*See also:* **II/Chromatography: Liquid:** Detectors: Mass Spectrometry; Detectors: Ultraviolet and Visible Detection; Nuclear Magnetic Resonance Detectors. **Extraction:** Solid-Phase Extraction; Solvent Based Separation; Ultrasound Extractions. **III/Solid Phase Extraction with Cartridges.**

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# Thin-Layer (Planar) Chromatography

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## Introduction

The term explosive has two meanings. It is used for individual chemical compounds among which trinitrotoluene (TNT), 1,3,4-trinitro-1,3,5-triazacyclohexane (hexogen-RDX), 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane (octagen-HMX), pentaerythriol tetranitrate (pentrik-PETN) nitroglycerine (NG) and nitrocellulose (NC) are commonly known. This term is also used for mixtures of the above individual compounds and their mixtures with other, non-explosive substances; the type and amount of components in a mixture determines its properties (brisance, melting, plasticity and the like). Explosives have been classified in many ways according to different criteria. Most important is the kind of addition, the $NO_2$ group and type, and the velocity of the reaction involved. The first are divided into the following groups; nitro compounds containing the $C\text{-}NO_2$ group, nitrate esters with $C\text{-}O\text{-}NO_2$ group and nitramines with $C\text{-}N\text{-}NO_2$ group. The second criterion divides explosives into high and low explosives (HEs and LEs respectively).

The identification and quantification of the HEs or LEs are very valid and also present difficult analytical problems. These problems become evident especially during: (i) testing of environmental pollutants, (ii) forensic investigations, and (iii) checking technological processes and service conditions of munitions manufacture. From an analytical point of view, at least three groups of TLC applications in explosive investigations can be distinguished. The first of them, most often represented in the research literature, concerns qualitative (including screening methods) and quantitative analysis of explosives. The second is where TLC is applied as a clean-up technique. In this case analysis is completed by other analytical techniques. The last group of applications mainly covers the evaluation of LE stability.

Although, recently, more sophisticated methods such as thermal analyses and gas or liquid chromatography are commonly used in many laboratories, TLC is still in use. Starting from paper chromatography and improved over many years, TLC has become very effective in the analysis of explosives. Apart from high performance adsorbents, and the ability to perform separations in both normal and reversed-phase systems, the real advances are due to the development of densitometry and the spray-on technique of sampling.

### Analyses of Explosives

Early TLC analysis was performed on home-made chromatographic plates and involved the separation of classical HEs such as TNT, RDX,

**Table 1** Examples of TLC separation of explosives

| Analyes | Chromatographic system | | Type of elution |
|---|---|---|---|
| | Stationary phase | Moblie phase | |
| TNT, RDX, PETN, tetryl, NC, NG | Silica gel | Trichloroethylene–acetone (4 : 1) | One-dimensional, isocratic |
| TNT, RDX, HMX, PETN, tetryl | Silica gel HPTLC $F_{254}$ | Carbon tetrachloride–aceto-nitrile (8 : 1) | One-dimensional, isocratic |
| HMX, RDX, 2,4-DNT, TNT, NG, PETN, tetryl | Silica gel HPTLC $F_{254}$ | I petrol ether – acetone (3 : 1) II petrol ether – ethyl acetate (8 : 1) | Two-dimensional, isocratic |
| Trinitrobenzene, 2,4-DNT, TNT tetryl, NG, PETN, RDX | Silica gel HPTLC $F_{254}$ | Benzene - petrol ether–methanol (8 : 6 : 1) | One-dimensional, isocratic |
| TNT m-dinitrobenzene, trinitro-benzene, tetryl, 2,4-dinitrochloro-benzene, picramide, heksyl | Silica gel G-magnesium silicate (1 : 1) | Xylene | One-dimensional, isocratic |
| 2,3,4-TNT, 2,4,6-TNT: 1,2-DNB: RDX | Silica gel $HF_{254}$ | Benzene–hexane–pentane (5 : 4 : 1) | One-dimensional, isocratic |
| RDX, HMX, tetryl, PETN, TNT, 2,4-DNT, 2,6-DNT | Silica gel with chemical bonded octadecyl, RP-C-18 | | AMD, gradient elution |
| Hexyl, picric acid, RDX, HMX, 2,4,6-N-tetranitro-N-methyl-aniline, 2,4- and 2,6-dinitro-toluenes, TNT, 1,3-dinitrobenzene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene | Silica gel HPTLC | | AMD, gradient elution |

HMX and PETN (see Yinon and Zitrin in Further Reading).

