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Application of TLC for Analysis of Trinitrotoluene Residues in Environmental Samples

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Abstract: The results of researches on possibility of thin-layer chromatography (TLC) application in analysis of after-explosion residues of trinitrotoluene in environmental samples are presented. A testing stand for model charges detonation was designed and made. Methods of extraction were specified and recoveries were estimated (they were about 80%). Chromatographic system suitable for analyte separation from other explosives and matrix components was proposed. A linear response curve of changes of densitometric measured A values versus analyte mass c in chromatographic band was received ($A = 35\,051c - 838$). A limit of detection (70 ng) and maximal range of linear measurements (2800 ng) was defined. TLC was used as an analytical method and - in case of more complicated measurements – as a technique of sample preparing for GC/MS measurements.

Keywords: TLC, densitometry, high explosives, coupling TLC/TLC and TLC/GC/MS methods

Introduction

A crime surge that was observed during last years all over the world also moved across Poland. The sudden increase of thefts, robberies and armed assaults is being found. The real plague that posed the greatest problem of public security are attacks with explosive charges. In search for authors of these attacks, the effort concentrate among other things, on determining of the kind of explosives that were used, their composition and the way of initiation. Determination of an explosive residue in environmental samples is an element of crime detection analysis of an event place.

Identifying analysis of this part of explosive which was spread into environment during detonation are complicated because of the small quantity of the sample and large quantity of impurities, which can interfere with analytes. Tests have to be preceded by a careful preparation of a sample that consists in separation of the mixture components and extraction of analytes. That is why chromatographic methods are mainly used in such investigations.

There are lots of publications on the application of chromatographic methods of analysis for identification and determination of explosives [1-3]. Unfortunately, over recent years a considerable fall of interest in thin-layer chromatography (TLC) is observed. The method is perceived as semi-quantitative with a limit of detection in the order of micrograms, which can be used as a complementary technique in some simple explosive analysis [4]. It seems that this opinion does not correspond with the modern instrumental TLC [5-7]. That is why detailed researches were taken up; they headed for evaluation of TLC use for explosive analysis for the police. In this work we concentrated our attention on measurements of trinitrotoluene residue, as it is the most widespread high explosive.

Experimental

Only a few works devoted to measurements of an explosive after-detonation residue can be found. Baran's investigations [7] as one of the first, showed that not the whole explosive changed during detonation and it made possible to identify explosive in samples collected near an explosion place. Unfortunately, a quantitative estimation of this process (for example mass of an explosive which does not change during detonation, determination of the range of further after-detonation changes of the explosive, estimation of influence of types of matrixes on recovery and quantities of an explosive residue collected in them) is not recognised. That is why we concentrated our attention on estimation of these parameters.

Analysis course and results

Possibility of the identification and determination of an explosive residue getting into matrixes located in the direct neighbourhood of explosion (water, sand, wood, bricks and concrete) and their degrees of detonation reaction were investigated. Experimental part of this work consisted in:

- design and realization of a stand for small (in the order of a few dozen of grams) explosive charges,
- estimation of the efficiency of different extraction methods from the point of view of maximization of recovery and purity of a sample,
- selection of chromatographic systems which are necessary for the explosive separation and extraction of analytes from other matrix components which are in a solution,
- estimation of quantitative relations in densitometric measurements.

Apparatus and materials

Applicator Linomat IV (Camag); horizontal chromatographic chamber DS (UMCS Lublin); densitometer CS-9000 (Shimadzu); gas chromatograph HP 5890 with mass spectrometer HP 5972 (Hewlett-Packard) and capillary column HP-5MS (length 30 m, inside diameter 0.25 mm and film thickness 0.25 μm); SPE set (J. T. Baker); chromatoplate with silica gel 60 F_{254} HPLC (Merck, No. 1.05548) or silica gel with chemically bounded octadecyl (Merck, No. 1.05559); organic solvents (J.T. Backer, Merck); explosive standards (Department of Explosives and Explosion Physics WAT).

