Development and Validation of a Thin Layer Chromatography Method in Forensics, for the Analysis of Organic Gunpowder Residues after Firing Weapons

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Abstract:

Fast and accurate analysis for identification and separation of nitro-glycerine (NG) and nitrocellulose (NC) in gunpowder residues, with one and two bases after firing weapons, remains a challenge that forensic experts have to develop and prove in international forums and in low concentrations (nanograms or fentogram). Therefore, this scientific research intends to do this by developing a thin layer chromatography technique, as a simple, efficient and fairly accurate method.

Standard compounds are easy to prepare and entire procedure can be easily managed even by a staff with basic training, whereas, the results are reported by comparing the unknown sample with the standard compound or reference material, which will be compared with the Rf parameter, which means, if these values are the same, then the presence of the compound is reported, whereas, if we don't have approximate values of Rf, then the absence of the compound in question is reported.

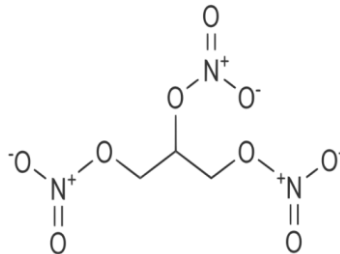**Key words:** TLC, nitro-glycerine, nitrocellulose, one and two bases, forensic

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Introduction

Burning – compressing material. When we mention this, we are referring to the gunpowder. First gunpowder used in firing weapons was the black gunpowder. From the moment of introduction of the smokeless gunpowder, the use of black gunpowder in small calibre guns decreased excessively. However, to this day, black gunpowder is still used for specific purposes.

Modern smokeless gunpowder for handguns almost exclusively contains Nitrocellulose (NC) as the main explosive component (with a single base). But, there may also be other explosive components, for example: glycerine Trinitrate (nitro-glycerine, NG) otherwise considered with two bases, or even Nitro guanidine (with three bases that are not desirable to be found in handguns ammunitions). In gunpowder with two bases, composition of components varies, from 60% to 80% Nitrocellulose and 40% to 20% nitro-glycerine.

Figure 1: Chemical structure of Nitro glycerine (NG)
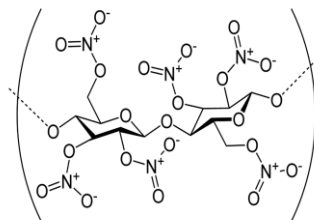
Figure 2: Chemical structure of Nitrocellulose (NC).

Experimental

Solvent

Analytical grade reagents (high purity reagents) were used for method development and validation. Below written reagents are from known manufacturers such as:

MERCK, SIGMA-ALDRICH, CARLO ERBALE.

1	Toluene	9	Alpha Naphthylamin
2	Cyclohexane	10	Acetic acid
3	Acetone		
4	Potassium hydroxide		
5	Ethanol		
6	Diphenylamine		
7	Sulphuric Acid		
8	Sulfanilic acid		

Standards:

Nitro-glycerine purchased from a known manufacturer with certificate of analysis (in concentration of 0.1 mg/ml).

Nitrocellulose mixed with Nitro-glycerine, taken by "K-9" Unit.

Equipments and materials:

1. Polyester micro beaker (lab dishware), for one use – by Fisher Scientific.
2. Test tubes for one use.
3. Thin-Layer Chromatograph plates (250 micron Silica Gel 60), of a credible manufacturer.
4. Lab Water Bath

Method development

Reagents used for the development of a thin layer chromatography method (TLC).

Spot visualization in a thin layer chromatography (TLC).

1. Visualization through UV lamp
2. Visualization through reagents: EtKOH, GRIESS.

The sample is extracted with a minimal amount of acetone which showed as more appropriate during the validation process.

With a capillary tube, a small amount of the extracted solvent is placed in the beginning of TLC plate.

Negative control (extractor) and positive control (referent samples of NG and NC) are applied in the beginning of the same TLC plate, beside or near the suspicious sample for comparative purposes.

The TLC plate is developed - by using Toluene - Cyclohexane (7:3) in the Lab Water Bath or another appropriate mobile phase.

Leave the plate to dry in the oven at 95 °C for 5 minutes, afterward visualization with the UV light is conducted, then spray it with "EtKOH" reagent and observe the change of colour and stains.

Leave the plate to dry in the oven at 95 °C for 5 minutes, and spray it with "GRIESS" reagent and observe the change of colour and stains.

The test is positive, when the appeared dot has Rf (travel factor) or is in the same line with the NG or NC standard, depending on the content of the test sample, whether it is with one or two bases. The relative error in percentage is calculated and presented with the expression:

$$E_r = \frac{x_i - x_s}{x_s} \cdot 100\%$$

Results and discussion

Selection of the mobile phase

Different combinations and different ratios of mobile phase are used, like i.e.: Toluene: Cyclohexane (7: 3 v / v), Toluene : Cyclohexane (6: 4 v / v), Toluene : Cyclohexane (5: 5 v / v), Toluene: Cyclohexane (3: 7 v / v), chloroform: acetone (2: 1),

acetone: methanol (3: 2). Mobile phase: Toluene : Cyclohexane (7: 3 v / v), showed as the more optimal in identifying and separating the NC and NG from gunpowder residues. The optimal mixing time is 2h and reaction occurs in 30 minutes, migrating for 70 mm in a room temperature (23 to 28 ° C). The separation and identification takes 30 minutes in a relative humidity of 30 to 37%.

