

**SEPARATION, IDENTIFICATION, AND QUANTIFICATION OF
EXPLOSIVES USING THIN LAYER CHROMATOGRAPHY
COUPLED TO MID-INFRARED LASER SPECTROSCOPY**

by
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ABSTRACT

The need for rapid instrumentation and methods for detection and identification of chemical and biological threat agents in national defense and homeland security settings has become an ever more important issue in modern society. Many of the samples collected for the sensing of explosives are contained in soil matrices. Soils from these media can contain mixtures of energetic compounds distributed heterogeneously. Other common samples that can be targeted for detection of explosives are debris surfaces; these surfaces can be metallic, plastic, wood, cardboard, fabric, etc. This work focused on the development of a new separation and identification technique using thin layer chromatography (TLC) hyphenated to mid-infrared (MIR) laser spectroscopy. The technique provides a practical, low cost, fast, robust, and reproducible method for screening of explosives, followed by identification and quantification.

Direct comparison of trinitrotoluene (TNT) spectra acquired by the new hyphenated technique and with Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) coupled to TLC was carried out. TNT solutions in a 0.39 – 100 μg of deposited mass were prepared and tested. The MIR laser method was evaluated by calculating the analytical figures of merit in terms of linearity of calibration curves, sensitivity, and precision. TNT spectra showed two characteristic bands of the explosive about 1350 and 1550 cm^{-1} when compared to spectra acquired by ATR-FTIR. The detection limit for TNT was 74 ng while the quantification limit was 224 ng. Multivariate analysis routines evaluated the spectroscopic data to find the sources of variation and determine how they were related. Partial least squares (PLS) regression analysis and PLS combined with discriminant analysis (PLS-DA) was used. Finally, to evaluate our hyphenated technique for detection

of explosives in soil, simulated contamination samples of TNT in soil were prepared and analyzed successfully. Experiments demonstrated that the method could serve as an excellent platform to devise analytical methods useful for the identification and quantification of chemical targets.

RESUMEN

La necesidad de instrumentos y métodos de rápida respuesta para la detección e identificación de agentes químicos y biológicos en entornos de defensa y seguridad nacional se ha convertido en un tema cada vez más importante en la sociedad moderna. Muchas de las muestras recolectadas para la detección de explosivos están contenidas en matrices de suelo. Estos suelos pueden contener mezclas de materiales energéticos distribuidos de manera heterogénea. Otras muestras comunes que pueden ser objetivos para la detección de explosivos son las superficies de escombros, superficies metálicas, superficies plásticas, superficies de madera, cartón o tela. Este trabajo se centró en el desarrollo de una nueva técnica de separación e identificación mediante cromatografía de capa fina (TLC) acoplada a espectroscopia láser de infrarrojo medio (MIR). La técnica proporciona un método práctico, de bajo costo, rápido, robusto y reproducible para la separación de explosivos, seguido de identificación y cuantificación. Se llevó a cabo una comparación directa de los espectros de trinitrotolueno (TNT) adquiridos por la nueva técnica y con la técnica de reflexión total atenuada con infrarrojo de transformada de Fourier (ATR-FTIR) acoplado a TLC. Se prepararon y probaron soluciones de TNT que dieron como resultado unas masas depositadas de 0.39 - 100 μg . El método láser MIR se evaluó calculando las figuras mérito en términos de linealidad de curvas de calibración, sensibilidad y precisión. Los espectros de TNT mostraron dos bandas características del explosivo de aproximadamente 1350 cm^{-1} y 1550 cm^{-1} en comparación con los espectros adquiridos por ATR/FTIR. El límite de detección para TNT fue de 74 ng, mientras que el límite de cuantificación fue de 224 ng. Las rutinas de análisis

multivariable fueron usadas para evaluar los datos espectroscópicos, encontrar las fuentes de variación y determinar cómo estos se relacionaban.

Se usó análisis de regresión de mínimos cuadrados parciales (PLS) y PLS combinado con análisis discriminante (PLS-DA). Finalmente, para evaluar nuestra técnica para la detección de explosivos en el suelo, se prepararon y analizaron muestras simuladas de suelo contaminado con TNT. Los resultados demuestran que el método puede servir como una excelente plataforma para diseñar nuevos métodos analíticos útiles para la identificación y cuantificación de estos agentes químicos.

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DEDICATORY

I want to dedicate this work to my family and friends. Thanks for all your love, help and support. You are my motivation to continue fighting every day to achieve my personal and professional goals.

I also dedicate this thesis to my dear friend, Jose Rafael Atilano-Lopez (1993-2017). I thank God for being able to meet you. Thanks for all the experiences we had together and for everything you showed me. Keep smiling in heaven as you always did.

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 INTRODUCTION

There is an urgent need for instrumentation that can sustain the development of rapid methods for the isolation, detection, and identification of traces of chemical and biological threat agents present in complex media. For anti-terrorist personnel, to first responders and law enforcement employees, police officers, airport screeners, and border patrol personnel, as well as members of the Navy, Army, Air Force, and National Guard, the risk of encountering explosives and other weapons of mass destruction is high. Many of the samples collected for the forensic detection of explosives come from complex matrices that contain soil, dirt and other interfering substances. Additionally, soils can be contaminated with explosives by several human activities, such as the use of explosives on training ranges, sites for the synthesis of explosives, as a result of conflicts between nations, from used waters and wastes in clandestine laboratories, and as a result of terrorist events, among others. Other common substrates that can be targeted for the detection of explosives include debris, metals, plastics, woods, cardboard, fabrics, and so forth.

Most studies that have been published on the detection of explosives are based on spectroscopic and chromatographic methodologies, which obtain very low limits of detection. However, the use of chromatographic techniques in field applications has been limited, primarily because of the lack of portable instrumentation. Conversely, spectroscopic techniques have the advantage of being tested in field applications,

facilitating the rapid acquisition of data and information, thereby leading to prompt decisions based on the results obtained and thus saving numerous lives and reducing casualties. Vibrational spectroscopy has been demonstrated to be valuable for the detection of high explosives, homemade explosives, and toxic industrial compounds. Infrared spectroscopy (IRS) has played a unique role in the detection of threat compounds and has been used for post-blast detection of energetic materials. The Mid-infrared (MIR) spectral region consists of the spectral window from approximately 350 to 4000 cm^{-1} . In this range, all molecules have characteristic vibrational signals that can be excited upon interaction with photons from the excitation source, enabling the detection of trace amounts of compounds. [1]

The proposed hyphenated technique: thin layer chromatography (TLC) coupled to Mid-infrared spectroscopy as a portable joined technique for the analysis of explosives, would be most commonly used in two situations: (1) post-blast examination and (2) identification of suspected explosive materials (pre-blast analyses on bulk material). In a post-blast case, preliminary results can lend support to the link between multiple incidents or between a terrorist incident and the organization potentially responsible for the incident. This portable coupled technique can provide critical information for the identification of a suspected explosive material. In these situations, portable instrumentation has a two-fold advantage: (1) the speed with which results can be obtained, and (2) eliminating the need to transport potentially dangerous materials to a laboratory. Identification at a scene enables informed decisions to be made concerning render-safe procedures and the transportation of materials. This is of importance when extremely sensitive explosives, such as organic peroxides, are suspected. When portable instruments are utilized during

searches authorized by search warrants, the preliminary results can be used to indicate areas where more efforts should be directed. The preliminary results can also provide advanced warning about which types of explosives may be encountered at a scene and hence enable searchers to be better informed of the safety risks at a scene.

1.2 OBJECTIVES

In the proposed investigation, a mid-infrared (MIR), laser spectroscopy methodology, coupled with thin layer chromatography (TLC) was employed to detect high energetic materials (HEMs). The technology proposed to detect these residues is based on the use of a portable quantum cascade laser (QCL) spectrometer. In this work, a methodology that allows for the detection of explosives, such as TNT, present in real-world samples (i.e., soils) and complex substrates using TLC-QCL is demonstrated. The tested methodology enabled the rapid and reproducible separation and identification of targeted explosives at near trace levels (~ ng) in the field in a short amount of time. The results show that TLC-QCL, as a coupled technique, is an excellent approach to use in the lab or the field. Multivariate analysis (MVA) routines were used to evaluate the spectroscopic data to find the sources of variation and determine how they were related. Partial least squares (PLS) regression analysis and PLS combined with discriminant analysis (PLS-DA) were used.

CHAPTER 2

FUNDAMENTAL PRINCIPLES

2.1 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a well-established method for separating compounds in non-volatile mixtures. It is considered a semi-micro to a micro qualitative analytical technique. When the technique is applied for chemical explosives residues, the minimum analyte detected currently can be of the order of micrograms. The technique provides a streamlined sampling and testing protocol that allows rapid, reproducible, separation and identification of drugs, explosives, and precursors, with extended use to a wide range of hazardous materials obtained from substrates, liquids, and solids for laboratory and field operations. Separated compounds can be detected by a chemical reaction with a chromogenic agent, response to UV light, or other physical means, and in the same way, it is possible to calculate the retention factor (R_f). The retention factor (R_f), describes the distance that each compound traveled through the column and each one is observed as a spot in the TLC. [2] TLC can be used to monitor the progress of a reaction, identify compounds in mixtures, and determine the purity of substances. Considering that the mobile phase can be manipulated, the organic compounds can be separate. In this research, the TLC contains Silica Gel as the stationary phase, and it will be coated as backing on an aluminum substrate. TLC function is a compound which has different affinities for the mobile and stationary phases, and this affects the speed where it migrates. The goal of TLC is to obtain well defined, well-separated spots. Specific examples of these applications include detection of pesticides or insecticides, in forensics, or identification of medicinal plants and their constituents.

2.2 INFRARED SPECTROSCOPY (IRS)

As a sensitive probe of important molecular motions, vibrational spectroscopy is defined as the study of the interaction of light with organic and inorganic molecules. [3] A breakthrough in the 1800s by Sir Frederick William Herschel provided documented evidence of the presence of infrared radiation. [3] However, the observation of molecular vibrational spectra started during the late 20th century before the invention of much more powerful excitation sources, such as lasers. The three regions of the infrared region consist of the near-IR (NIR: 12,500 to 4000 cm^{-1}) can excite harmonic, and overtone vibrations, the mid-IR (MIR: 4000-400 cm^{-1}) may be used to study the fundamentals vibrations and the far-IR (FIR: 400-40 cm^{-1}) near the microwave region and has lower energy [4].

The MIR region provides significant fingerprint qualitative information of chemical structures from 650-1400 cm^{-1} . Therefore, both academia and the industry have implemented and developed this technique. The results are evidenced upon the broadened areas of research which have described the molecular structure, conformation, and dynamics in recent years. The technique provides three main data collection setups: attenuated total reflection (ATR), transmission, and reflection [5]. In recent years, infrared spectroscopy has been coupled with multivariate analysis (chemometrics) due to how powerful the coupling has become. In the branch where this combination has been most used is in the pharmaceutical industry due to how powerful chemometrics is when it comes to extracting relevant information from the data.