Selection of chromatographic system and quantitation

High explosives which are used in the army such as TNT (2,4,6-trinitrotoluene), RDX (1,3,5-trinitro-1,3,5-triazacyclohexane), HMX (1,3,5,7-tetranitro-1,3,5,7-tetraazacyklooctane), PETN (pentaerythritol tetranitrate) and tetryl (2,4,6-trinitrophenyl-*N*-methylnitramine) were chosen for researches. The first step included finding of a chromatographic system, which made their separation possible in order to their identification and estimation of analytical parameters. All experiments were conducted using standard solutions; 20 μL of acetone solutions and their mixture of 10 ng/μL concentration were placed on a start line of chromatoplates (spraying technique, band in width 4 mm). Chromatograms were developed on 5 cm in length in horizontal chamber DS. The correctness of indications was estimated by observation of quenching of fluorescence. The most profitable separation (Figure 1) was gained in the case of following chromatographic system: stationary phase – high-performance silica gel 60 F_{254}, mobile phase – carbon-tetrachloride-acetonitrile 8:1 (v/v).

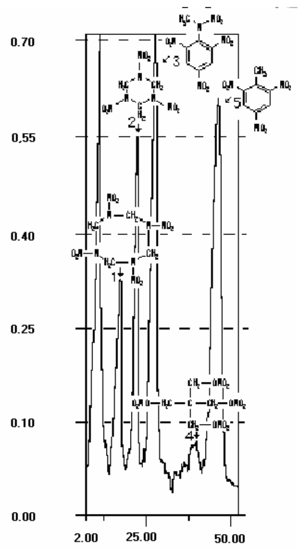

Figure 1. High explosive separation; Y-axis – absorbency in relative units of apparatus; X-axis – range of elution.

In order to increase reliability of analyses absorption UV spectrums of separated mixture components were measured *in situ*, getting information about a wave length λ_{max} which corresponds to an absorption maximum. The third measure of the explosive identification (beside the comparison of R_f and λ_{max}) were also proposed. It was a coloured reaction with suitable reagents. It was assumed that the above mentioned parameters of qualitative analysis are the sufficient information for unequivocal identification of an analyte against the rest of explosives.

The following parameters of quantitative analyses of TNT in the above mentioned chromatographic system were proposed: R_f = 0.71, λ_{max} = 232 nm, spraying chromatogram with: (a) – methyl solution of *N*,*N*-diethyl-*p*-phenylenediamine or diphenylamine and then UV exposure, (b) – Gries reagent in alkaline medium or (c) – 10% methyl solution of potassium hydroxide. Each of them reacts with TNT giving a coloured band [8].

The estimation of quantitative relations *A* = *f(c)* (where: *A* – area of densitometric peak in relative units of the apparatus, *c* – TNT mass [ng] in chromatographic band) conducted also with standard solution. Variable volumes (5 - 500 μL) of the solution of 10 ng/μL concentration were placed on a start line on chromatoplate and than the chromatograms were developed in accordance with the technique described above. The chromatograms were scanned by *zig-zag* technique at a wavelength corresponding to λ_{max}. The results averaging from five measurements allowed for specification of the equation of an analytical curve ($A = 35\ 051c - 838$) and estimation of the limit of detection (70 ng) and the maximal range of linearity of detector indications (2800 ng). The received results were used for estimation of TNT recovery in extraction processes and for verification researches.

Extraction

Searching for a method of efficient getting TNT from matrixes various extraction techniques were applied. In the case of solid matrixes liquid-solid extraction (LSE) in Soxhlet apparatus and LSE assisted by ultrasonic were used. In the case of water TNT was extracted in liquid-liquid system (LLE) or solid phase-extraction technique (SPE) with columns filled with C-18 silica gel, increased polarity octadecyl (C-18 Polar Plus) and columns with styrene-divinylbenzene copolymer (SDB-1) are used.