One-dimensional ascending or horizontal techniques have usually been applied for the development of chromatograms in a closed chamber (**Table 1**). Multiple and two-dimensional development techniques have seldom been used but automated multiple development (AMD) and gradient development are becoming more frequently used.

In TLC much attention is paid to the methods of analyte visualization, especially to search for specific reactions which can confirm the presence of an analyte in a spot or band. Confirmation of the identity of an analyte is thus based on at least two criteria,

$R_F$ and colour reaction. The majority of more or less specific explosive visualization schemes have been worked out long ago (**Table 2**). Exhaustive reviews of those methods can be found in the publication by Yinon and Zitrin, mentioned above, and in York *et al.* (see Further Reading).

Most investigations on the analysis of explosives by TLC have been confined to environmental protection. In such research, classical TLC has been applied mainly as a screening method and has served to provide essentially qualitative analysis; instrumental TLC provides both qualitative (screening) and quantitative determination. Environmental analysis of explosives is justified by the fact that nitrotoluenes and

**Table 2** Examples of visualization of explosives

| Visualization method | Analyte | Quantification | |
|---|---|---|---|
| | | Yes | No |
| UV absorption | 2,3,4-TNT, 2,4,6-TNT, 1,2-DNB, RDX | x | |
| NaOH in methanol acetone (1 : 1) | Nitroaromatic compounds | | x |
| π complex formation with *o*-toluidine, N,N-dimethyl-aniline, m-chloroaniline or anthracene | m-DNB, TNB, TNT, tetryl, 2.4-dinitrochloro benzene, picramide, heksyl | | x |
| Griess reagent | RDX, HMX | x | |
| UV followed by 5% DPA in methanol acidified by $H_2SO_4$ | RDX and nitrates | | x |
| 5% DPA in methanol | Nitroaromatic compounds | | x |
| NaOH/ Griess reagent | NG, NC | x | |
| 0.2% Würster's salt in methanol | NC, NG, PETN | | x |

especially trinitrotoluenes are highly toxic compounds. The aromatic amines formed by their biodegradation are suspected to be carcinogenic. Due to careless handling during the manufacture, loading, and packing etc., of explosives, groundwater, surface water and soil may be contaminated with these compounds. Occasional plant accidents, and residues from World War II are also sources of pollution.

Screening methods, which most often use colorimetric visualization reactions, serve to lower the cost and are less time-consuming for the analysis of a large number of samples. An excellent example which illustrates the advantages of TLC for such applications is provided by Haas and Stork. The most interesting information in this work lies in the method of sample preparation (liquid–liquid extraction) and the technique of separation of large numbers of analytes. Water samples taken from wasteheaps, containing post-production residues of TNT, were extracted with isopropyl ether under a range of pHs. At pH 4 aromatic amines were retained in the aqueous phase and phenols and nitro compounds were extracted into the organic phase (ether extract A). To enable extraction of amines into the organic phase, the pH was adjusted to 8 (ether extract B). Phenols were isolated from extract A by treating with 1M NaOH (nitrotoluenes remained in the extract). The aqueous phase, of extract A was then re-adjusted to pH 4 and re-extracted to transfer phenols to the organic phase (extract C). After drying and concentration, the three extracts are analysed by normal phase chromatography using two-dimensional isocratic elution. Analytes are identified by the quenching of fluorescence or by colour reactions. In extract A (nitrotoluenes) 25 compounds were found and 2,4-, 2,6-dinitrotoluene and TNT were identified. In extract B (nitroamines) 26 compounds were found. In extract C, containing phenols, 28 compounds were found and 2,4-dinitro-6-methylophenol and 2,6-dinitro-4-methylphenol were identified. Confirmation of TLC results and quantification were performed using spectrophotometry and gas chromatography.