2.3 QUANTUM CASCADE LASERS

Quantum cascade lasers (QCLs) were designed at Bell Labs by Federico Capasso, Alfred Y. Cho, and their collaborators (1994). [6] A QCL, is a semiconductor laser that differs fundamentally from diode lasers. This is because QCL relies on one type of carrier: electrons. The QCL is also called unipolar semiconductor laser, which electronic transitions between conduction band states convert electrical energy into electromagnetic radiation, essentially in the entire MIR and into the FIR regions. These semiconductor lasers are based on superlattice heterostructures comprised by indium, gallium, and arsenic (InGaAs) or indium, aluminum, and arsenic (InAlAs). [3,4]

The QCL spectrometer used in this study contains a highly sensitive, thermoelectrically cooled mercury-cadmium-telluride (MCT) detector as part of the complete system. The use of quantum cascade laser spectroscopy (QCLS) has proven to be an effective tool to measure traces of organic materials. [7] QCLs can be tuned to operate *in situ* in standoff detection (outside the sample compartment), they often present detection potentials on a varied of substrates that may mask the signals of the chemical or biological organism of interest. [7] Detection limits (DLs) for QCLs instruments have been reported as low as parts-per-billion. [8] Another advantage of QCLs is portability. Multivariate analyses (MVA), such as principal component analysis (PCA), partial least squares (PLS) regression, discriminant analysis (DA) and combined MVA algorithms, such as PLS-DA, constitute branches of chemometrics that can be used to generate models that have very high values of sensitivity and selectivity. [9,10]

2.4 HIGHLY ENERGETIC MATERIALS (HEMs)

Explosives are substances that react chemically evolving a large amount of heat and exerting a high pressure of its surroundings if exposed to friction, impact, flame, heating or any other application in where it has high sensitivity. The explosives can be classified either as a low and high explosive [11]. In this project, the primary purpose is to get the optimization of a mobile phase in which a good resolution in the R_f values can be reached after separation of the explosives. Through the years, there has been an interest in the research topic of detecting and identifying different types of explosives or well known as a highly energetic material (HEM). The term HEM is used for any material that can be reached a highly energetic state mostly by synthesis or analysis in chemical reactions [12]. The number of reports on the use of these materials (HEM) in recent years has grown exponentially. The most used HEM are 2, 4, 4- trinitrotoluene (TNT), hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX or also known as Research Department Explosive), pentaerythritol tetranitrate (PETN), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and 2,4,7,9-Tetramethyl-decyne-6-diol-4,7 (TMDD). [11] These compounds play important roles in military applications. They provide energetic compounds that can be useful to defense community designing new weapons for military operations using shells, grenades, demolition operations, guns or gas-generators, ammunition, and underwater blasting. Developing this method, we can detect as fast as possible if there is any trace of HEMs on any surface. The project seeks to find the efficient and fast way to separate mixtures of HEMs. Subsequently, by combining TLC with QCL spectroscopy, these samples can be analyzed in the field.

CHAPTER 3

CHEMOMETRICS AND MULTIVARIATE ANALYSES

3.1 CHEMOMETRICS AND MULTIVARIATE ANALYSES

Chemometrics has been defined as a chemical discipline that uses statistical and mathematical methods to design or select optimum procedures and to provide or extract maximum chemical information. Chemometrics as a sub-division of MVA is an emerging field of study within analytical chemistry to present and understand data deviating from classical models. [13] It is based on applications of MVA routines and algorithms. In chemistry and biology, the objective of controlling as many variables as possible is work-intensive and not transferrable to the application. With emerging technologies, univariate analyses may not always prove feasible. Chemometrics is an extension of the univariate analysis to handle multiple variables simultaneously. Using MVA, noise, detector performance, controls, and background signals can be taken into consideration as unwanted signal contributions. For this reason, the use of algorithms to separate these variables according to the instrument response is necessary. Typical problems that can be successfully handled by chemometrics methods are the determination of the concentration of complex mixtures, classification, predictions, recognition, and evaluation of complex data. The inclusion of chemometrics will provide the analyst with information about the system under study, the covariance and the correlation of the data could agree with the experimental objectives.

In 2009, Varmuza and Filzmoser defined chemometrics based on multivariate analyses techniques, as a chemical discipline that makes the use of mathematics and statistics to obtain the most significant chemical information on a massive dataset [14]. Recently, the

use of multivariate analysis has increased in combination with spectroscopic techniques to carry out models of systems of interest and power to discriminate analytes with higher confidence intervals even for a single sample. [15] When chemometrics routines are utilized, complex data can be analyzed, and accurate results can be obtained in a short time. There are many methods of chemometrics analyses that can be used to determine how your data sets are interrelated and to find sources of variation. The principal component analysis (PCA) is an advantageous method frequently used to determine the causes of the system's variation under scrutiny [16]. PCA considers all the variables and is considered an exploratory data analysis. Another method is partial least squares (PLS) which is useful to make a concentration model and then very helpful to predict the concentration of unknown samples. Some advantages in the use of PLS: (a) it can be used even when the spectral bands overlap, (b) much faster, and (c) may be used for non-concentration properties, among other advantages [16].

3.2 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis is the most common method used in MVA. It is an exploratory method. The aim of PCA is the reduction of dimensionality of the problem. PCA is an algorithm that recognizes the spectra as variation in patterns, performs a mathematical decomposition of the patterns, and reconstructs the patterns using orthogonal vectors. PCA performs multivariate data reduction by transforming the data into orthogonal components (uncorrelated to each other) which are linear combinations of the original variables. PCA is mainly used for: visualization of the data, the

transformation of the latent variables (LVs) and separation of valuable information from noise. The maximum variance of the scores is defined by the first principal component (PC1) and the second principal component (PC2) is defined as an orthogonal direction to PC1 and again having the maximum possible variance. PCA is recommended for use before doing applying any other method due to the sensitivity that it has to detect outliers which is advantageous to know if one is on the “right path”.

3.3 PARTIAL LEAST SQUARES -DISCRIMINANT ANALYSIS

Chemometrics analysis methods, such as PLS and PLS-DA, were used to develop a multivariate sensor. This allowed achieving a more robust spectral identification, classification, and quantification of target chemical compound. In this study, PLS Toolbox, v. 8.1 (Eigenvector Research Inc., Manson, WA, USA) for Matlab (The MathWorks, Inc., Natick, MA, USA) was used to analyze the spectral data. Partial least squares combined with the discriminant analysis is a supervised pattern recognition method and one of the most widely used chemometrics tools, mainly when the goal is to discriminate, classify, and identify spectral similarities in a multivariate dataset. [17-20]. It is a linear classification method that combines the properties of PLS regression with the discrimination power of a classification technique. The method is based on the PLS regression algorithm, which searches for LVs with a maximum covariance between a descriptor matrix \mathbf{X} and a response matrix \mathbf{Y} (containing the membership of samples to the \mathbf{G} classes expressed with a binary code: 1 or 0). The primary advantage of PLS-DA is that the relevant sources of data variability are modeled using LVs, which are linear combinations of the original variables. Thus, the MVA methodology allows the graphical

visualization and understanding of the various data patterns and relations using LV scores and loadings. The scores represent the coordinates of the samples in the LV projection hyperspace. [19, 20]

The first step is to arrange the spectral data in a matrix with dimensions of $n \times m$ (n rows and m columns), where n represents the number of samples used in the calibration set and m represents the number of spectral variables (wavenumbers in units of cm^{-1}) in the study. In this study, the matrix dimensions were 170×3056 for the calibration set. The second step is to apply the required preprocessing handling of the data. The purpose of preprocessing the data is to remove or to decrease the effect of the interference from the background and substrate, thereby enhancing the vibrational signatures of the TNT. We used PLS-DA to decompose the original matrix into two new matrices: one that contained most of the relevant information including the most significant variability in the data and a second one that provided information not as appropriate to the data, generally termed noise. The matrix containing the information of interest had a dimension of $n \times p$, where p is a new column matrix called the LV, which is the product of the transformation from the original variables. The LVs are orthogonal vectors that are linear combinations of the original data. In general, two or three LVs are enough to capture most of the variability in the data. In the LVs, each sample that was initially represented by an IR spectrum is now represented by only one value, termed the score of the sample. These scores describe how the samples are related to each other. Score plots were obtained by graphing LV2 against LV1. These plots provide essential information regarding how various samples are associated with each other [17]. Finally, the original matrix with dimensions of $170 \times$

3056 for the calibration set tested was reduced to an array with dimensions of 170 x 2 using two LVs.

In PLS-DA, the optimal number of LVs that offers the best classification for the calibration set is determined using cross-validation procedures. We used the Venetian Blind (VB) technique with 10 group splits to divide the calibration set into cross-validation groups. We applied several preprocessing steps to the data to generate multivariate models capable of discriminating among and clustering the spectra based on chemical similarities, (i.e., Explosive, and No Explosive). The preprocessing steps used were auto-scaling (AS), mean centering (MC), standard normal variate (SNV), first and second derivatives, and combinations of them. Different spectral ranges comprised between 1000-1600 cm^{-1} were analyzed. Of all the multivariate models generated, the ones that performed best were those that used the spectral range (1302.4 - 1388.4 cm^{-1}).

For PLS-DA, classification parameters such as sensitivity (SEN) and specificity (ξ), derived from the confusion matrix from the calibration, cross-validation, and prediction sets, to evaluate the performance of the classification models were used. Sensitivity describes the ability of the model to correctly recognize samples that belong to that class, and specificity describes the model's ability to reject samples of all other classes. These can be calculated as follows:

$$SEN = TP / (TP + FN) \quad (1)$$

$$\xi = TN / (TN + FP) \quad (2)$$

where TP is the number of true positives, TN is the number of true negatives, FP is the number of false positives, and FN is the number of false negatives. When dealing with two classes A and B, where A is positive (a specific HEM, In this study TNT) and B is

negative (another HEM or the clean substrates), TP is the number of members of class A that have been correctly classified, FP is the number of members of class B that have been incorrectly predicted as class A, TN is the number of members of class B that have been correctly classified, and FN is the number of members of class A that have been incorrectly predicted as class B. Both SEN and ξ take values between 0 and 1, where 1 is the desired result. [18]

CHAPTER 4

LITERATURE REVIEW

4.1 LITERATURE REVIEW

This investigation was based on several previous contributions, which served to generate the ideas for this project. There are several contributions where spectroscopy coupled with TLC have been used. A small portion of these is included as reference:

In 1993, Nawrocki et al. evaluated the chemistry associated with zirconia and its use in chromatography. Moreover, the authors discovered that zirconia could be used as a stationary phase in TLC coupled to IR and produced even better results than silica and alumina as a stationary phase. [21]

In 2011, Sabino et al. investigated the used of TLC coupled to easy ambient sonic-spray ionization mass spectrometry (EASI-MS) for the analysis of cocaine and crack. In this study, Sabino and collaborators concluded that the combination of TLC with EASI-MS inspection seems to provide a powerful combination for forensic investigation of illicit drugs reducing the risks of false positives and false negatives. [22]

In 2011, Erçağ et al. Rapid detection of nitroaromatic/nitramine explosives PVC tablets on chromatographic paper and reflectometry sensing on. Discovered a quantitatively and qualitative method using chromatographic paper using sprayed reagents to produce colored spots. Target explosives TNT, Tetryl, DNT, RDX, and HMX. [23]

In 2013, Yao et al. studied the possibility of separating, identifying and carrying out a rapid determination of organophosphate pesticide methidathion in tea leaves by TLC–surface-enhanced Raman scattering (TLC-SERS). Moreover, the results presented herein demonstrated that the proposed technique is an excellent candidate for simultaneously detecting various organophosphate pesticides in tea, both qualitatively and quantitatively [24]

In 2014, Zhang et al. published an article entitled “Thin Layer Chromatography Coupled with Surface-Enhanced Raman Scattering as a Facile Method for On-Site Quantitative Monitoring of Chemical Reactions.” They developed a TLC-SERS method for on-site quantitative monitoring of chemical reactions. [25]

CHAPTER 5

ANALYTICAL TECHNIQUES AND EXPERIMENTAL SETUP

5.1 PROJECT DESCRIPTION

The main objective of this contribution was to design methodologies for detection, identification, and quantification of various types of explosives: TNT, PETN, and RDX. Figure 1 shows the analytical techniques included in this study.

5.2 MATERIALS AND METHODS

Reagents that used were HEMs such as PETN, RDX, and TNT. PETN and RDX were prepared in the laboratory. The other explosive 2,4,6-trinitrotoluene (TNT min. 30 wt.% water) was being acquired from Chem Service (West Chester, PA, USA). Acetone (99.5%, GC grade) was purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used as solvent to prepare standard HEMs solutions. All solvent used as mobile phases were reagent grade and included acetone, hexane, ethyl acetate, toluene, methanol, and 1,2-dichloroethane purchased from Sigma-Aldrich or Thermo-Fisher Scientific. Silica gel coated TLC plates with aluminum support (Merck, TLC Silica gel 60 F254) were used for chromatographic runs.