Samples of municipal water of 500 mL volume were spiked with150 μg TNT and then an analyte was extracted (shaking during 15 min) with two portions of methylene chloride and ethyl acetate (2 : 1) mixture, each of them of 25 mL volume. Connected organic layers were dried by anhydrous magnesium sulphate, filtered and concentrated first with an evaporator to about 5 mL and next in nitrogen stream to 500 μL. The received solutions were spotted (5 μL) at a start line of chromatoplates. SPE columns were rinsed by 2 × 1 mL of hexane and 2 × 1 mL of methanol, conditioned by 2 mL of water and then a water sample was passed at 1 L/h. Subsequently the columns were dried for 15 min in nitrogen atmosphere. Analyte was eluted withtwo portions of acetone, each of them of 1 mL volume. Received extracts were concentrated in nitrogen atmosphere to 500 μL and analysed according to the procedure.

Samples of bricks, concrete and sand of 20 g each were powdered and spiked with 150 μg of TNT; samples of wood (sawdust) were contaminated in the same way but because of extraction thimble volume only 10 g samples were used for analysis. An analyte was extracted withtwo portions of acetone (each of 12 mL) in an ultrasonic water bath (30 °C, 25 min) or in Soxhlet apparatus (150 mL of acetone, 5 exchanges). Next stages of the extracting and determination of the analyte (concentration, chromatographic analysis and densitometric measurements) were identical as in the case of the water extracts. After developing of chromatograms the number of the analytes in bands were estimated (conditions of chromatographic analysis and scanning were the same like during quantity relation measurements).

The most beneficial effects, in a sense of the possibility of analyte extraction from other components of a sample, which can move to a solution in extraction processes, were obtained in the case of water extracts (Figure 2a). The eluent was only insignificantly contaminated and a chromatogram closed to the one obtained during standard measurements. Less advantageous results were obtained during analysis of sand, brick and concrete samples (Figure 2b-d). Considerable amount of impurities moves into extract but they do not interfere with the applied chromatographic set. Thanks to this, a confirmation of the analyte presence and estimation of its amount in a chromatographic band is possible. Definitely the least profitable effect was got in the case of wood samples. The amount of impurities, which moved into extract, was very large. They interfere with TNT, making analyte identification impossible.

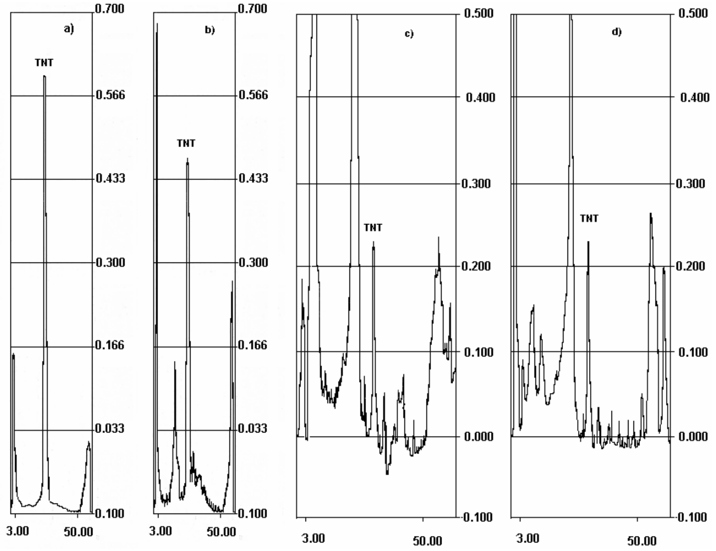

Figure 2. Resolution of mixture components received after extraction a) – water, b) – sand, c) – concrete, d) – bricks. TNT peak is marked. The other designations as in Figure1.

Some improvements of the resolution of wood extract components by changing mobile phase composition and chromatogram developing by two-way elution technique did not show any results. A great number of interference with similar retention properties made measurements impossible. That is why we attempted TLC/TLC coupling and separate TNT in two different phase systems.