On-site TLC screening methods for explosives in soil have been reviewed by Nam who suggested that the results obtained using two colorimetric-based methods (TNT and RDX-methods), commonly used for on-site analysis of explosives in the USA, are not perfect. The first of the methods, is based on the reaction of an acetone soil extract of TNT with base, to produce reddish coloured Jankowsky ions. For the RDX method, the soil extract is first acidified and reacted with zinc to reduce the RDX to nitrous acid; the solution obtained is reacted with Griess reagent to produce a reddish coloured azo dye. Unfortunately,

these are not specific reactions and require other techniques for confirmation. The work describes the separation techniques for nitroaromatics, nitroamines and nitrate esters, classical, colour visualization methods and also the costs of analysis. Laboratory analytical methods for the most commonly found components of explosives, and environmental transformation of these substances in a soil matrix, have been developed in the work of Jenkins *et al.*

More recently gradient automated multiple development (AMD) HPTLC using a normal phase system has been applied to the determination of explosives and their biodegradation products in contaminated soil and water. The subjects of examination were hexyl, picric acid, RDX, HMX, 2,4,6-N-tetranitro-N-methylaniline, 2,4- and 2,6-dinitrotoluenes, TNT, and other by-products such as 1,3-dinitrobenzene, 2-amino-4,6-dinitrotoluene, and 4-amino-2,6-dinitrotoluene found in former ammunition site waste in Germany. The use of optimized gradient elution and an AMD system allowed the separation of analytes from environmental samples and quantification at the 5–20 ng level; humic substances presented in water samples do not interfere. In the case of samples of soil with a high concentration of humic substances, a clean-up technique is necessary. The superiority of TLC is displayed by the fact that similar analyses using GC or HPLC require (even for water samples) very careful sample clean-up or the application of size exclusion chromatography.

Modern TLC equipment has also been applied to the quantitative determination of HE residues in soil and water using a reversed-phase system with automated multiple development gradient elution after liquid–liquid and solid-phase extraction. Quantification was carried out by UV or visible absorption light measurement *in situ*. The work demonstrated that the application of AMD was effective for the isolation and separation of relative complex HE mixtures in one analytical process (**Figure 1**). Most of the examined HEs absorb UV light, which allows their quantification by densitometry (**Table 3**).

