# Investigations of Thin Layer Chromatographic Techniques Used for Forensic Explosives Analysis in the Early 1970s

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**ABSTRACT:** The article describes three brief experimental studies of the thin layer chromatographic (TLC) techniques used for forensic explosive investigations at Royal Armament Research and Development Establishment at Woolwich (RARDE Woolwich) in 1974. The first study demonstrated that 200 ng amounts of nitroglycerine (NG) and pentaerythritol tetranitrate (PETN) in TLC spots visualized using the sodium hydroxide/Griess system can be recovered and reanalyzed using a second TLC eluent. In the second study the characteristics of a titanium(III) chloride/4-dimethylaminobenzaldehyde visualization system for nitrotoluenes were investigated. In the third study it was shown that under certain conditions the rate of PETN spot color development after Griess reagent spray could be very slow in comparison with that of NG.

**KEYWORDS:** criminalistics, forensic science, thin-layer chromatography, explosives, nitroglycerine, pentaerythritol tetranitrate, Griess reagent, titanium(III) chloride, dimethylaminobenzaldehyde.

At the first phase of a Public Inquiry (The Guildford and Woolwich Inquiry), which took place in London in May and June 1990, Sir John May investigated the 1976 convictions of the Maguire family and others for the possession of nitroglycerine. The convictions related to incidents in December 1974. In an interim report [1] Sir John expressed his belief that the convictions were unsound. The Home Secretary subsequently referred the case to the Court of Appeal which, after hearing evidence, quashed all seven of the convictions. During September and October 1991, second and third phases of the Public Inquiry examined the instigation of the prosecutions and the various reviews of the case between 1976 and 1990. The work described in this article was carried out between the first and second phases of the inquiry, in order to provide a solid experimental foundation for subsequent evidence. It is reported in part because of its intrinsic interest, and in part because thin-layer chromatography (TLC) remains an important technique for explosives trace analysis.

Identification of nitroglycerine (NG) traces by means of thin layer chromatography (TLC) was a crucial part of the prosecution evidence presented in the original trial. The

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<sup>&</sup>lt;sup>1</sup>Principal Scientific Officer, Forensic Explosives Dept., Defence Research Agency, Fort Halstead, Sevenoaks, Kent, UK.

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analyses had been carried out at the Royal Armament Research and Development Establishment at Woolwich (RARDE Woolwich). The initial phase of the inquiry therefore examined the techniques used very closely indeed. The Interim Report [1] concluded, among other things, that the TLC method used could not have distinguished between NG and pentaerythritol tetranitrate (PETN). Although PETN is also an explosive the distinction was of significance because the indictments referred specifically to the possession of nitroglycerine. The report noted evidence suggesting that such a distinction could have been made by scraping the detected spots from the initial plate, recovering unchanged material from the scrapings, and reanalyzing the recovered material in a different TLC system.

The inquiry established that, in addition to the tests that had given positive results for NG, a second set of TLC analyses was carried out in an attempt to detect any mononitrotoluenes (MNTs) or dinitrotoluenes (DNTs) present. These substances are present together with NG in some gelignite explosives. The nitrotoluenes tests gave negative results.

During preparatory work for the study of NG and PETN spot recovery a TLC plate with NG and PETN spots was lightly sprayed with sodium hydroxide then heated on a hotplate which had not yet reached the intended 100°C. The plate was nevertheless sprayed with Griess reagent and it appeared that the PETN spot color developed much more slowly than normal. When the other studies had been completed it was decided that this apparent effect deserved further investigation. It was of significance because RARDE Woolwich had maintained at the Maguire trial that PETN could be distinguished from NG on the basis that PETN spots appeared more slowly than those of NG. During tests carried out by Thorburn-Burns [4], and by me, prior to the first phase of the inquiry no such distinction had been observed.

The TLC techniques employed at RARDE in the early 1970s had been developed in part from an original method of Lloyd [2] and were described by Jenkins and Yallop in 1970 [3]. By the time of the Maguires case in 1974 benzene eluent had been replaced by toluene (known as 'System 1'). An excellent review of TLC methods for explosive analysis, covering the period in question, has been given by Yinon and Zitrin [5].