5.3 EXPERIMENTAL SETUP

The spectral range covered by the QCL spectrometer was 990-1650 cm^{-1} . The type of setup evaluated was the direct measurement from QCL to sample, in back-reflectance mode. Comparison of the spectra obtained by the QCL with spectra obtained with a

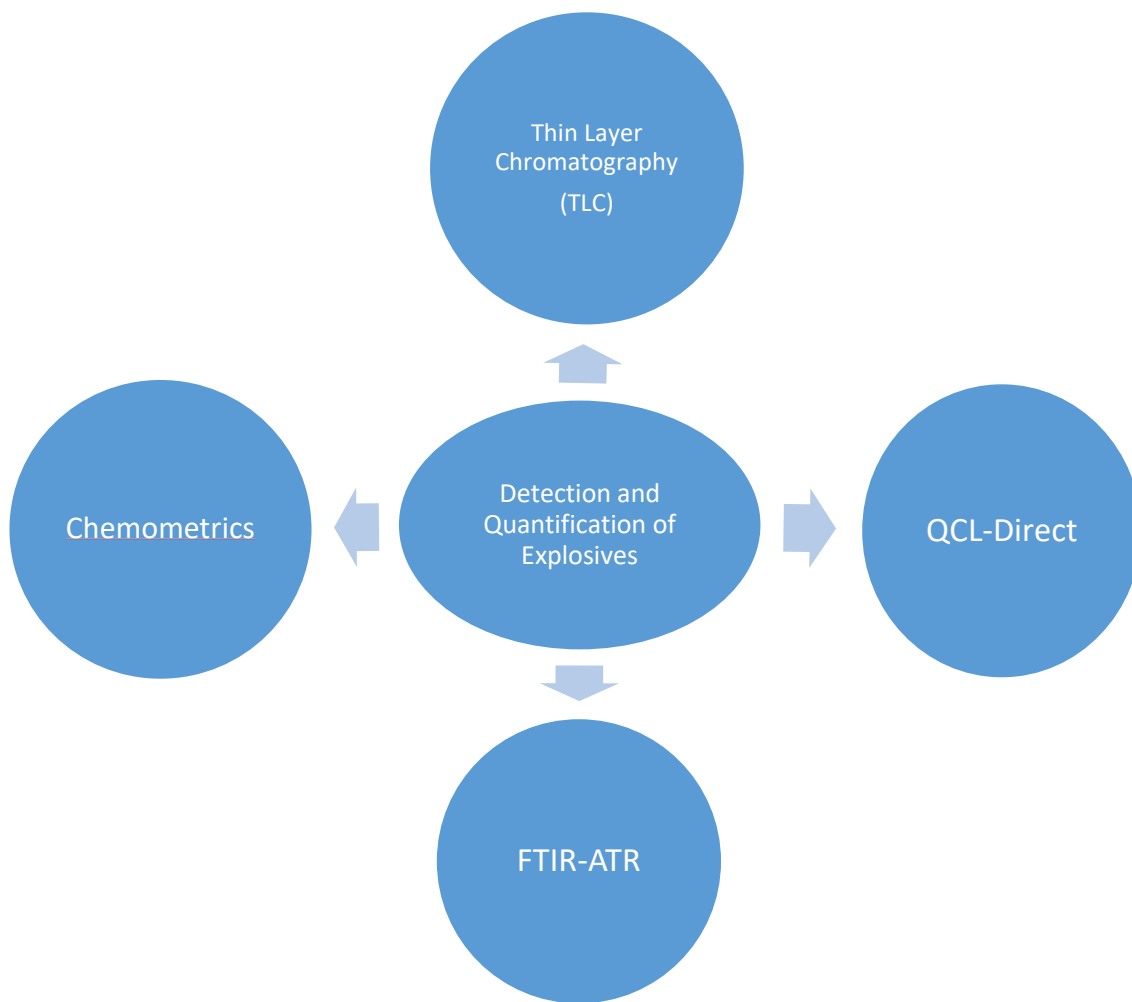


FIGURE 1. Analytical techniques used in this study.

Spectrum Two Spectrometer ATR-FTIR (Perkin Elmer). Spectra were acquired at the focal point (~ 6 in. from the QCL lens) for direct measurements. The surface was examined with two co-adds per TLC plate repeating the step to obtain 25 spectra for each sample type. Spectra will be exported to OPUS®, MATLAB and Unscrambler® for the application of corresponding MVA statistical methods.

5.4 MULTIVARIATE STATISTICAL TOOLS

Several calibration models were generated using OPUS v. 6.5 spectroscopic suite for data acquisition and analysis (2007, Bruker Optics, MA, USA). These models were based on partial least squares (PLS) regression algorithm, allowing the prediction of surface concentration values of the chromatographic “spots”. Also, principal component analysis (PCA) was used to understand the target system: HEMs, mixtures, and substrates or stationary phases. Discrimination models were carried out using the combined algorithms of partial least squares-discriminant analysis (PLS-DA). These are part of the PLS_Toolbox 7.9 software package (Eigenvector Research, Inc., Wenatchee, WA, USA) running on the MATLAB® 7.13 platform (R2011b; The Math Works, Inc. Natick, MA, USA).

CHAPTER 6

HEMs ANALYSIS USING TLC-MIR LASER SPECTROSCOPY

6.1 ABSTRACT

This work focused on the development of a new technique separation and identification technique using thin layer chromatography (TLC) hyphenated to mid-infrared (MIR) laser spectroscopy. The technique provides a practical, low cost, fast, robust, and reproducible method for screening of explosives, followed by identification, and quantification. Direct comparison of trinitrotoluene (TNT) spectra acquired by the new hyphenated technique and with attenuated total reflection-Fourier transform infrared (ATR-FTIR) coupled to TLC was also carried out. TNT solutions in a 0.39-100 μg of deposited mass were prepared and used in the chromatographic experiments. The MIR laser spectroscopy (MIRLS) method was evaluated by calculating the corresponding analytical figures of merit (FM) in terms of linearity of the calibration curves, the sensitivity of the developed methodology, and the precision when compared to what is considered a primary method: ATR-FTIR. TNT spectra showed two characteristic bands of the explosive about 1350 cm^{-1} and 1550 cm^{-1} when compared to spectra acquired by ATR/FTIR. The detection limit for TNT was 74 ng while the quantification limit was 224 ng. MVA routines were used to evaluate the spectroscopic data to find the sources of variation and determine how they were related. Partial least squares (PLS) regression analysis and PLS combined with discriminant analysis (PLS-DA) was used.

Finally, to evaluate the developed hyphenated technique in the detection of explosives in soil matrices, simulated contamination samples were prepared and analyzed using the TLC-MIRLS, demonstrating that the methodology can serve as an excellent platform to devise analytical methods useful for identification and quantification of chemical targets, such as chemical agents, illicit drugs, insecticides, persistent organic pollutants, and other chemicals of interest.

6.2 INTRODUCTION

From decades ago published scientific literature on explosives detection has been mostly based generally on the spectroscopic based method of analysis. [26-31] A critical contribution of these general methods is that each one separately could obtain very low limits of detection. Concerning the applications and use of chromatographic techniques in the field, they have been both, because of their non-portability of the instrumentation required to implement the various separation techniques, and partly due to the high cost of the detection schemes needed for coupling to the detection schemes coupled to them. Thin layer chromatography (TLC) is characterized by providing a streamlined sampling and testing protocol that allows rapid, and reproducible, separation of a wide range of hazardous materials (drugs, explosives, and precursors) taken from surfaces, liquids, and solids for laboratory and field operation.

Spectroscopic techniques on the other end have has the advantage of being tested in standoff/remote sensing scenarios, facilitating the acquisition of data quickly, leading to prompt decisions based on the information obtained, thus reducing casualties and saving numerous lives. Vibrational spectroscopy has demonstrated to be valuable for a variety

of applications related to the detection and characterization of threat compounds such as chemical warfare agents, high variety of explosives, including homemade explosives, and toxic industrial compounds [26, 32-41].

The MIR region of the electromagnetic spectrum is the domain of vibrational spectroscopy and comprises the spectral window from approximately 350 to 4000 cm^{-1} . In this spectral range, all molecules have characteristic vibrational signals that can be excited upon interaction with photons from the energy sources, enabling the detection of trace amounts of compounds. [42] The limitation of MIR spectroscopy has been related to the lack of polychromatic sources of high brightness (optical power). The search for more powerful MIR encountered an important milestone in 1994 when scientists at Bells Labs invented quantum cascade lasers (QCLs) which included as properties: highly monochromatic, collimated, coherent, and polarized. [43,44] Nowadays, QCLs are widely tunable commercially available sources, and portable setups based on them allow for detection of chemical and biological threat compounds of high interest in the field. [45-49]

When reference spectra are absent, valuable information about the molecular structure of the analyzed compound may be obtained by spectral interpretation. Coupling broadly tunable MIR lasers to TLC can provide valuable information for the identification of unknown materials that may be encountered at a crime scene, including unexploded explosives, and hence enable personnel to be better informed about the safety risks to be taken. The QCL-TLC focusing as a portable hyphenated technique for explosives analysis has not been reported yet. This hyphenated instrumentation would be most useful under two possible situations: (i) pre-blast analyses on bulk material, (ii) post-blast examination. Overall, portable instrumentation has a two-fold advantage: (a) the speed

with which results can be obtained and (b) avoiding the need to transport potentially dangerous materials back to a central laboratory.

Thus, the primary objective of this work was to couple IRS as a detection/identification technique to TLC which arises from the need to identify separated components reliably. The first *in situ* FTIR detection of chromatographic spots on a TLC plate was reported in 1975 by Percival and Griffiths. [50] A thin layer (100 μm) of adsorbent on an IR transparent AgCl support allowed IR transmission measurements of dyes and amino acids at the 1-10 μg level. Thus IRS-TLC technique changes from a qualitative analysis (TLC used alone) to a confirmatory, quantitative analysis when TLC was coupled to IRS.

For the last four decades, detection limits were improved by application of mulling oil to TLC plates to reduce IR scattering [51, 52]. In 1978 Fuller and Griffiths [53] demonstrated the viability of diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy for the measurements of methylene blue on a silica plate. Since then, several studies have been conducted to explore the potential of TLC-DRIFT analysis [54-57]. These studies, extensively reviewed by Brown and Beauchemin [58], revealed that various conventional TLC phases, such as silica, alumina, cellulose and reversed-phase materials used in combination with DRIFT yielded minimum identifiable quantities (identification limits) down to about 1 μg . The major difficulty encountered in using DRIFT as an *in-situ* detection method for TLC is the strong absorption background of the adsorbent which causes serious interferences spectral regions. For example, silica gel absorbs strongly in the areas from 3700 to 3100 cm^{-1} and 1600 to 800 cm^{-1} , obscuring possible analyte absorptions at these frequencies.

As an important application of the developed hyphenated methodology, a study the separation, identification, and quantification of a HEM contained in a simulated complex contamination mixture of TNT in soil matrices which resemble the real world was carried out. The acquired data were processed with chemometric routines. The approach allowed for rapid and reproducible separation and identification of targeted explosive at near trace level (~ng) in the field, in a short time.

6.3 EXPERIMENTAL

6.3.1 Materials and Reagents

Reagents used in this investigation included highly energetic materials (HME) or explosive such as 2,4,6-trinitrotoluene (TNT) acquired from ChemService (West Chester, PA, USA), pentaerythritol tetranitrate (PETN; prepared in the lab), solvents used in preparing HME solutions: methanol (99.9%, HPLC grade), dichloromethane (CH₂Cl₂, HPLC grade), and acetone (99.5%, GC grade) and the mobile phases for the chromatographic separations: acetone, hexane, cyclohexane, ethyl acetate, toluene, methanol, petroleum ether, dichloromethane, acetonitrile, 1,2-dichloroethane. All solvents used were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Aluminum TLC plates coated with silica gel used for chromatographic runs were acquired from Merck (TLC silica gel 60 F254; Merck & Co., Kenilworth, NJ, US). Chromogenic agent diphenylamine (DPA) (≥99%, ACS reagent, Sigma-Aldrich) was employed as visualization means for TNT spots when required.

6.3.2 Sample Preparation

Developing chambers used for the TLC runs were clear glass jars with lids (5 cm diam., 10 cm height), to which the solvents or solvents mixes were transferred (~5 mL) to a depth of just less than 0.5 cm, for saturation with solvent vapors and were allowed to stand. TLC plates used were cut to a size of 2 cm width x 9 cm tall and were carefully managed to avoid damage or contamination of the adsorbent layer. A reference mark was traced at the bottom of each plate by drawing a line across at 1.5 cm height using a pencil. Ten microliters (10 μ L) aliquots of HEM solutions were added using a capillary tube and gently touching the plate at the reference mark, allowing the solvent to evaporate. The prepared TLC plates were placed in the developing chambers and covered. The average diameter size of the spot was estimated as 3 - 4 mm after of deposition. Several solvents and mixtures were tested as a mobile phase: acetonitrile, dichloromethane, acetone, isopropyl alcohol, butyl alcohol, benzene, toluene, petroleum ether, hexane, and cyclohexane. The TLC plate was allowed to develop until the solvent level was about 0.5 centimeters below the top of the plate. Immediately was removed the plate from the developing chamber and the solvent front was marked with a pencil and allowed to dry. Spots containing separated analytes were detected, marked (with pencil), and described (shape and size). Non-colored spots were detected using a UV lamp. Distances traveled by solvent and HMEs were measured and R_f values calculated.