During the measurement of PETN, Wurster's salts were applied (organic nitrate esters oxidize Wurster's salts yielding the so-called 'Wurster's cations' which are intensely coloured). It has been proved that this reaction can also be used for quantitative analysis. The extraction of HEs from water samples, using solid-phase extraction (SPE) cartridges filled with SDB-1 phase (recoveries $\approx 90\%$), was shown to be more effective than liquid–liquid extraction. Slightly lower values of recoveries during extraction from soil (77.0–84.4%) were obtained but these result from the more complex procedure for their preparation for analysis (necessity of purification).
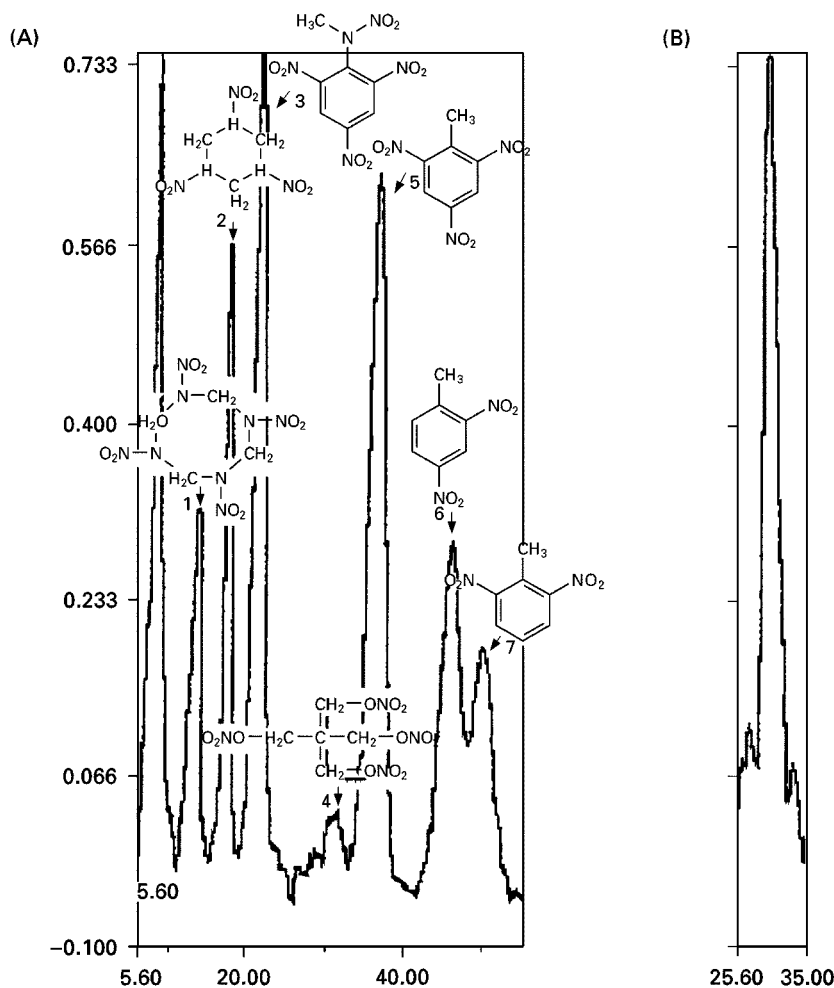
**Figure 1** Chromatogram of high explosives: 1 = octagen, 2 = hexogen, 3 = tetryl, 4 = pentrit, 5 = trotyl, 6 = 2,4-DNT, 7 = 2,6-DNT: (A) scanning at wavelength 220 nm, (B) pentrit, scanning at 600 nm. Abscissa – absorbance, ordinate – range of the spots in mm. (Reproduced with permission from Błądek J *et al.* 1998.)

The proposed technique for the elimination of the majority of non-polar impurities from soil extracts using SPE is very effective for the identification and quantification of the analytes examined (**Figure 2**).

Post-blast analyses, or identification of explosive residues in forensic investigations, are directly associated with combat, and criminal or terrorist activity. Results of such analyses may give information about the type, and sometimes also about the source of

**Table 3** Parameters of quantification

| High explosive | $\lambda_{max}$, nm | Calibration curve | | | Detection limits, ng |
| --- | --- | --- | --- | --- | --- |
| | | $A*.10^{-3} =$ | Correlation coefficient | Max, range of linearity, ng | |
| Octagen | 215 | 8.8c − 0.1 | 0.9861 | 1600 | 200 |
| Hexogen | 220 | 10.7c + 0.6 | 0.9858 | 1600 | 200 |
| Tetryl | 220 | 28.5c + 3.1 | 0.9989 | 1000 | 50 |
| Pentrit | 600 | 30.9c + 0.3 | 0.9899 | 1600 | 200 |
| TNT | 220 | 12.5c + 50.5c − 0.6 | 0.9989 | - | 50 |
| 2.4-DNT | 240 | 29.7c − 1.0 | 0.9378 | 1000 | 100 |
| 2.6-DNT | 220 | 10.7c − 0.7 | 0.9728 | 2000 | 200 |