Some wood extract was applied to starting lines of chromatoplates coated with silica gel with chemically bounded octadecyl, amine group or octyl, search for some phases which could reduce the number of interference with comparable R_f value. The best results were got in the following phase system: stationary phase – silica gel with chemically bounded octadecyl (Merck catalogue number 1.05559), mobile phase - acetonitrile - water 5 : 3 (v/v). This system was used for purification of the wood extract. The wood extract sample was placed at a start line in the form of six strips of 20 μL volume each. After chromatogram developing analytes with R_f value close to TNT were removed along with the adsorbent (the chromatoplate was used in this case for preparation purposes). The adsorbent was moved to an extraction column of SPE set and the analytes were eluted with two portions of acetone, 1 mL of volume each. The received extract was concentrated in nitrogen atmosphere to 500 μL of volume and then spotted at the start line (100-300 μL in one band). The amount of impurities, which accompanied TNT, was considerably smaller than in the initial extract and large volume samples did not overload a plate. Chromatograms were developed in previously described NP system (Figure 3).

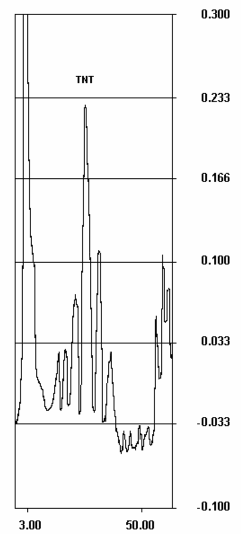

Figure 3. Separation of wood extract components by TLC/TLC.

Having a technique for TNT separation from any matrix the experiments were repeated and matrixes were contaminated with known (100-200 μg) amounts of analytes. Averaging results of measurements (n = 5) were used for calculation of recovery (Table 1, 2). It was also shown that the analyte recovery does not depend on its concentration in the matrix in the discussed range of concentrations.

Table 1. TNT recovery from water

Extraction technique		Recovery [%] (n = 5)	Standard deviation [%]
LLE		90.2	5.0
SPE:	C-18	80.3	5.1
	C-18 Polar Plus	83.5	4.8
	SDB-1	92.9	4.4

Table 2. TNT recovery from solid matrixes (LSE)

Matrix	Soxhlet apparatus		Ultrasounds	
	Recovery [%] (n = 5)	Standard deviation [%]	Recovery [%] (n = 5)	Standard deviation [%]
Sand	59.2	6.1	55.3	5.2
Wood*	64.4	7.6	56.0	7.6
Brick	54.9	5.9	43.6	5.3
Concrete	55.5	5.6	44.9	5.9

*Recoveries estimated after TLC/TLC coupling

Verification researches

These researches consisted in measurements (according to p. 2.1) of TNT residue in samples received after standard charge explosion. The question was if and in what range proposed method of researches can be used for:

- identification analysis,
- estimation of an explosive which does not undergo explosion transformation,
- measurements of degree of possible after-detonation explosive transformation,
- evaluation of matrix influence on the amount of explosive residue accumulated in it.

Standard charge explosion stand

Measurements of after-explosion explosive residues are usually made in firing grounds, after detonation of a charge weighting 1-2 kg, and conditions are close to those in place of an accident. These tests are expensive, dangerous, time consuming and not environmentally friendly. Trying to make them simpler, a stand for explosions of small standard charges was designed and realized. Experiments were conducted in a steel tube with inside diameter of 120 mm, wall thickness of 30 mm and length of 800 mm. The tube was placed on a steel plate having a thickness of 10 mm and a diameter of 200 mm and being an element of a lock of a tube base (Figure 4). TNT charge of 25 g weight surrounded by a material performing a role of a base (matrix) was placed axially inside the tube at 1/3 length of the tube (measured from the base). Matrixes (parameters given in Table 3) were placed in paper containers or foil bags and then fused with blasting cap *erg* type (Figure 5).

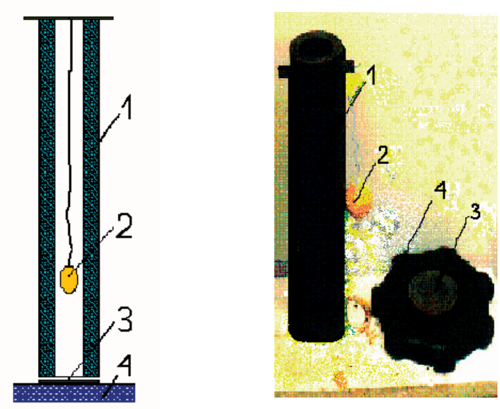

Figure 4. Scheme of explosion simulation set: 1 – steel tube, 2 – explosive charge and matrix, 3 – lock of the tube base, 4 – steel plate.