# **Materials and Methods**

# TLC Materials and Conditions

TLC plates were Macherey-Nagel Polygram SIL G UV<sub>254</sub> 200 mm  $\times$  200 mm and 50 mm  $\times$  200 mm with a layer thickness of 0.25 mm, supplied by CAMLAB, Cambridge, UK. They were used as supplied without activation. Solutions were applied to the plates using disposable glass micropipettes of 1, 2, and 5  $\mu$ L sizes, supplied by CAMLAB.

Elution was carried out vertically in plain unlined glass tanks. The eluents were prepared from reagent (GPR) or analytical grade solvents. 'System 2' eluent consisted of 20% ethyl acetate, 40% 40 to 60°C petroleum ether and 40% 60 to 80°C petroleum ether.

Visualization reagents were applied using commercial aerosol propellant sprays. Table 1 summarizes the compositions of the three Griess reagents used. Griess reagent 1 consisted of 10 g sulphanilamide, 1 g naphthylethylenediamine hydrochloride (NED) and 20 mL 85% orthophosphoric acid made up to 250 mL with water. Griess reagent 2, consisting of 2.5 g sulphanilic acid, 0.25 g NED and 75 mL glacial acetic acid made up to 250 mL with water was used only for the some of the PETN rate of color development tests. Griess reagent 3, which was also only used for the PETN rate of color development tests, was prepared from stock solutions containing 1 g of sulphanilic acid in 100 mL of 33% v/v glacial acetic acid and 0.5g  $\alpha$ -naphthylamine in 100 mL of 33% v/v glacial acetic acid acetic acid

Reagent Number	Composition	
1	10 g sulphanilamide + 1 g naphthylethylenediamine hydrochloride + 20 mL 85% orthophosphoric acid—made up to 250 mL with water.	
2	2.5 g sulphanilic acid + 0.25 g naphthylethylenediamine hydrochlo- ride + 75 mL glacial acetic acid—made up to 250 mL with water.	
3	Solution A—1 g sulphanilic acid in 100 mL 33% v/v glacial acetic acid.	
	Solution B-0.5 g $\alpha$ -naphthylamine in 100 mL 33% v/v glacial acetic acid. ( <i>Caution</i> $\alpha$ -naphthylamine is known carcinogen)	
	Mix 10 mL of Solution A and 10 mL of Solution B with 80 mL of 80% v/v glacial acetic acid shortly before use.	

TABLE 1—Compositions of Griess reagents.

solution and 10 ml of  $\alpha$ -naphthylamine solution with 80 mL of 80% v/v glacial acetic acid. (*Caution*: Because  $\alpha$ -naphthylamine is a known carcinogen special precautions were taken to avoid any exposure of personnel to this reagent). DMAB/HCl reagent (Ehrlich's reagent) consisted of 1g 4-dimethylaminobenzaldehyde and 3 mL concentrated hydrochloric acid dissolved in 180 mL n-butanol and 30 mL ethanol. Titanium(III) chloride (titanous chloride) was a 10 wt% solution in 20 to 30 wt% hydrochloric acid used directly as supplied from Aldrich, Dorset, UK.

After the completion of visualization plates were kept in clear nylon bags. This is of particular importance for plates which have been sprayed with Griess reagent since these quite rapidly discolor on continued exposure to air.

# Recovery and Reanalysis of NG and PETN

TLC spots were scraped and collected using tubes prepared as follows: 50 mm lengths of soft glass 'melting point' tubing approximately 1.3 mm O.D. and 0.9 mm I.D. were cut. One end of each length was gently flamed until only a small hole remained. Vacuum was applied at this end while the other end was twisted and pushed through two layers of Whatman GF/A glass fibre paper. The small discs cut from the paper were drawn into the tubes, forming porous plugs at the flamed ends. The prepared tubes were washed with ethyl acetate before use.