6.3.3 MIR Laser Spectroscopy Spectral Acquisitions

A LaserScan™ pre-dispersive spectrometer (Block Engineering, Southborough, MA, USA) was used to obtain the MIR spectra of each spot detected. This system was

designed to operate in back-reflection mode. The mid-IR spectra were recorded in spectral range 990 - 1600 cm^{-1} , at 6 inches as the distance from the substrates. A total of 4 spectral acquisitions were taken for each sample spot (3 co-add/acquisition) at a spectral resolution of 4 cm^{-1} . The experiments were performed at room temperature (~ 21 °C). Spectra using both backgrounds, TLC silica-gel without sample and aluminum surface (Al) were acquired and compared.

6.3.4 Univariate Analysis

A univariate method of analysis was used to evaluate the performance of the TLC-QCL based methodology by calculating the analytical descriptors or figures of merit (FM) of the results of the experiments. These Figure Merits are defined in terms of linearity of calibration curves, sensitivity, and precision according to the International Conference on Harmonisation (ICH) guidelines [59]. To determine the linearity of the method developed, nine TNT standard solutions were prepared in the concentration range that would produce chromatographic spots of deposited mass between 100 and 0.390 μg , in addition to a blank solution (no TNT present). The chromatographic experiments were implemented in six different analyses. Linear regression analysis was performed by plotting the average peak areas obtained at each concentration. The sensitivity was estimated by calculating the detection limit (DL) and the quantitation limit (QL), from the calibration curve using concentrations that showed a linear trend with the spectral response. The precision of the method was evaluated through the repeatability and intermediate precision (inter-day). The repeatability of the measurements of peak areas was determined by analyzing three deposited mass levels (high 100 μg , medium 25 μg and low 1.56 μg) four times without

changing the position of TLC plates. The intermediate precision (inter-day) of the method was determined to evaluate six replicas of each of the three concentrations levels already specified over a short period (four days). Peak areas were used to calculate the mean, standard deviation, and coefficients of variation (CV).

6.3.5 Multivariate Analysis

All spectra were stored in the Thermo-Galactic SPC format (Thermo Fisher Scientific, Inc., Madison, WI, USA) and analyzed using PLS and PLS-DA chemometrics models generated using PLS Toolbox, v. 8.1 (Eigenvector Research Inc., Manson, WA, USA) for Matlab (The MathWorks, Inc., Natick, MA, USA). The samples tested corresponded to the same samples used in the univariate analysis.

6.4 RESULTS

6.4.1 TLC separation of TNT in a TNT/PETN Binary Mix

Several organic solvents were used as mobile phases to separate two nitro explosives in a lab prepared simulated Pentolyte formulation (a HEM binary mixture of 50%TNT:50%PETN typically used for military purposes). Solvents used as mobile phase were toluene, methanol, hexane, acetone, ethyl acetate, dichloromethane, ether, and their mixes. The best mobile phase to separate TNT ($R_f = 0.56 \pm 0.01$) and PETN ($R_f = 0.45 \pm 0.01$) was the binary mixture hexane (1): toluene (4). Other solvent systems also worked well, but the determined R_f value was either too low or too high. The spot diameter of the sample on the TLC plate was 4 ± 1 mm (PETN) and 6 ± 1 mm (TNT). The average time for the TLC separation of the mixture of HEM was $\sim 10 \pm 1$ min.

Fig. 2 shows the spots formed for TNT at different deposited mass (0.39 - 6.25 μg) when developed by chromogenic reagent DPA to generate characteristic orange-brown color. This is the method currently used in field separations. It should be noted that lower deposited mass of 1.56 μg are not possible to detect by visual inspection of the presence of the nitroaromatic explosive. This justifies the necessity to couple TLC to a technique capable of providing characteristic vibrational information of chemical compounds such as MIR laser spectroscopy or Raman scattering.

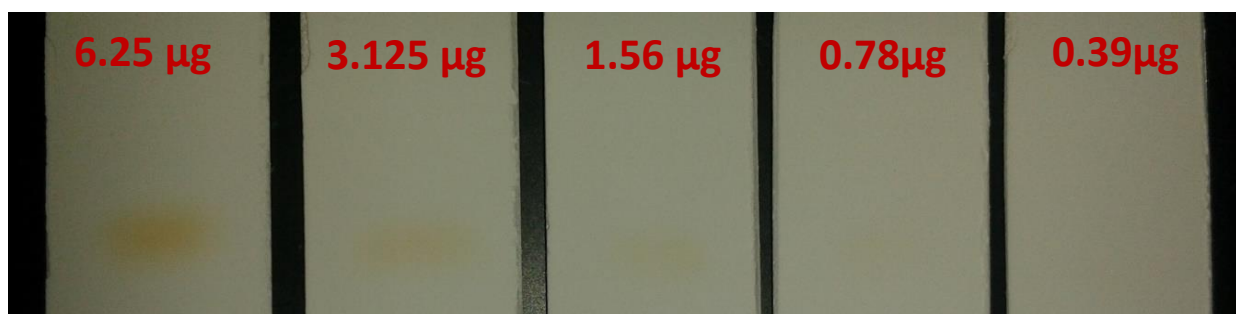


Figure 2. TNT spots on silica gel TLC plates at several deposited mass after using DPA reagent to generate characteristic orange-brown color for TNT.

6.4.2 Spectral Profiles of TNT

The hyphenation of TLC and IRS was approached in two ways. In the first approach, FTIR measurements are performed *in situ*; that is, the separated compounds are analyzed directly on the TLC plate. Spectral interferences are expected as almost all materials used as stationary phases have intense absorption bands in the MIR region. The second approach is usually more laborious and involves the transfer of analyte from the TLC plate to an IR-transparent substrate before FTIR measurements are made. Over the years,

research has shown that *in situ* as well as transfer methods can be practical and useful, each having specific advantages and limitations [60].

Spectral measurements were acquired *in situ* on the silica gel-based TLC plates. For the background single beam spectrum without the sample, two alternatives were considered, aluminum substrates (Al) and silica gel of the TLC plates as shown in Fig. 3. The goal was to determine which of the two background materials provided neater and more informative IRS spectra. Fig. 3A showed the spectra when Al substrates were used to acquire the background single beam spectra. The spectrum of TLC (Fig. 3A-a) was acquired from the silica gel of the TLC plates. Fig. 3A-b shows the spectrum of TNT/TLC. The difference spectrum (TNT/TCL–TCL) is shown in Fig. 3A-c. This procedure was done to remove the IR vibrational signals from the substrate and obtain the infrared vibrational characteristics signals of TNT. The resulting TNT spectrum shows an essential and distinctive band of the explosive around 1350 cm^{-1} , other IR bands are not appreciated using this method when compared with the reference spectrum of TNT (Fig. 3A-d).

Fig. 3B shows spectra when a TLC of silica gel is used as a background. The procedure followed was a single-beam spectrum of a clean area of the TLC plate was obtained and stored as background. Immediately after without moving the TLC plate another single-beam spectrum was measured and rationed against the stored background spectrum resulting in the “100% LINE” spectrum (Fig. 3B-c). This allowed revealing how the noise level varied across the range, the flatness of the 100% LINE indicated that the QCL spectrometer was stable during sample and reference spectral acquisitions periods [61,62]. The single-beam spectrum of the sample spot was then measured and rationed against the stored background spectrum, to get TNT spectrum on

TLC silica gel (Fig. 3B-a). This method for recording the MIR spectra of TLC spots is quicker in contrast to the methodology previously explained. Also, as mentioned by Percival and Griffiths [50], allowed compensating interfering bands due to silica gel and water vapor lines. The resulting TNT spectrum (Fig. 3B-a) shows two intense and representative bands of the HEM at 1350 cm^{-1} , 1551 cm^{-1} , and others minor intensity bands when compared with the reference spectrum of TNT (Fig. 3B-b). Using this protocol an excellent spectral compensation of silica gel and water vapor bands was achieved.

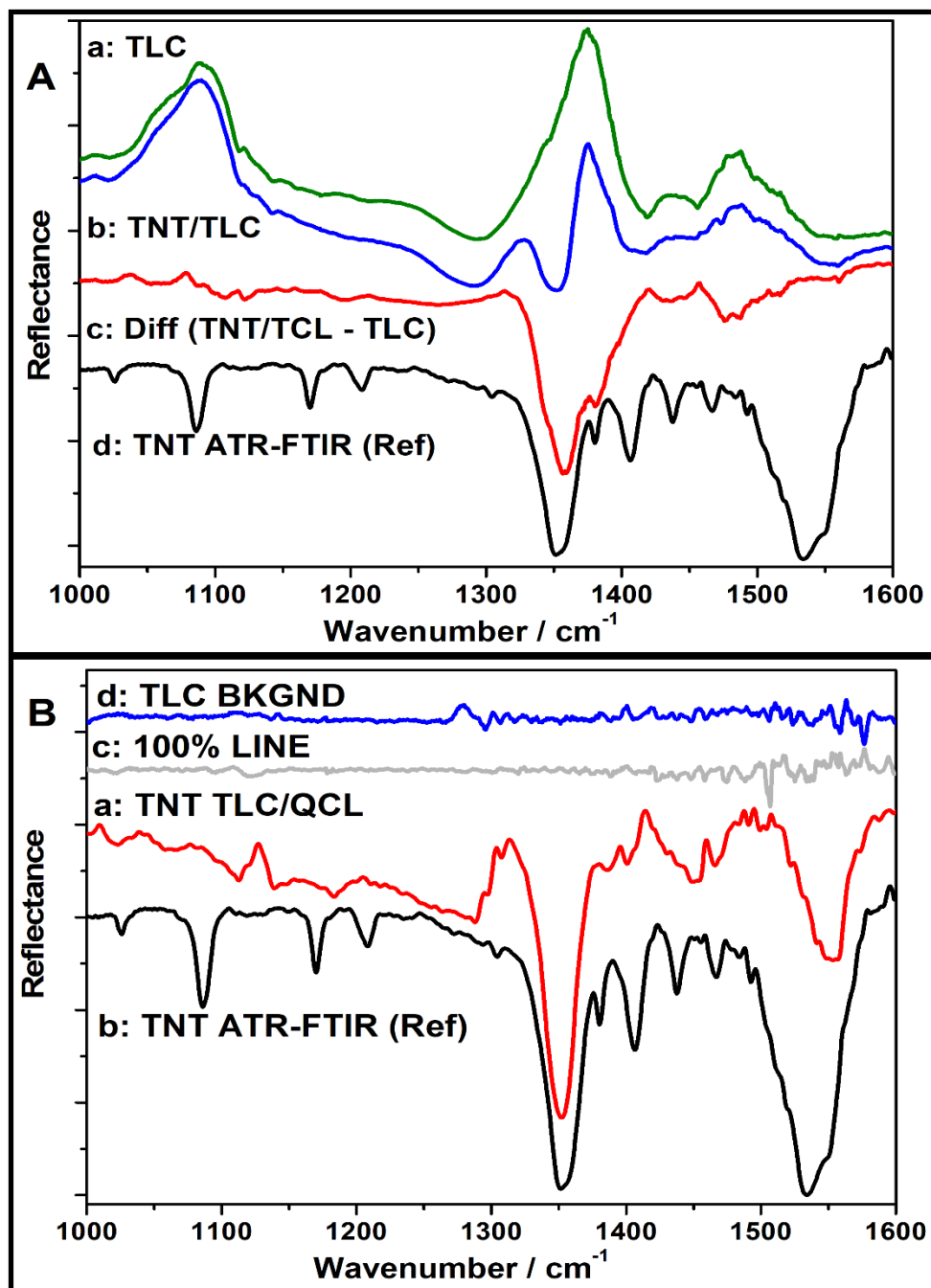


Figure 3. A. (a) Reflectance spectrum of silica gel (TLC) using Al substrate as background; (b) TNT/TLC with Al background; (c) difference spectrum: TNT/TLC - TLC with Al as background; (d) TNT reference spectrum measured by ATR-FTIR. B. (a) the background form was aluminum surface. In the bottom, (B) the background form was silica gel TLC free of sample.