Table 3. Properties of explosive base (matrix)

Parameter	Matrix				
	Water	Wood	Sand	Brick fragments	Concrete clods
Mass [g]	200	100	300	300	300
Refinement	Tap water	Sawdust	Grains < 2 mm	Compact fragment with a hole for explosive	Regular clods of 50-60 g

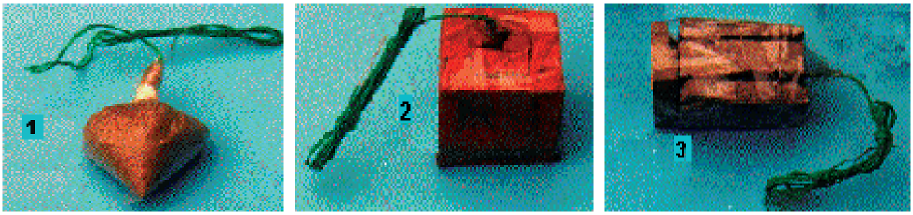

Figure 5. Examples of fused TNT charges surrounded by matrixes 1 – sand, 2 – brick, 3 – concrete.

After explosive detonation the tube inside was swept with a bore brush, the tube was taken off the base and some matrix fragments that remained on it (treated like representative samples) were given to analyses. In the case of water the lock of the base was replaced by a steel plate with a pit, and liquid condensed inside it (about 50 mL) was analysed. Regardless of an explosive or matrix type, and analytical purposes of the experiment, this operation was repeated five times.

Measurements of TNT residue after detonation

At first analyses were performed identically as in the case of the recovery analyses. Unfortunately no satisfactory results were obtained. Analyte quantities were – regardless of the type of matrix – insufficient for their identification and determination. Attempts at dosing larger volumes of extracts (200-300 μL) also failed. As a matter of fact analyte masses dosed at start lines were larger in that case but masses of components which were not determined were also larger and that made chromatograms close to these showed in Figure 3. Further experiments were conducted – as in the case of wood extracts – by TLC/TLC technique, using alternately inverted and normal phase system.

Extracts were placed at start lines of chromatoplates covered by silica gel with chemically bound octadecyl in the form of six bands, each of them of 20 μL volume. Mobile phase was a binary mixture of acetonitrile - water 5 : 3 (v/v), and elution range was 6 cm. Next the adsorbent and the analyte were removed and placed in a column of SPE set and eluted with two portions of acetone, each of them of 1 mL volume. The extracts were analysed in NP system, applying to start lines 360 μL. Received chromatograms (with distinctly extracted TNT bands) insignificantly differed (Figure 6). Only wood extract contained (like in the case of the recovery from this matrix) considerably more peaks, but they did not disturbed densitometric measurements. The number of the analytes in individual matrixes (Table 4) was estimated with external standard, taking into account before estimated recoveries.

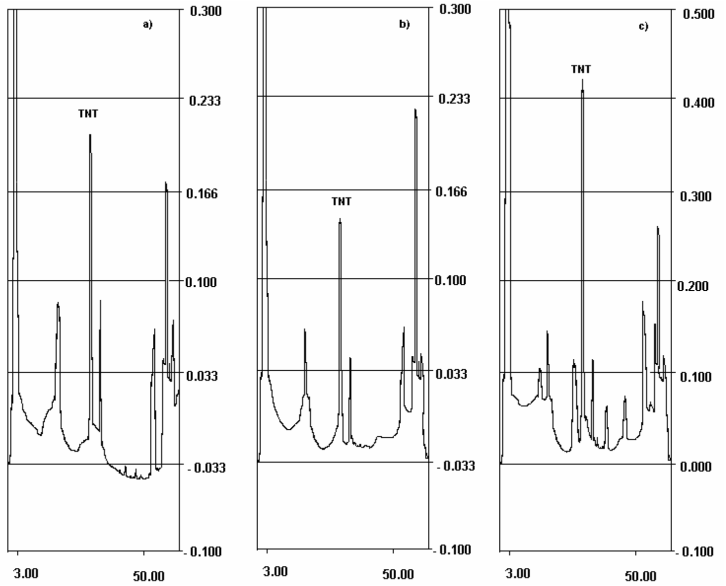

Figure 6. Chromatograms of extracts after TNT explosion. TLC/TLC coupling: a) – sand, b) – concrete, c) – wood. The other designations as in Figure 1.

