracted from carpet, wood, concrete, and drywall. The method utilized isocratic flow rate with electrospray ionization and three ions representative of HMTD in multiple reaction monitoring (MRM) mode. The limit of detection for carpet was 0.18 µg, wood was 0.60 µg, concrete was 0.19 µg, and drywall was 0.14 µg. Carpet extractions had the highest percent of HMTD recovered (81%), while drywall (32%), wood (18%), and concrete (8%) extractions showed much lower recovery. This study indicates that in an area where HMTD may have been produced the best samples to be collected for the identification of HMTD are those of carpet, as carpet will yield the highest amount of HMTD for the longest amount of time when compared to wood, concrete, and drywall. If carpet is unavailable for collection, other building materials should be collected. After carpet samples, drywall has the highest amount of HMTD recovered; however, wood samples yield detectable HMTD for a longer period of time. Concrete samples yield the lowest amount of HMTD throughout the study and showed a slightly shorter timeframe for detection when compared to drywall, making it the least likely of the materials to contain HMTD residues in an investigation.

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