*A, densitometric peak area; c, amount of the pesticide in the band.
(Reproduced with permission from Błądek J *et al.* 1998.)
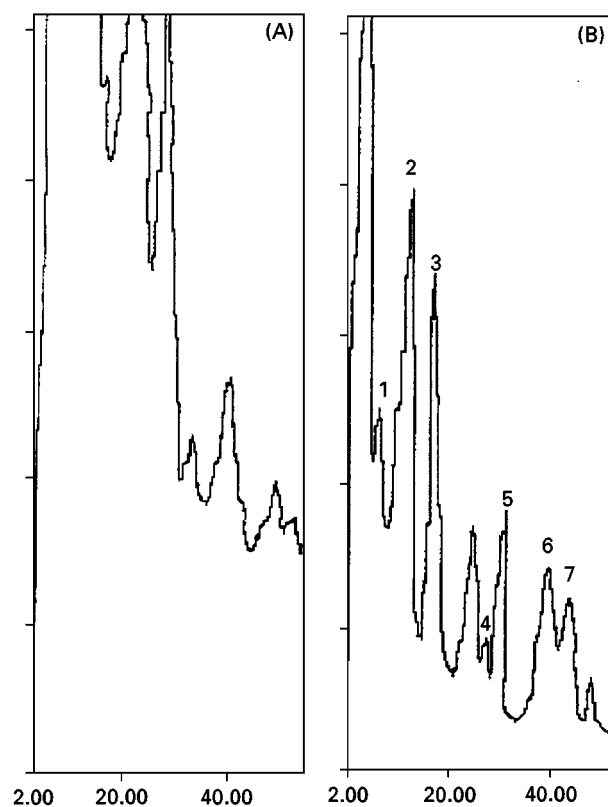
**Figure 2** Chromatograms of soil sample: (A) before purification, (B) after purification by SPE. Notation as in Figure 1. (Reproduced with permission from Błądek J *et al.* 1998.)

explosives or about possible contact with explosives. From an analytical point of view, these methods are similar to investigations of explosives in environmental matrices; the difference concerns isolation from the matrix (debris, bodies and surfaces). These matrices are usually extracted in two ways: by water and by organic solvent. The water extract is then analysed for inorganic ions and the organic extract for the explosives. Yinon and Zitrin have described the complexity of such analysis (see Further Reading).

## TLC as a Clean-up Method

Forensic and environmental analyses are usually performed in several stages, one of the most important being sample preparation. This stage covers the isolation, concentration, and purification of analytes for further analysis. Three basic methods of purification of analytes may be distinguished: liquid–liquid extraction, solid-phase extraction and preparative thin layer chromatography. For preparative TLC, the analytes are spotted as a long band on the start line. After developing chromatograms the plates are dried, and cut, if plastic or aluminium, or scored, if glass, so that they contain the standard lanes and part of the sample. Classic, preparative TLC has been rarely used for the purification of explosives. Easy access and a variety of stationary phases for SPE make it more useful than preparative TLC in this application.

**Table 4** Examples of TLC separation and visualization of stabilizers

| Stabilizer | Chromatographic system | | Type of elution | Visualization |
|---|---|---|---|---|
| | Stationary phase | Mobile phase | | |
| DPA and its derivatives | Silica gel HPTLC | *1 step*: petrol ether–benzene (1:1) *2 step*: benzene *1 dimension*: benzene-1,2-dichloroethane– carbon tetrachloride (10:5:6) | One-dimensional, two-steps, isocratic | UV or VIS absorption |
| DPA and its derivatives | Silica gel | *2 dimension*: ethyl acetate–petrol ether (2:8) | Two-dimensional, isocratic | UV or VIS absorption |
| EC and its derivatives | Silica gel | *1 dimension*: benzene *2 dimension*: benzene–diisopropyl ether (3:1) | Two-dimensional, isocratic | UV or VIS absorption |
| DPA and its derivatives | Silica gel with zinc powder | *1 dimension*: petrol ether–benzene– acetone (99:99:2) *2 dimension*: petrol other–ethyl acetate (4:1) | Two-dimensional, isocratic | 0.25% ethanol solution of *p*-diethylaminebenzaldehyde in 0.25 M HCl |
| EC and its derivatives | Silica gel with zinc powder | *1 dimension*: 1,2-dichloroethane *2 dimension*: petrol ether–ethyl acetate (4:1) | Two-dimensional, isocratic | EC: 0.003% dichlorofluorosceine in ethanol followed by UV; derivatives: 0.25% ethanol solution of *p*-diethylaminebenzaldehyde in 0.25 M HCl |