Table 4. TNT residue that penetrate into matrix after detonation of 25 g charge

Matrix	TNT content	
	Mass* [μg]	Standard deviation [%]
Water	0.260	6.1
Sand	0.384	5.8
Brick	0.178	6.1
Concrete	0.161	6.4
Wood	0.208	7.1

*Weight averaging from 5 measurements.

Even superficial analysis of the results compiled in Table 4 show that the quantities of TNT residue are insignificant (they are about 0.1%). It is noticed that (regardless of the type of the matrix) there are distinct differences between the extract compositions that were received from matrixes in explosion zone and the extract compositions from matrixes, which were not in this zone. Chromatograms of the first one contain additional bands, which are absent from the matrix contaminated by TNT, but which was not in the explosion zone. These observations suggest that this part of TNT, which did not undergo any chemical changes during a detonation, undergo further chemical reactions just after the detonation.

Analysis of products of inexplosive TNT changes

Striving for confirmation of chemical changes of TNT residue, which did not react in detonation way, the following experiment was conducted. A sand sample of 300 g weight was contaminated with acetone solution of the analyte in proportion which corresponded to 1% of TNT content (with reference to sand), and then it was placed around 25 g RDX charge (RDX oxygen balance is negative, just like TNT one). During this experiment TNT contamination of sand was equivalent to this part of TNT charge, which was not undergo detonation. After RDX detonation TNT content in the sand sample was analysed. Only 24.6% of TNT (the result averaged from 5 explosions, taking into consideration before estimated recovery) was detected. A substantial TNT loss and appearance of additional chromatographic bands (the same R_f values as these observed during TNT charges detonations) indicate that they derive from products of inexplosive TNT changes.

The most intense chromatographic band (not observed during analyses of the matrixes which were not in the explosion zone), distinctly yellow, was analysed in detail by GC/MS. This band of R_f value was equal 0.55 (the value estimated after extract resolution in a reversed phase set). It was removed from a chromatoplate and the adsorbent was placed in SPE cartridge. The analytes were eluted with acetone (2 × 1 mL) and the received extract was concentrated in nitrogen atmosphere to 500 μL volume. It was injected with 2 μL of extract to a sample injector. The analyse conditions were following: temperature of sample injector system – 250 °C; of transfer line 250 °C; of mass analyser – 160 °C; temperature program – 40 °C/2 min → 10°C/min → 280 °C/5 min; carrier gas – helium; ion source closure delay – 4.7 min; ion detectability range – 40-400 a.m.u.; ionisation energy – 70 eV; dosage method – split/splitless, splitless = 0.48 min; split flow - 50 mL/min. Analysed sample contained two components. They were identified by a comparison of the received mass spectra with these were from the spectrum library. These were 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene.

Conclusions

Thin-layer chromatography presented a suitable method for researches of explosives included in complex environmental matrixes; it can be used directly for analyses of these substances or as a preparatory technique (purification). An instrumentation of the method created a possibility of dissemination of TLC/TLC coupling, which was rarely used so far. Researches done confirmed usability of this technique for getting explosive traces included in wood, sand, brick, concrete or water samples. The developed method allows qualitative and quantitative estimation of explosives included in matrixes remaining in the close vicinity to the explosive charge. That is why it is possible to estimate not only a type but also a quantity of an explosive used in a place of an accident.

Noticing of after-explosion changes of explosives we consider the greatest achievement of this work. Analysis of the products of these changes can be an additional, increasing certitude of crime detection diagnosis, element of an accident place examination.

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