6.4.3 Comparison between TLC-QCL and TLC-FTIR

To evaluate the ability of the MIR laser spectroscopy as a technique of vibrational identification on silica gel, TNT was selected as a target. Some of the vibrational bands that were tentatively assigned to TNT were at 1024 cm^{-1} corresponding to CH_3 -deformation, at 1086 cm^{-1} assigned to C-H ring in-plane bending, at 1350 cm^{-1} attribute to symmetric stretching of nitro groups, and 1551 cm^{-1} to asymmetric NO_2 stretching [63]. For benchmarking purposes, a comparison was made between the MIR spectra acquired with TLC-ATR/FTIR and with the proposed new technique. Typical TNT spectra obtained by TLC-QCL (Fig. 4-a), TLC-ATR/FTIR (Fig. 4-b), ATR/FTIR (Fig. 4-d) reference are shown in Fig. 4. Silica gel QCL reflectance spectrum (Fig. 4-c) is also illustrated to show the level of interference from the stationary phase. This figure demonstrates that TLC-QCL can be a useful analytical method and it is much more powerful several other spectroscopic techniques. As can be observed, that the intensity that occurs in the band at 1350 cm^{-1} , characteristic of nitro group vibrations using TLC-QCL (Fig. 4-a) is much more intense than that obtained by the ATR/FTIR (Fig. 4-b). The total integrated band intensity observes it was approximately 154x the intensity of the FTIR based method.

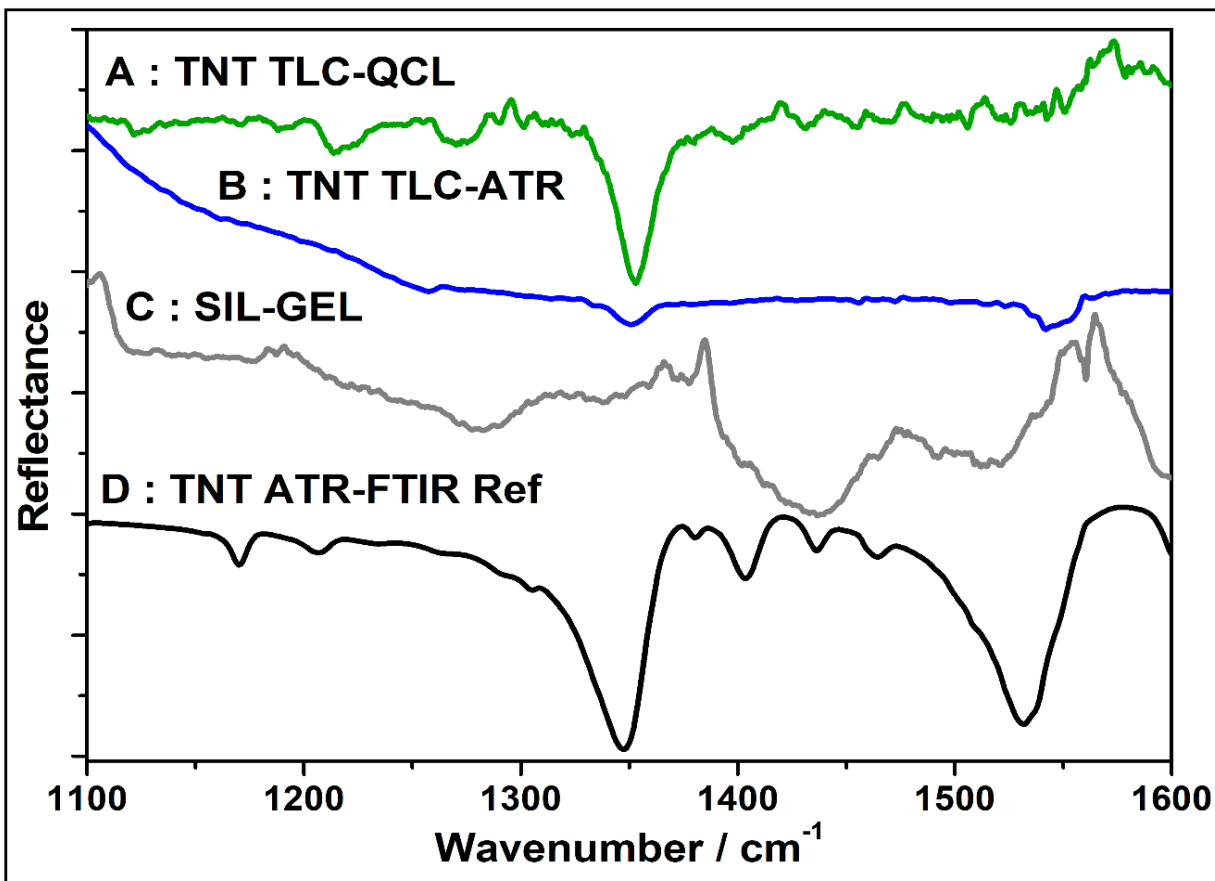


Figure 4. Normalized MIR spectra of TNT and silica gel with various spectroscopic techniques: (A) TNT laser reflectance spectrum (TLC-QCL); (B) TNT spectrum obtained using TLC-ATR-FTIR; (C) silica gel QCL spectrum; (D) TNT reference spectrum obtained by ATR-FTIR.

6.4.4 Quantification Profiles of TNT using Silica Gel TLC

Fig. 5 demonstrates that TLC/QCL can be used to build robust analytical methodologies for both purposes identification and quantification of the chemical targets selected. By depositing TNT on silica gel TLC in a deposited mass range from 0.39 to 12.5 μg , the spectrum band shapes were very similar to the TNT reference spectrum. In Fig. 5, as the concentration decreases, the intensities of the IR vibrational bands decrease according to Beer's law, at the same time IR bands ($\sim 1550 \text{ cm}^{-1}$) disappear at deposited mass less

than 3.12 μg , this fact may be occurred due to the effect of the rovibrational band of water starting from 1400 cm^{-1} . Another important reason is prominent to the strong absorption bands from silica gel displayed over the region of 1400 – 1600 cm^{-1} . As a matter of fact, at low concentrations of TNT deposited on silica gel plate, the IR band at 1351 cm^{-1} (attributed to nitro group vibrations) is notably present. Thus, the six spectra are shown in Figure 5 demonstrate that TLC-QCL hyphenated technique can be applied successfully for the identification of explosives.

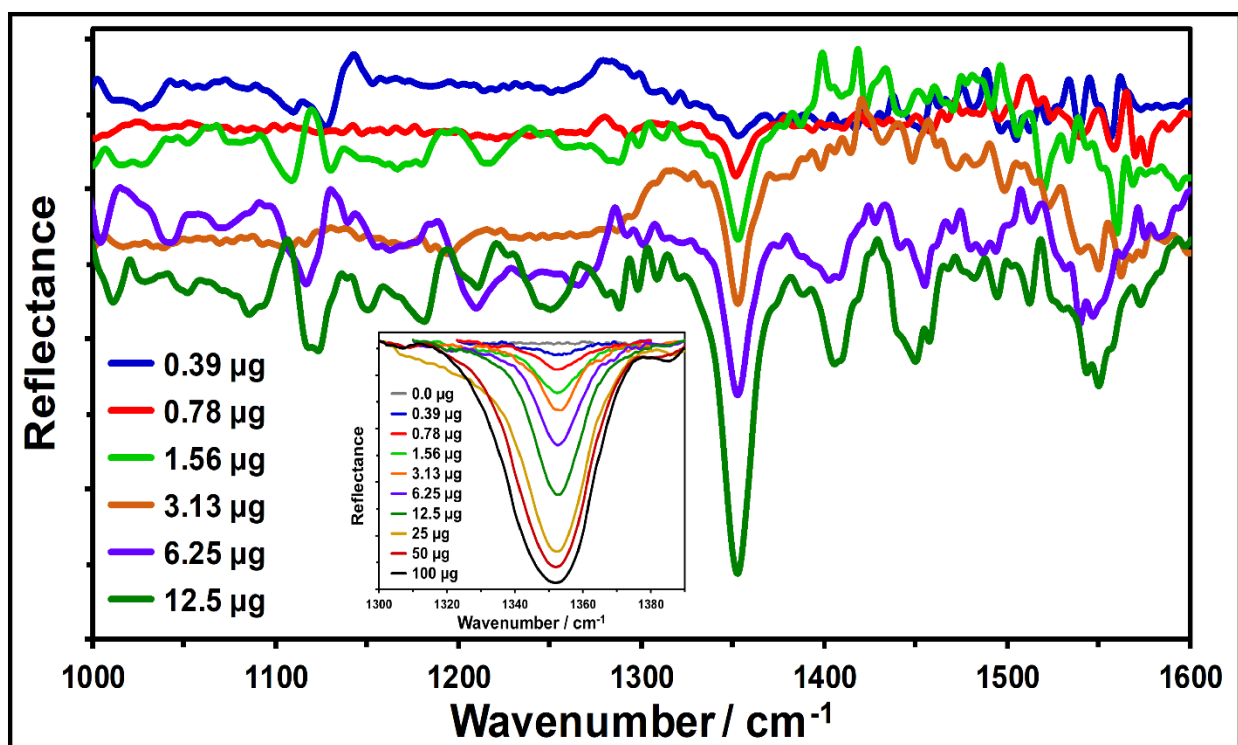


Figure 5. TNT spectra at different concentrations on TLC silica gel. Baseline correction and smoothing (25 pts.) were applied to each spectrum as a preprocessing signal.

6.4.5 Quantification by Univariate Analysis

To determine the viability of TLC/QCL for quantitative analysis, a univariate study was made of peak areas as a function of the quantity of TNT (μg) deposited on the silica gel

plates (μg). The nitro group symmetric stretch band at 1351 cm^{-1} in the TNT reflectance spectrum illustrated in Fig. 5 was selected for the study. Inset Fig. 5 shows the spectral window of $1300\text{-}1396\text{ cm}^{-1}$ with baseline correction applied (OPUS v. 6.0, Bruker Optics, Billerica, MA, USA). A plot of peak areas vs. the amount of TNT deposited is shown as an insert picture in Fig. 6. A non-linear dependence on the TNT spot deposited mass for the full range tested (0 to $100\text{ }\mu\text{g}$) was observed. Nonlinearity at high sample deposited mass can be attributed to effects of saturation of the sample, according to Beer's law, the linear response as a function of analyte concentration holds only for low concentrations, establishing the linear dynamic range of the analytical curve. The linear dependence with high confidence found was 0 to $1.56\text{ }\mu\text{g}$. This result is illustrated in Fig. 6. Analysis of the data and values for some of the analytical FM obtained from the least squares linear regression analysis are shown in Table 1. The lowest quantity of explosive that could be detected (DL) for TNT was determined as $0.074\text{ }\mu\text{g}$ (74 ng), and the value for QL for TNT was $0.224\text{ }\mu\text{g}$ (224 ng) using Equations 3 and 4:

$$\text{DL} = 3.3 \cdot \sigma / m \quad (3)$$

$$\text{QL} = 10 \cdot \sigma / m \quad (4)$$

Where σ is the standard deviation of intercept and m is the slope, both from the calibration curve (Table 1). The smallest amount of sample measured was $0.39\text{ }\mu\text{g}$ under the experimental conditions used, which resulted in a signal to noise ratio (S/N) of 25.8. The band intensities (in the range of $1336\text{-}1366\text{ cm}^{-1}$) were taken as the average height of the six spectra. The noise level (N) was taken as the average root mean square (rms) values of the six spectra measured in the range of 1200 cm^{-1} to 1220 cm^{-1} , that is, the standard

deviation of all data points in this region [61]. The values for the CV (%) of the spectral response expressed as peak areas were less than 2%, mostly for inter-day precision and repeatability in the three concentration levels tested, indicating that the method was precise. Values of precision are also included in Table 1.