## TLC in Research

This work mainly concerns LEs (propellants). Nitrate ester-based propellant compositions decompose under normal storage conditions producing nitrogen dioxide as a primary decomposition product. This $NO_2$ is the source of further, autocatalytic decomposition of propellants, limiting their safe storage life. In order to reduce the rate of deterioration of propellants, stabilizers (usually diphenylamine – DPA) or centralite I-(N,N'-diethyl-N,N'-diphenylurea) –EC, which react with the primary decomposition products, are usually incorporated, to inhibit degradation. The amount of stabilizers added varies, but is typically less than 1% (in the case of DPA) and 3% (in the case of centralite). The stabilizers react with nitrogen oxides forming different degradation products and whilst some of these products also act as stabi-lizers others contribute to stabilizer depletion. Examples of the application of TLC to stabilizer measurement are presented in **Table 4**.

One example of the application of TLC for propellant stability research uses three chromatographic systems for the isolation of stabilizers (**Figure 3**) from single-base, free emulsion double base and emulsion propellants (**Table 5**). Samples are divided into 2 parts. One is heated for 1 h at 120°C in an open vessel and the other left unheated. All samples are then dissolved in acetone (this procedure is more effective then the extraction of stabilizers) and analysed to observe the differences between the state of chemical change of the stabilizer and its reaction products before and after ageing. Analyses are performed using a liquid–crystal detector, and confirmation was done using densitometry.
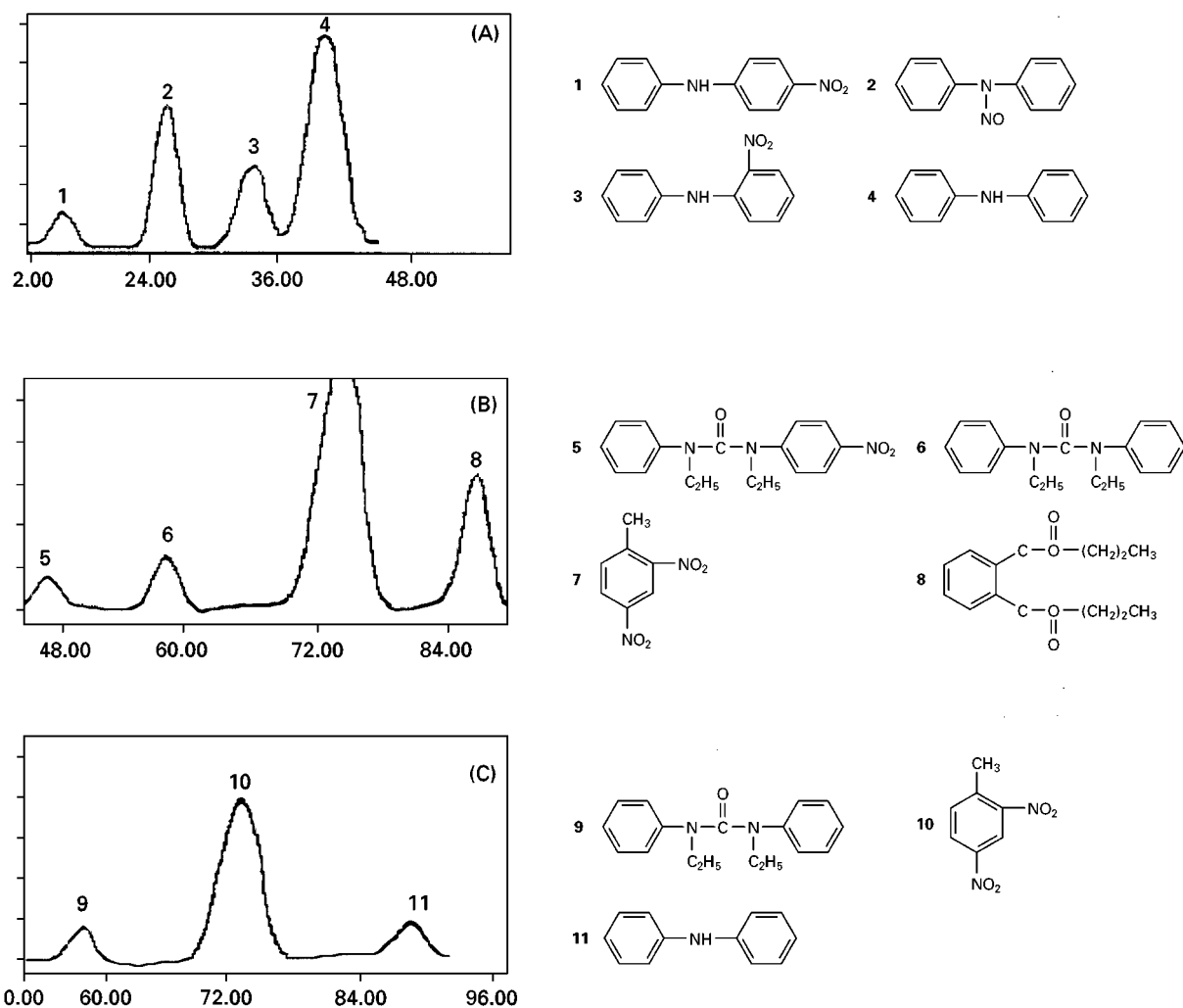