Solutions of NG, PETN, and a mixed standard were applied 20 mm apart along a line 20 mm from the edge of a 200 mm square TLC plate in the order indicated in Table 2. The plate was eluted in toluene for a distance of 100 mm beyond the application line.

Spot No.	Sample	Spot colors after Griess	Rf values
1	Mixed standard	Pink	0.08 (RDX)
		Pink/yellow center	0.60 (NG, PETN)
2	200 ng NG	Pink/yellow center	0.59
3	500 ng NG	Pink/brown center	0.60
4	1 μg NG	Pink/brown center	0.60
5	200 ng PETN	Pink	0.63
6	500 ng PETN	Pink/yellow center	0.63
7	1 μg PETN	Pink/yellow center	0.63
8	5 µL solvent blank	No spots	
9	Mixed standard	Pink	0.08 (RDX)
		Pink/yellow center	0.61 (NG, PETN)

TABLE 2-Initial analysis in 100% toluene eluent. Plate Sil G UV254.

Mixed standard contained 200 ng each of NG, PETN, TNT, and RDX.

After elution and drying the plate was lightly sprayed (two to three sweeps) with molar sodium hydroxide solution, heated at 100°C for 10 min, then sprayed with Griess reagent 1. NG and PETN spots appeared almost immediately as recorded in Table 2.

All PETN and NG spots were clear and the larger quantities showed centers of differing color, a common observation with this visualization reagent. RDX in the standard mixture appeared as a pink spot at Rf 0.08. Under these conditions 200 ng of 2,4,6-trinitrotoluene (TNT) did not produce a detectable pink spot, but was observed before spraying as a UV absorbing spot at Rf 0.75. PETN and NG in the standard mixture were not resolved, the two spots appearing as one.

Each of the spots in lanes 2 to 7 was separately recovered for reanalysis. To recover each spot the plugged end of one of the prepared collection tubes was connected to vacuum. The open end was then used to scrape the silica comprising the spot from its plastic substrate. The freed silica was drawn into the tube as scraping proceeded and collected above the porous plug. When the spot had been recovered vacuum was disconnected from the tube and 10 to 20  $\mu$ L of ethyl acetate was injected into the open end from a microsyringe. Under gravity this was allowed to percolate through the collected silica. As the first 5 to 10  $\mu$ L slowly emerged they were directly spotted onto a second TLC plate. The tube was then discarded. The entire process took about 5 min per spot to complete. It required care and precision, but was not unduly difficult.

The recovered samples were applied to a second plate as indicated in Table 3. As a blank 5 to 10  $\mu$ L of ethyl acetate that had been passed through an unused vacuum recovery tube was applied to the plate. The plate was eluted in 'System 2,' ethyl acetate/ petroleum ether mixture, to a distance of 100 mm and allowed to dry. It was sprayed heavily (until visibly wet) with molar sodium hydroxide, heated as before and sprayed with Griess reagent 1.

#### Visualization of Nitrotoluenes

1  $\mu$ g quantities of the three isomers of mononitrotoluene, a variety of dinitrotoluene isomers and 2,4,6-trinitrotoluene (TNT) were applied to a plate as indicated in Table 4. The plate was eluted in toluene to a distance of 100 mm and allowed to dry. It was inspected under short wavelength UV then sprayed with titanium(III) chloride reagent, which is a deep mauve color. After approximately 2 h the plate was dry, but retained a mauve color. It was sprayed with DMAB/HCl reagent and faint yellow spots appeared for TNT and some of the DNT isomers, but not for the MNT isomers. After a further

Spot No.	Sample	Spot colors after Griess	<b>RF</b> values
1	Mixed standard	Mauve	0.02 (RDX)
		Mauve	0.38 (NG)
		Faint Mauve	0.53 (PETN)
2	NG rec. from 200 ng spot	Mauve	0.38
3	NG rec. from 500 ng spot	Mauve	0.37
4	NG rec. from 1 µg spot	Mauve	0.37
5	PETN rec. from 200 ng spot	Mauve	0.50
6	PETN rec. from 500 ng spot	Mauve	0.50
7	PETN rec. from 1 µg spot	Mauve	0.51
8	Solvent blank	No spots	
9	Mixed standard	Mauve	0.02 (RDX)
		Mauve	0.38 (NG)
		Faint Mauve	0.51 (PEŤN)

TABLE 3-Reanalysis in 20% ethyl acetate/80% pet ether eluent. Plate Sil G UV254.