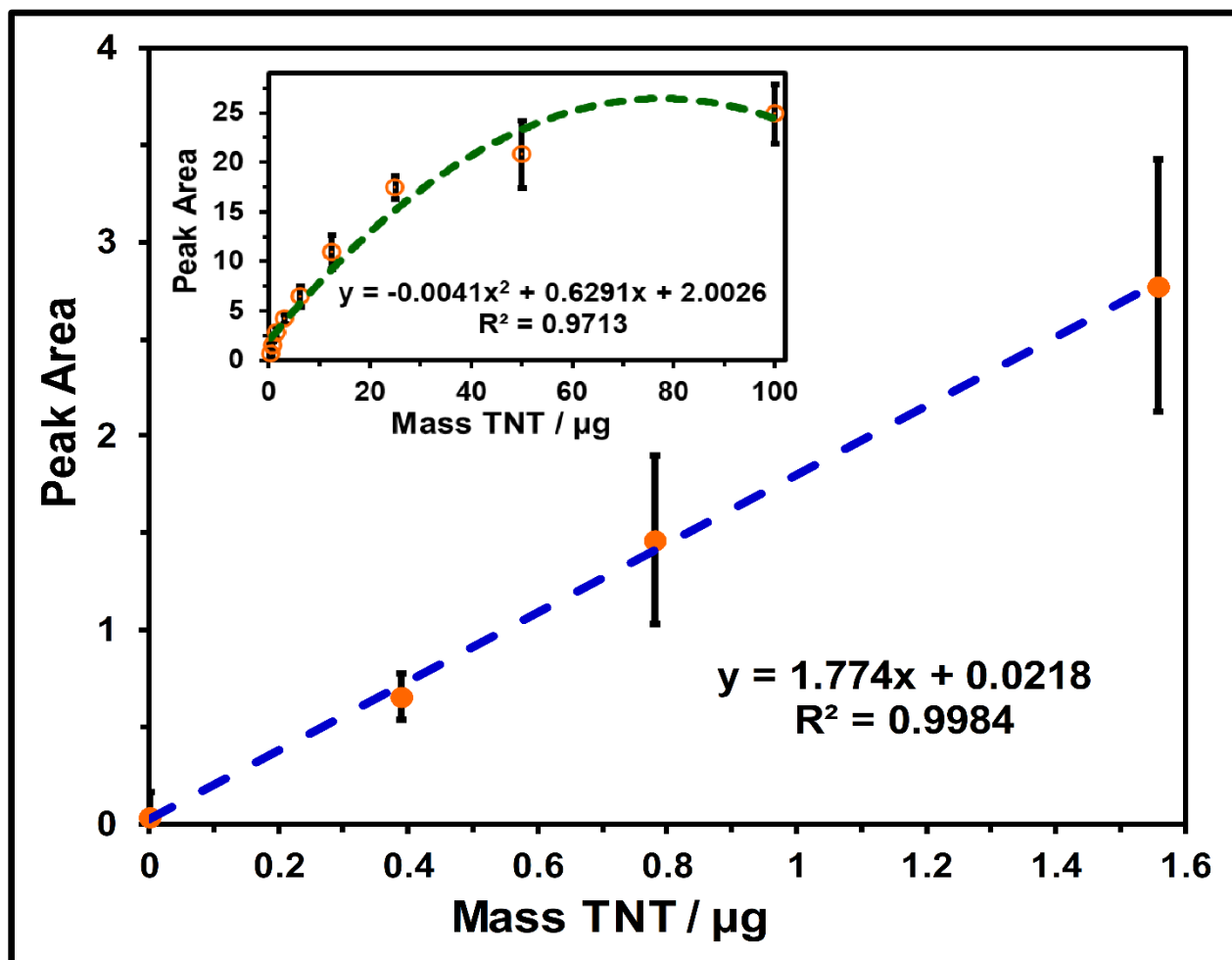


Fig. 6. Calibration curve for TNT at different masses deposited from 0.0 to 1.56 μg on the silica gel TLC plates. Inset is the calibration curve at concentrations from 0.0 to 100 μg .

Table 1 Regression analysis of calibration curve and merit figures for TNT detection

| Parameters | Value |
|-------------------------------------|---|
| Linearity range (μg) | 0-1.56 |
| Slope (m) | 1.774 |
| Standard deviation of slope | 0.050 |
| Intercept | 0.022 |
| Standard deviation of intercept | 0.045 |
| Regression equation | Peak area = 1.774 (μg) + 0.022 |
| Determination coefficient (R^2) | 0.998 |
| DL (μg) | 0.074 |
| QL (μg) | 0.224 |
| Precision inter-day (CV, %) | |
| for 100 μg (n=6) | 1.090 |
| for 25 μg (n=6) | 1.369 |
| for 1.56 μg (n=6) | 3.492 |
| Repeatability (CV, %) | |
| for 100 μg (n=4) | 0.809 |
| for 25 μg (n=4) | 1.005 |
| for 1.56 μg (n=4) | 2.083 |

6.4.6 MVA of the TLC-QCL Based Methodology

Taking advantage of the data present in the full MIR reflectance spectra of TNT recorded by TLC/QCL, chemometrics analysis methods, such as PLS and PLS-DA, were used to develop a multivariate sensor. This allowed achieving a more robust spectral identification, classification, and quantification of our target chemical compound. In this study, PLS Toolbox v. 8.1 for Matlab was used to analyze the spectral data.

We divided the data into two groups for multivariate analysis: A Calibration set and a prediction (or Test) set. The Calibration Set contained approximately 70% of the data,

and the Test Set comprised the remainder of the data. PLS-DA was applied to the spectral data to discriminate, classify, or group all the spectra as Explosive or No Explosive. Some main steps in this part are to arrange the spectral data and apply the required preprocessing to the data. The purpose of preprocessing the data is to remove or to decrease the effect of the interference from the background and substrate, thereby enhancing the vibrational signatures of the TNT. We used PLS-DA to decompose the original matrix into two new matrices: one that contained most of the relevant information including the most significant variability in the data and a second one that provided information not as appropriate to the data, generally termed noise.

In PLS-DA, the optimal number of LVs that offers the best classification for the calibration set is determined using cross-validation procedures. We used the Venetian Blind (VB) technique with 10 group splits to divide the calibration set into cross-validation groups. We applied several preprocessing steps to the data to generate multivariate models capable of discriminating among and clustering the spectra based on chemical similarities, (i.e., Explosive, and No Explosive). The preprocessing steps used were auto-scaling (AS), mean centering (MC), standard normal variate (SNV), first and second derivatives, and combinations of them. Different spectral ranges comprised between 1000-1600 cm^{-1} were analyzed. Of all the multivariate models generated, the ones that performed best were those that used the spectral range (1302.4 - 1388.4 cm^{-1}).

For PLS-DA, classification parameters such as sensitivity (*SEN*) and specificity (ξ), derived from the confusion matrix from the calibration, cross-validation, and prediction sets, to evaluate the performance of the classification models were used. Sensitivity describes the ability of the model to correctly recognize samples that belong to that class,

and specificity describes the model's ability to reject samples of all other classes. Both SEN and ξ assume values between 0 and 1, where 1 is the desired result [18]. Table II presents a summary of the results obtained from applying PLS-DA to the QCL spectra of the TNT on substrates of TLC for the calibration, cross-validation, and prediction sets.

Table 2. Summary of PLS-DA results of QCL spectra of TNT deposited on TLC

| Parameters | Data Set | |
|-----------------------------|-----------|--------------|
| | Explosive | No Explosive |
| Sensitivity (Cal): | 1.0000 | 1.0000 |
| Specificity (Cal) | 1.0000 | 1.0000 |
| Sensitivity (CV) | 1.0000 | 1.0000 |
| Specificity (CV) | 1.0000 | 1.0000 |
| Sensitivity (Pred) | 1.0000 | 1.0000 |
| Specificity (Pred) | 1.0000 | 1.0000 |
| Classification Error (Cal) | 0.0000 | 0.0000 |
| Classification Error (CV) | 0.0000 | 0.0000 |
| Classification Error (Pred) | 0.0000 | 0.0000 |
| RMSEC | 0.0764 | 0.0764 |
| RMSECV | 0.0963 | 0.0963 |
| RMSEP | 0.0690 | 0.0690 |
| R ² Cal | 0.9437 | 0.9437 |
| R ² CV | 0.9109 | 0.9109 |
| R ² Pred | 0.9529 | 0.9529 |
| Number of LVs | 3 | |
| Variance captured in Y (%) | 94.37 | |

Figures 7 and 8 show the score plots resulting from the PLS-DA runs. The score plots allow visualization of the clustering of the spectral data and it demonstrates that the best

results were obtained for the models generated after preprocessing was applied. For the MVA of the MIR vibrations of the HEMs deposited on TLC using QCL, we obtained the best chemometrics models for PLS-DA by taking the SNV and applying MC. The class predictions of explosive on TLC from the cross-validation set derived from the PLS-DA models for the discrimination of each spot with HEM (Explosive) on TLC from the clean spot (No explosive) are shown in Figure 7. Three LVs were required to obtain the best multivariate classification model with *SEN* and ξ equal to 1 (see Table 2) for the HEM tested from the calibration, cross-validation, and prediction data sets. The optimum chemometrics models were obtained by taking the SNV and applying MC. The variance captured from matrix Y was 94.37%, which is enough for a good classification of the predicted spectra set on TLC. For the multivariate analysis of the HEM (TNT) on TLC, six LVs were necessary to capture 80% of the total variance in the spectral data. As shown in the score plots in Fig. 8, two LVs with 52.75% of the spectral variance were sufficient to obtain an excellent classification to distinguish the spot with HEM (TNT) from the clean spot (without TNT). In this model, the spectra from the prediction set (in Fig. 8, Test_Expl, and Test_No_Expl) were well grouped according to chemical characteristics with the spectra from the calibration set.

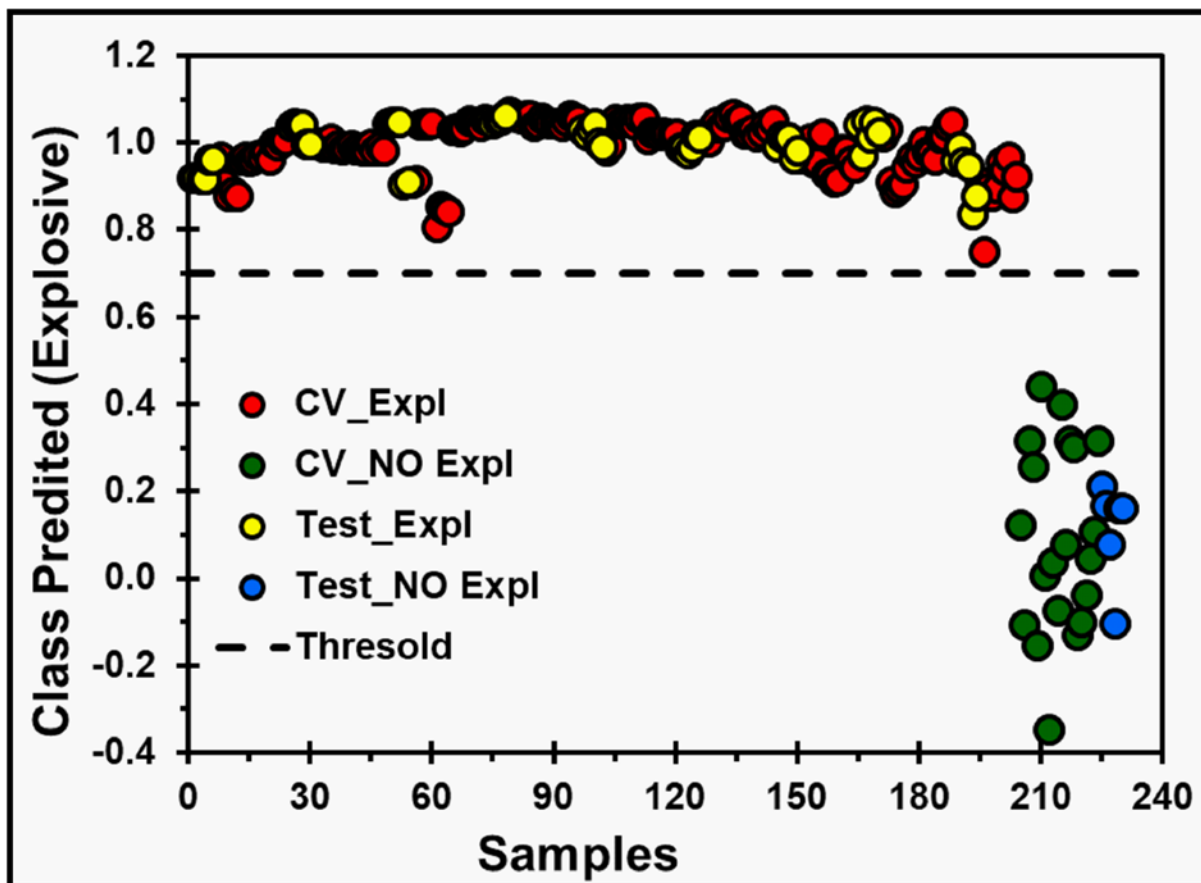


Figure 7. The PLS-DA model for discriminating HEM on TLC. Class prediction for TNT. Preprocessing steps applied were SNV and MC. The red dotted line represents the threshold for discrimination and a 95% confidence level for clustering.