**Figure 3** Fragments of propellant chromatograms: (A) single-base, (B) double base, (C) emulsion propellant. Abscissa – absorbance, ordinate – range of the spots in mm. Peaks notation: 1 = 4-nitrodiphenylamine, 2 = N-NO-diphenylamine, 3 = 2-nitrodiphenylamine, 4 = diphenylamine, 5 = 4-nitro centralite, 6 = centralite, 7 = 2, 4-dinitrotoluene, 8 = dibutylphthalate, (c) 9 = centralite, 10 = 2,4-dinitrotoluene, 11 = diphenylamine. (Reproduced with permission from Błądek J *et al.* 1993.)

**Table 5** Characteristic compositions of propellant samples

| Propellant | Concentration [%] | | | | | | |
|---|---|---|---|---|---|---|---|
| | NC | EC | NG | DPA | DNT | DBP | Others |
| Single-base | 96–99 | | 0.7–2.0 | | | | 0.0–1.8 |
| Emulsion-free double- base | 56–66 | 20–45 | | 0.7–3.0 | 7–12 | 0.0–5.0 | 0.0–1.5 |
| Emulsion | 56–70 | 10–20 | 0.5–1.0 | 3–4 | 10–15 | | 0.0–1.5 |

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In three lots of single-base propellants, stored for 48, 47 and 41 years consumption of DPA in the range 75–100% was determined (before heating). The main products of chemical change are N-NO-DPA, 4-$NO_2$-DPA, 2-$NO_2$-DPA and also traces of dinitro-DPA. The most often observed differences in the concentrations of DPA before and after heating were in the range 0.2–0.6% (m/m). In most stable double-base propellants during storage of up to 50 years, a small decrease of 5–10% EC content was observed. Rarely, traces of 4-$NO_2$-EC were found. This work also found that in some unstable double-base propellants (stored for 40 years) and mortar propellants (stored for 38 and 23 years), 90–100% of the EC had changed into its reaction products mostly into mononitro-EC. Studying chemical changes of EC stable propellants before and after heating, there were determined differences in EC concentrations in the range 0.2–0.8% (m/m). As in single-base propellants, the dynamics of stabilizer changes in double-base propellants, both under natural and accelerated ageing varies up to ten times under the same conditions of ageing. It means, that the quality, especially of basic components of the propellants, has greater influence on their decomposition than the 'age'. The use of TLC methods to determine the stabilizers and their reaction products in the propellants seems to be suitable and reliable.