Mixed standard contained 200 ng each of NG, PETN, TNT, and RDX.

Spot No.	Sample	Color after TiCl <sub>3</sub> /DMAB	Color after Heating	Rf values
1	Blank	No spots	No spots	_
2	1 μg 2-NT	No spots	VFt yellow	0.80
3	1 μg 3-NT	No spots	Yellow	0.80
4	1 μg 4-NT	No spots	Yellow	0.78
5	1 μg 2,6-DNT	No spots	Yellow	0.74
6	1 μg 2,5-DNT	VFt yellow	Orange	0.77
7	1 μg 2,3-DNT	Ft yellow	No spots	0.58
8	1 μg 2,4-DNT	VFt yellow	Yellow	0.67
9	1 μg 2,4,6-TNT	VFt vellow	Yellow/brown	0.77

TABLE 4—Analysis of nitrotoluenes.

Ft = Faint VFt = Very faint.

4 h the mauve background had disappeared and spots for 3-nitrotoluene (3-NT) and 4nitrotoluene (4-NT) could just be discerned, but all spots were still faint. The plate was kept overnight, but no improvement resulted. It was finally heated for approximately 5 min at 100°C. This briefly produced an overall orange/brown color, which quickly faded leaving much enhanced spots.

In order to determine the detection sensitivity of the system for dinitrotoluenes quantities of 2,6-DNT and 2,4-DNT varying between 1 ng and 200 ng were applied to a TLC plate as indicated in Table 5. The plate was eluted in toluene and after drying observed under short wave UV light. Only the 200 ng spot of 2,6-DNT was faintly discernable. The plate was sprayed heavily with titanium(III) chloride, allowed to dry as before, then sprayed with DMAB/HCl reagent. No spots could be seen until the plate was inspected the following morning, approximately 16 h after spraying, when yellow spots could just be seen for the larger quantities, down to 50 ng. After 25 h the spots were still very faint so the plate was heated at 100°C for approximately 5 min. As before the plate briefly became discolored, but quickly returned to near white with enhanced spots.

# Rate of PETN Spot Color Development

 $1 \mu g$  quantities of NG and PETN were applied alternately to a 50 mm  $\times$  200 mm TLC plate at 10 mm intervals (two spots of each). A second plate was similarly prepared.

Spot No.	Sample	Color after TiCl <sub>3</sub> /DMAB/Heat	Rf values
1	5 μl solvent blank	No spots	
2	1 ng 2,6-DNT + 1 ng 2,4,-DNT	No spots	
2 3	2  ng  2,6-DNT + 2  ng  2,4,-DNT	No spots	
4	5  ng  2,6-DNT + 5  ng  2,4,-DNT	No spots	
5	10  ng  2.6-DNT + 10  ng  2.4-DNT	Just visible orange	0.65 (2,4-DNT)
-		Just visible vellow	0.75 (2,6-DNT)
6	20 ng 2,6-DNT + 20 ng 2,4,-DNT	VFt orange	0.65 (2,4-DNT)
0		VFt yellow	0.74 (2,6-DNT)
7	50 ng 2,6-DNT + 50 ng 2,4,-DNT	VFt orange	0.66 (2,4-DNT)
,	50 mg 2,0 2111 · 00 mg =, ., 211-	VFt yellow	0.74 (2,6-DNT)
8	100 ng 2,6-DNT + 100 ng 2,4,-DNT	Ft orange	0.66 (2,4-DNT)
0	100 mg 2,0 2101 1 100 mg -, 0, -101	Ft yellow	0.74 (2,6-DNT)
9	200 ng 2,6-DNT + 200 ng 2,4,-DNT	Ft orange	0.66 (2,4-DNT)
	200 ng 2,0 2101 / 200 ng 2,0, 2101	Ft yellow	0.74 (2,6-DNT)

TABLE 5—Detection sensitivity for dinitrotoluenes.