Method Validation

Specificity:
During the establishment of the method different mobile phases were used, different extractors, different sample quantities with different concentrations, also negative samples were used by specialist 1 and specialist 2, such as: "Urea" (chemical fertilizers), "Urine", "3x15" (chemical fertilizers), "Kan" (chemical fertilizers) and "Hand grease", all of which gave the same negative reactions under the same conditions. Therefore, after all these analyses we concluded that as more specific for identification and separation of NG and NC from the gunpowder residues after firing the gun without identified interferences of other factors, are: Toluene: Cyclohexane (7: 3), stationary phase, silicate gel plates, silica gel 60, F254, acetone extractor, quantity 10 μl, reagent for spraying EtKOH and Griees.

Precision (Repeatability/ Reproducibility)
Measurements were conducted by specialist 1 and 2 for the four above mentioned samples in Table 5 and 6, of three drops from each sample with a same concentration, a same 10μl quantity, under same conditions and on the same day, by specialist 1 and 2 in the same laboratory. Measurements were made to determine the Rf.

Based on the measurements, we conclude that the coefficient o variability (CV%) deriving from the relative

standard deviation (RSD or Er) for measurements of the same plate on the same day and from different specialists, does not exceed 2%. Whereas, the coefficient of variation (CV %) derived from the relative standard deviation (RSD or Er) for measurements of different plates, on different days and from different specialists does not exceed 8%.

Determination of the detection/sensitivity limit for NG and NC:

Initially the mobile phase is prepared for the development of a thin layer chromatography. In this context the mobile phase is prepared as follows:

1. Toluene : Cyclohexane - 7:3

The solvent is also prepared for the plate visualization (1.EtKOH and 2.GRIESS).

As a sample for analysis we took the NG Standard in different concentrations: Measurements were conducted by specialist 1 and 2 for the four mentioned samples in Table 5 and 6, with three drops from each sample with a same concentration, a same 10µl quantity, under same conditions and on a same and different days, by specialist 1 and 2 in the same laboratory. Measurements were made for determination of the Rf.

Based on the measurements, we conclude that the coefficient of variability (CV%), which derives from the relative standard deviation (RSD or Er) for measurements of the same plate on the same day and from different specialists, does not exceed 2%. Whereas the coefficient of variability (CV %), which derives from the relative standard deviation (RSD or Er) for measurements from different plates, on different days and from different specialists, does not exceed 8%.

Quality Control:

In the quality control were included: positive control (NG standard and the sample taken by "K-9" for NC) and the

negative control. We conclude that in all conducted tests, during the validation process, both, positive and negative controls have functioned.

Matrix:

Matrix or mixing of the sample with possible interfering compounds, and as a result, we conclude that they don't influence on other compounds in the separation and identification of NC and NG. The testing factors were: Urea (chemical fertilizer), Urine, KAN (chemical fertilizers), methanol, acetone, cotton and hand creams.

Adoptability:

During the establishment of the method hundreds of measurements were conducted, with the same method but with different parameters, until we managed to set optimal parameters, which influenced in better separation and identification of the NC and NG of gunpowder residues, after firing weapons. We conclude that the most appropriate parameters and conditions are those that are mentioned in the procedures section.

Reliability and interpretation of results:

Based on the data and results obtained during the validation process (Tables: 1, 2, 3, 4, 5 and 6) of the analyzed samples (143), can be concluded that these results are reliable and credible. All samples were prepared and analyzed by 2 specialists and the results were also checked and confirmed by 2 specialists.

During the validation process for the interpretation and comparison of results, statistical method for determining the relative standard deviation (coefficient of variability) is used. Values for determining the relative standard deviation (coefficient of variability) were taken from the obtained results, presented in a tabled form in Tables 5 and 6. After calculation

of these results, the value of the relative standard deviation (coefficient of variability) appeared to be approximately 3% for the same plate and the same sample of the same concentration and on the same day.

Based on the above mentioned data, we recommend that the value of the relative error is not higher than ± 3%

Also during the validation process, the results obtained for different plates and same sample of the same concentrations, on different days were compared. After calculation of these results, the value of the relative standard deviation (coefficient of variability) appeared to be approximately 8%.

REFERENCES:

1. Validation and implementation of (new) methods, ENFSI on 4 November 2006.
2. The Development and Validation of Thin Layer Chromatography Densitometry Method for the Analysis of Diclofenac Sodium Tablets, Pharmaceutical Analytica Acta 2013.
3. Validation of thin Layer Chromatographic Screening methods for Pesticide Residue Analysis.
4. Forensic Investigation of Explosions. CRC PRESS 1998, A.Berveridge.
5. YINON, Jehuda; Zitrin, Shmuel. Modern Methods and Applications in Analysis of Explosives. Israel 1996.
6. BELL, Suzanne. Forensic Chemistry. Pearson Education, New Jersey 2006.
7. Identification of the nitrate esters of five alkyl alcohol by thin-layer chromatography. 99. Kohlbeck JA.
8. Determination of nitro glycerine and resorcinol in double-base propellant following separation by TLC. Anal Chem. 1965; 37:1282-3. 96. Booker JI.

9. A method for the identification of smokeless powders and their residues by TLC of their minor constituent. J Forensic Sci. Soc. 1973; 13:199-201.83. Twibell JD, Wright T, Sanger DG, Bramley RK, Lloyd JBF, Downs NS.

10. The efficient extraction of some organic explosives from hand swabs for analysis by gas liquid and TLC, J Forensic Sci. 1984;29:277-83. 30. Peak SA.

11. A thin-layer chromatographic procedure for confirming the presence and identity of smokeless powder flakes. J Forensic Sci. 1980; 25:679-81. 31. Archer AW.

12. Separation and identification of minor components in smokeless powders by TLC. J Chromatog 1975:108:401-4