## Conclusions

There are a large set of analytical needs connected with the analysis of explosives. The importance of this problem can be appreciated by looking at the statistics on recent bombings in the world. Besides the tasks mentioned above, there is a need for vehicles or mail screening, bomb search, protection of special infrastructure features, and so on. Of course, not all analysis can be performed using TLC.

In the history of TLC for analysis of explosives the 1960s and 1970s was a time of very intensive use. The development of modern analytical methods has caused a decrease in interest in TLC. An increase of interest in TLC, as applied to the analysis of explos-

ives, can be observed in the 1990s and is connected with the intensive development of the method.

TLC, like all other chromatographic techniques, is a comparative method. It is impossible to obtain information about the structure or identification of unknown compounds or mixtures. In such cases there exits the possibility of combining TLC with other, mainly spectroscopic, analytical techniques (e.g. TLC-MS). Such combinations will probably start widening the application of TLC in the analysis of explosives.

*See also:* **II/Chromatography: Thin-layer (Planar):** Densitomery and Image Analysis; Instrumentation; Layers; Mass Spectrometry; Modes of Development: Conventional; Preparative Thin-layer (Planar) Chromatography; Spray Reagents; **Extraction:** Solvent Based Separation; Solid-Phase Extraction. **III/Explosives:** Gas Chromatography; Liquid Chromatography. **Humic Substances:** Liquid Chromatography.

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# EXTRACTION: PRESSURIZED FLUID EXTRACTION

# FATS

## Crystallization

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### Introduction

Fats, as mostly represented by triacylglycerols, are employed in foods, cosmetics, pharmaceuticals, etc. as the main bodies of end products, or as the matrices in which fine chemicals are dispersed. The crystallization behaviour of fats has two major industrial implications: (a) processing of the end products made of fat crystals, such as chocolate, margarine and shortening, whipping cream, etc. and (b) separation of specific fats and lipids materials from natural resources, mostly from vegetable or animal fats and oils, which contain various molecular species having different chemical and physical properties. As for the separation technology of crude fat resources, such as palm oil, milk fats, hydrogenated vegetable oils, etc. it may be worth noting that current market demands raise a great necessity to develop the fractionation of high-melting and low-melting fats and lipids through dry fractionation. The main causes for this are the replacement of hydrogenation by dry fractionation, a new regulation of usage of fat materials for confectionery fats, better functionality of physically refined vegetable oils compared to conventional materials, etc. This review highlights the basic information on recent research on the crystallization of fats, with a specific emphasis on the separation phenomena.

The specific characteristics of the crystallization of fats are, on the one hand, polymorphism, and molecular interactions on the other. No long-chain compound without polymorphic modifications is present, and this property is more enhanced in triacylglycerol (TAG) crystals. The molecular interactions in TAG crystals are operative mostly through van der Waals forces between hydrophobic aliphatic chains, in which geometrical and steric hindrance is critical between the glycerol groups.

### Polymorphism in Fats

Polymorphism is defined as the ability for a chemical compound to form different crystalline or mesophase (liquid crystalline) structures. The melting and crystallization behaviour differs from one polymorph to others, since the different crystal structures correspond to different Gibbs energies.

Polymorphic crystallization is primarily determined by the rate of nucleation, as determined by thermodynamic and kinetic factors. If the first crystallizing form is less stable, it converts to more stable polymorphs through the solid matrix or liquid mediation. As a consequence, morphology of fat crystals is a function of the crystal structure itself, and the thermal processes of the crystallization and sub-