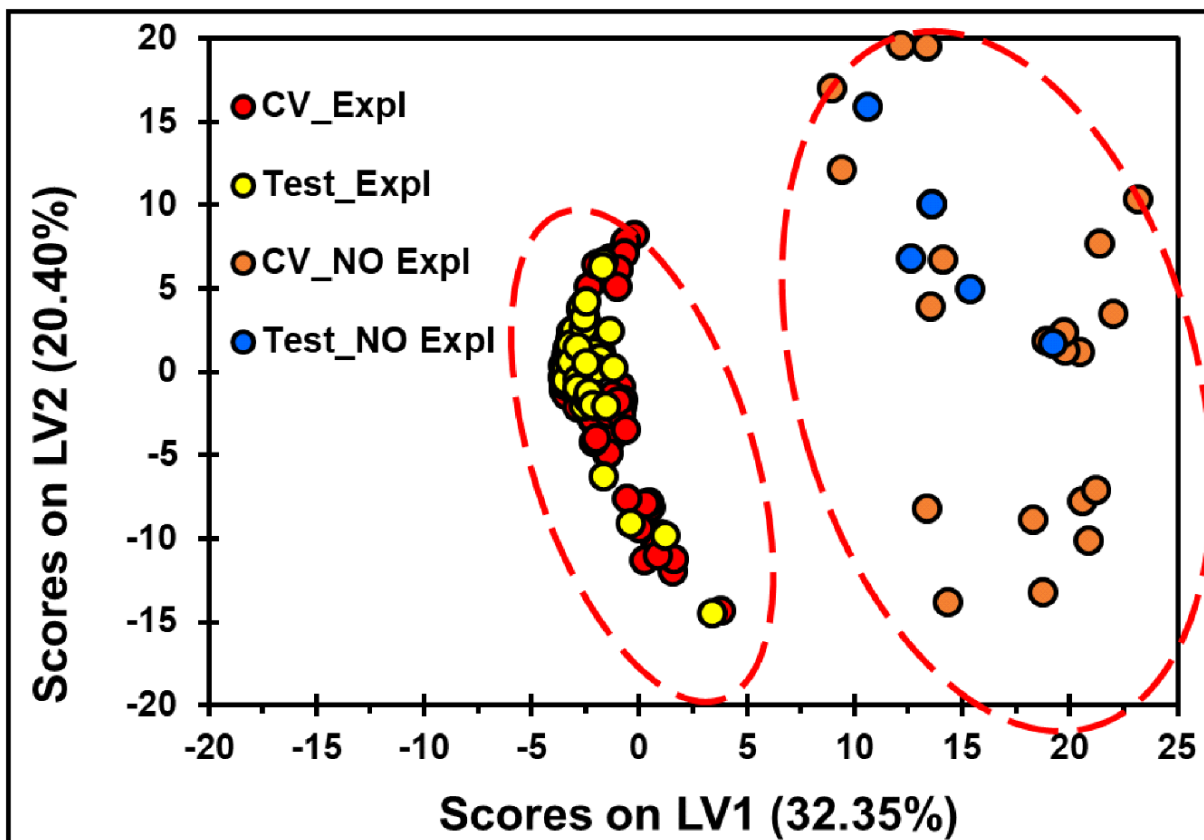


Figure 8. The PLS-DA model for discriminating HEM on TLC. Score plots of LV2 versus LV1 for the detection of TNT on TLC. Preprocessing steps applied were SNV and MC. The red dotted line represents the threshold for discrimination and a 95% confidence level for clustering.

In the PLS clustering analysis for the QCL spectra of the HEM on TLC, six LVs were required to capture 80% of the total variance in the spectral data using the first derivative (15 points) and MC as the preprocessing steps. Two LVs corresponding to 61.73% of the variance were enough to obtain excellent spectral classification by the amount of explosive deposited between 0 - 3.13 μg as can be observed in Fig. 9. Using this model, spectra from the prediction set (0 - 3.13 μg Test) were grouped with spectra from the

calibration set according to vibrational signatures in function of the amount of TNT deposited.

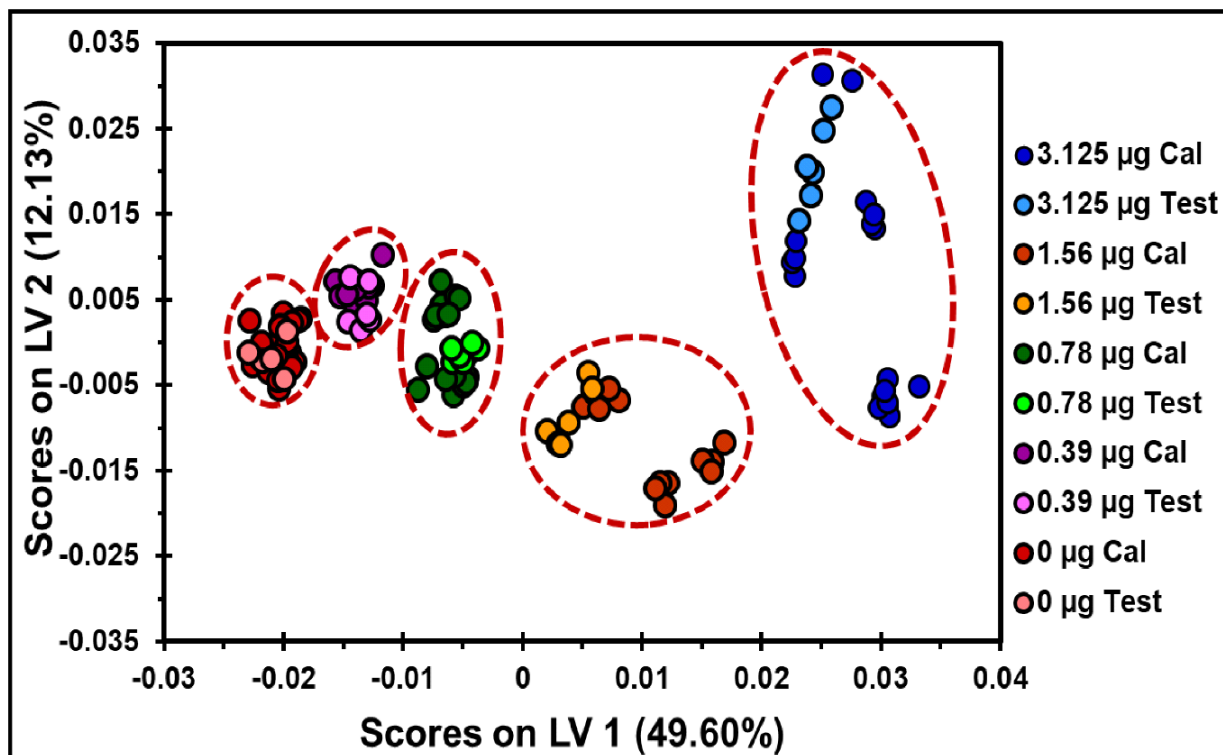


Figure 9. The PLS model for quantifying HEM on TLC. Score plots of LV2 versus LV1 for the quantity of TNT on TLC. Preprocessing steps applied were the first derivative (15 points) and MC. The red dotted line represents the threshold for discrimination and a 95% confidence level for clustering. The spectral range used 1302.4–1388.4 cm^{-1} .

PLS regressions were used to analyze the spectral data and to find the best correlation between the multivariate spectral information and the TNT concentrations (in $\mu\text{g spot}^{-1}$). Also, the root mean square error of cross-validation (RMSECV), root means square error of prediction (RMSEP), the coefficient of determination from cross-validation ($R^2\text{-CV}$) and coefficient of determination from prediction ($R^2\text{-Pred}$) were calculated and used as indicators of the quality of the obtained spectral correlations. Chemometrics models were

obtained based on PLS regressions using the spectral range measured (1302.4–1388.4 cm^{-1}) and the same sample concentrations as those in the PLS-DA analyses. As a preprocessing step, we applied the first derivative and MC to each spectrum from the spectral dataset. Fig. 10 shows a PLS model plot for TNT on TLC. The best results for RMSECV, RMSEP, R^2 -CV, and R^2 -Pred, including the number of LVs required in the PLS model, are shown in Table 3. Three LVs were needed to obtain the best PLS models, resulting in determination coefficients higher than 0.98.

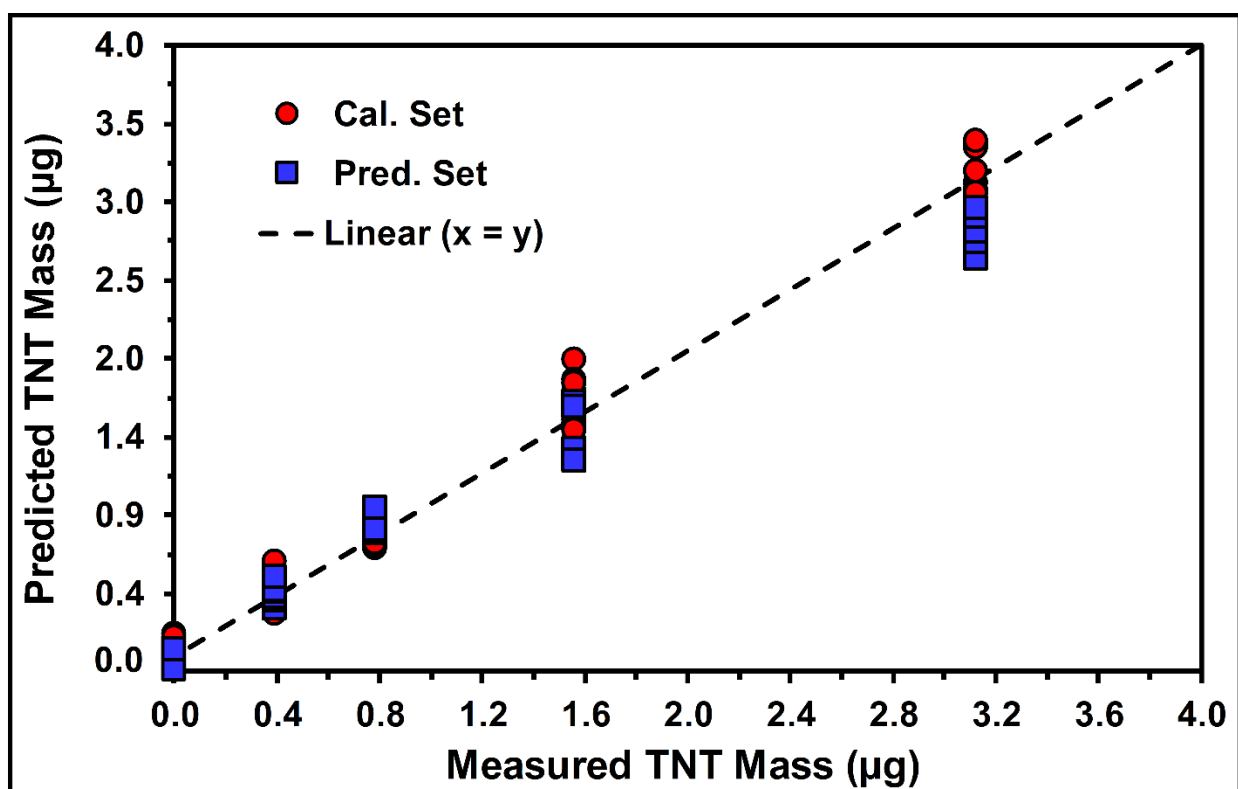


Fig. 10. PLS regression plot of the predicted TNT mass vs. measured TNT mass of deposited onto the TLC substrates. The preprocessing steps applied were the first derivative (15 points) and MC. The spectral range was 1302.4–1388.4 cm^{-1} .

Table 3. Statistical parameters of the PLS calibration model for TNT deposited on TLC

| Parameters | Value |
|--|-----------------|
| Deposited mass Range (μg) | 0 - 3.125 |
| Wavenumber Range(cm^{-1}) | 1302.4 - 1388.4 |
| Number of LVs | 3 |
| Variance Captured (%) | 98.63 |
| RMSEC | 0.135356 |
| RMSECV | 0.15467 |
| RMSEP | 0.209829 |
| R ² CV | 0.982124 |
| R ² Pred | 0.978144 |

6.4.7 Detection of TNT in Soil Matrices Using TLC-QCL

To evaluate the performance of the hyphenated technique in real-world applications of detection of explosives, contaminated soil samples were prepared and analyzed with TLC-QCL. Samples of 0.5 g dry of 3 soil types (sandy, clayish, organic) were transferred to glass vials (7 mL) with lid (Fig. 11A) and 2 mL of TNT solution (0.05/ μL or 5 $\mu\text{g}/\mu\text{L}$) in methanol. Then, the samples were shaken manually for 2 min and left uncovered to allow the solvent to evaporate (Fig. 11B). Subsequently, 2 mL of methanol was added to extract the TNT from the soil matrices. Then, 10 μL aliquots were obtained from the supernatant liquid and transferred to silica gel TLC plates. Then, the solvent was allowed to evaporate, and the plates were placed in the developing chamber to perform the chromatographic runs as described before. Visualization of separated spots was carried out using a UV lamp (see Fig. 11C-E), although this was not required since the R_f value for TNT ($0.56 \pm$

0.01) is highly reproducible. Finally, the IR vibrational detection on the spot was performed using MIR laser spectroscopy. Fig. 12 shows the IR spectra of TNT from the three different soil types at the concentration levels of $50 \mu\text{g spot}^{-1}$ and $0.50 \mu\text{g spot}^{-1}$.

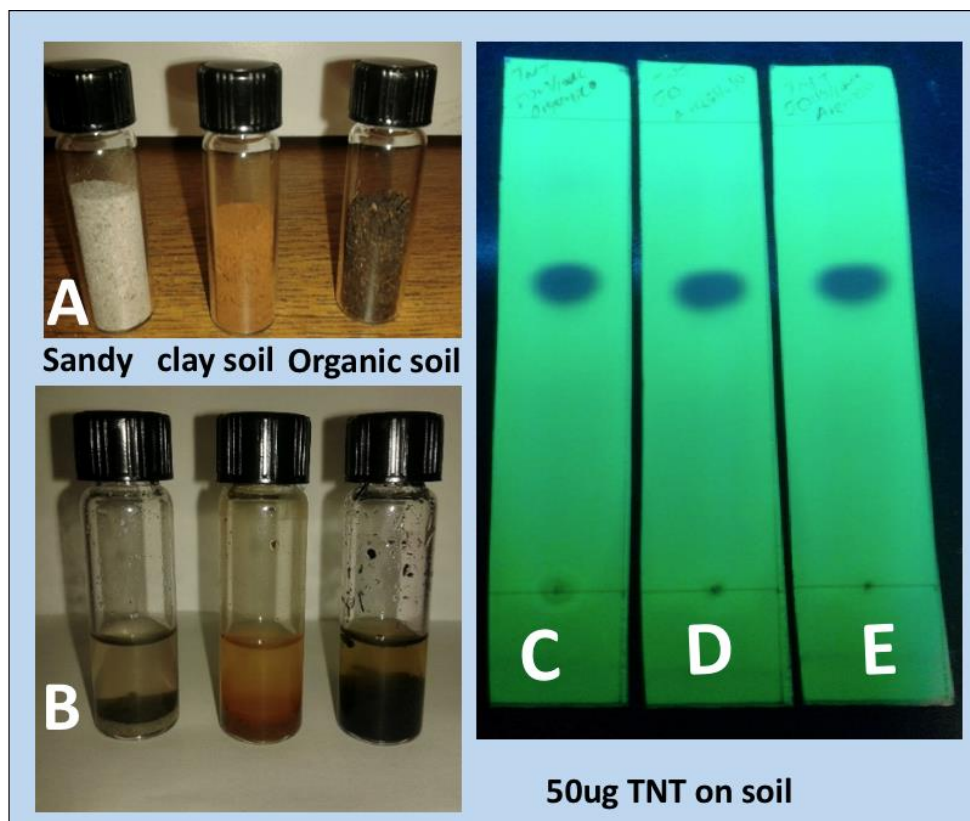


Figure 11. Simulated sample TNT in soil, (A) types of soil investigated: sandy, clay and organic soil; (B) mixture of TNT solution and soil, once repose 5 min; sample-spot visualization using UV lamp from different soils: (C) sandy, (D) cay soil and (E) organic soil.

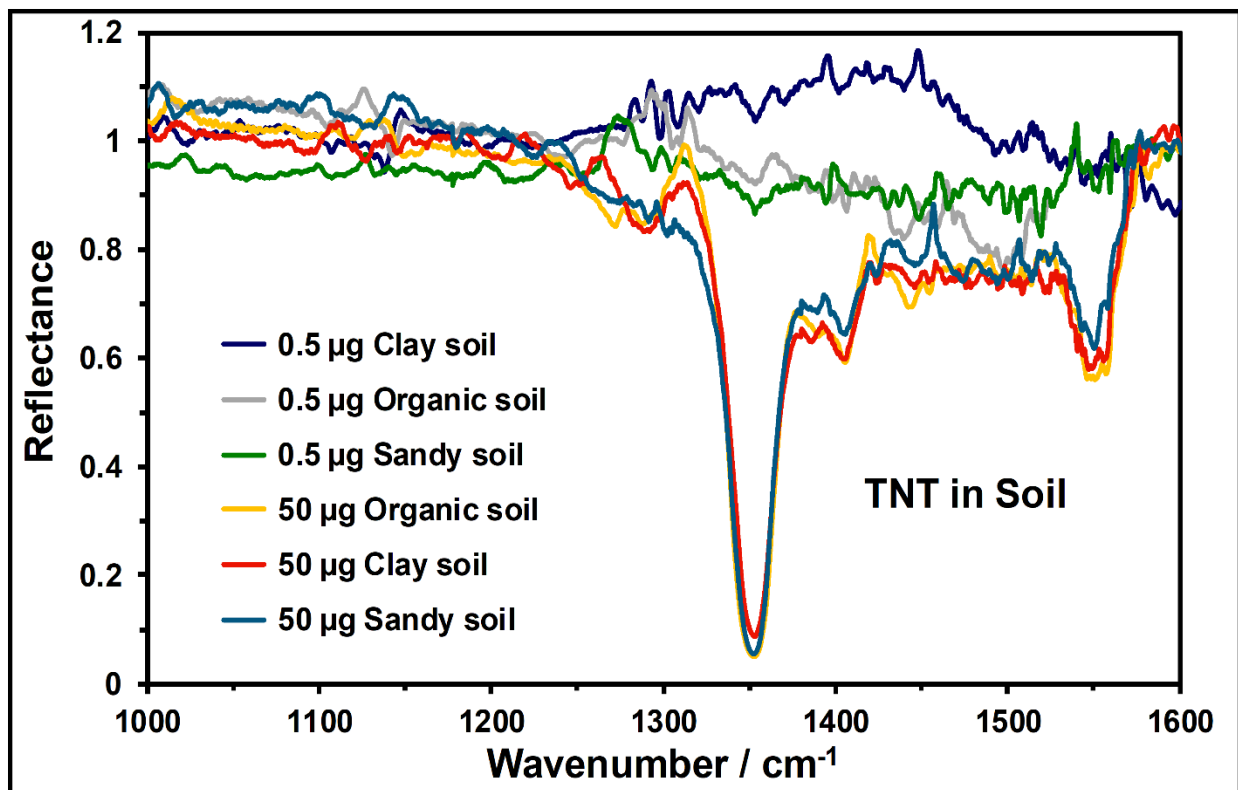


Figure 12. TNT spectra after separation of a soil matrix using silica-gel TLC at two concentrations. Soil types investigated were clay, sandy and organic. Concentrations tested were 0.5 and 50 μg spot⁻¹.

The spectra shown in Figure 12 demonstrate that TLC-QCL can serve as an excellent platform to develop analytical methodologies useful for the identification and quantification of chemical targets. TLC-QCL spectra of TNT at various deposited masses (from 0.50 and 50 μg) were very similar to TNT reference spectrum obtained from the literature and measured by ATR/FTIR. Even at low concentrations as 500 ng, the nitro band at 1351 cm^{-1} could be observed.

6.5 CONCLUSIONS

A new hyphenated technique that couples a quick, easy to implement, and low-cost separation technique (TLC) with a powerful, laser-based MIR reflectance technique are described. TLC-QCL can be employed for rapid separation, identification, and quantification of analytes of interest, such as HEMs at low concentration levels. The optimum mobile phase for separating the target HEM selected (TNT, $R_f = 0.56$) from other HEMs and environmental media, was the binary solvent mix composed of hexane (1): toluene (4). The spot diameters of the samples were ~ 0.5 cm, as determined by UV fluorescence. This information was crucial because it allowed locating samples quickly and was very useful for *in situ* MIR laser sensing. TNT spectra acquired exhibited several characteristic bands in the range of $1350\text{-}1550\text{ cm}^{-1}$ when compared to reference spectra, measured by direct back reflectance. *In situ* spectral measurements using silica-gel TLC plates as background allowed to achieve the best MIR vibrational signals of TNT, when compared with those obtained using Al substrates as background. A DL of 74 ng and a QL of 224 ng were achieved when using the linear range of the calibration curve. A comparison of the technique with TLC-ATR/FTIR demonstrated that the new hyphenated technique is much more sensitive (154 fold) for the same experimental conditions.

Chemometrics based MVA assisted in the detection of HEMs deposited on TLC and required only taking the first derivative and applying MC as preprocessing steps for PLS regression. Robust results for RMSECV, RMSEP, $R^2\text{-CV}$, and $R^2\text{-Pred}$ were obtained. Preprocessing steps for PLS-DA included SNV and MC. These were enough to obtain efficient models with sensitivity and specificity equal to 1.0.

CHAPTER 7

GENERAL CONCLUSIONS

A new hyphenated technique that couples a quick, easy to implement, and low-cost separation technique (TLC) with a powerful, laser-based MIR reflectance technique are described. TLC-QCL can be employed for rapid separation, identification, and quantification of analytes of interest, such as HEMs at low concentration levels. The optimum mobile phase for separating the target HEM selected (TNT, $R_f = 0.56$) from other HEMs and environmental media was the binary solvent mix of hexane (1): toluene (4). The spot diameters of the samples were ~ 0.5 cm, as determined by UV fluorescence. This information was crucial because it allowed locating samples quickly and was very useful for *in situ* MIR laser sensing. TNT spectra acquired exhibited several characteristic bands in the range of $1350\text{-}1550\text{ cm}^{-1}$ when compared to TNT reference spectra, measured by direct back reflectance. *In situ* spectral measurements using silica-gel TLC plates as background allowed to achieve the best MIR vibrational signals of TNT, when compared with those obtained using Al substrates as background. A DL of 74 ng and a QL of 224 ng were achieved when using the linear range of the calibration curve. A comparison of the technique with TLC-ATR/FTIR demonstrated that the new hyphenated technique is much more sensitive (154 fold) for the same experimental conditions.

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CHAPTER 8

ACCOMPLISHMENTS AND SCIENTIFIC CONTRIBUTIONS

This work provides the basis for a robust method that could be implemented in the agencies to reduce the number of explosives events that had occurred in recent years. This methodology provides a rapid detection, identification, and quantification of these HEM's. Previous works focused on the detection and quantification of explosives by chromatographic separation techniques, trained canine teams, mass spectrometry, infrared absorption spectroscopy, surface-enhanced Raman spectroscopy, X-ray imaging, thermal neutron analysis, electrochemical procedures, and ion mobility spectrometry (IMS). These techniques have some advantages, but also some of them have disadvantages such as that they consume much time. These are techniques with expensive and delicate instruments. In this research, a new simpler and faster methodology are proposed to detect and quantify the traces of these explosives. This was possible by coupling Thin Layer Chromatography (TLC) with MIR laser spectroscopy. The methodology of the investigation was based on the use of a QCL spectrometer operated in direct mode, pointing where the explosive spot is located. The figures merits, detection limit (DL), quantification limit (QL) and other parameters were presented to demonstrate the great potential of the technique.

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