Ft = Faint VFt = Very faint.

Both plates were eluted in toluene and after drying sprayed lightly with molar sodium hydroxide solution. Both plates were then heated in an oven at 80°C ( $\pm$ 1°C) for 10 min, the temperature being monitored by a mercury-in-glass thermometer placed alongside the plates. After cooling one of the plates was sprayed with Griess reagent 1 (sulphanil-amide, phosphoric acid and naphthylethylenediamine) whilst the other was sprayed with Griess reagent 2 (sulphanilic acid, acetic acid and naphthylethylenediamine). The plates were observed at intervals thereafter for a period of 4 h. In both cases intense NG spots with dark centers appeared immediately upon spraying with Griess reagent. The PETN spots were not visible at first, but gradually appeared over the observation period. At the end of the time the PETN spots were moderately intense.

A similar experiment was carried out in which the temperature of heating was 90°C rather than 80°C. Again the PETN spots increased in intensity over a period of 2 h 15 min. In an initial attempt to repeat this experiment for photography an apparent effect of daylight upon the intensity of the PETN spots was noted. The plates were kept in a dimly lit photographic studio after spraying and the PETN spots did not increase in intensity as expected over a period of 1 h 30 min. However, when the plates were removed into daylight for closer inspection the PETN spots rapidly increased in intensity.

In a third experiment 1  $\mu$ g and 2  $\mu$ g quantities of NG and PETN, both separately and in combination, were applied to two plates. Both plates were eluted in toluene and sprayed lightly with molar sodium hydroxide. One was heated at 100°C and the other at 82°C both for 10 min. Both were sprayed with Griess reagent 1. After initial observation the plate which had been heated at 82°C was exposed to daylight by placing it, within its nylon bag, horizontally outside for 15 min. The weather was overcast with no direct sunshine. Photographic records of these plates were made and are discussed below. This experiment was repeated using Griess reagent 3, and in this instance the plate that had been heated at 100°C was also exposed to daylight.

#### **Results and Discussion**

# Recovery and Reanalysis of NG and PETN

Figure 1 is a photograph of the TLC plate on which the recovered samples were reanalyzed and Table 3 lists the observed spots. All of the recovered samples produced easily detectable spots and the separation between NG and PETN in this eluent is clear. It will be noted that the color of the spots is recorded as mauve rather than pink. This is of course merely a subjective record but does indicate a shift in hue (which may have been caused by the increased intensity of sodium hydroxide spray applied to this plate).

Sufficient material was recovered from both 200 ng NG and 200 ng PETN spots for reanalysis using a second eluent. The analysis in 'System 2' produced, as expected, a clear separation between NG and PETN. There was no indication of cross-contamination between the NG and PETN spots, and the blank solvent produced no spots. The apparatus and procedure used to achieve this were quite straightforward and there appears to be no reason why this method or something very similar could not have been applied in 1974.

In addition to producing a separation of NG and PETN such reanalysis would have provided further evidence regarding the identity of the spots detected using toluene elution. There is a good probability that such a reanalysis would have distinguished between NG and any other interfering substance that may yet be discovered.

#### Visualization of Nitrotoluenes

Table 4 records the spots observed on the first nitrotoluenes plate and Table 5 records the results from the detection sensitivity plate. The spots were observed in transmitted daylight, looking through the plate towards a window.

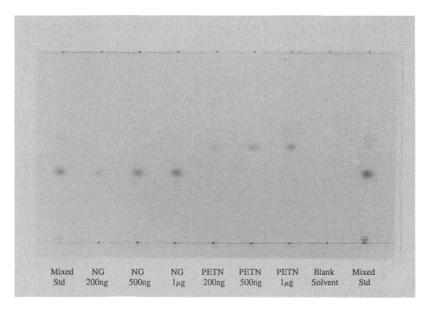


FIG. 1—TLC of extracts from recovered spots. The quantities shown are those of the original spots. Eluent System 2. Griess reagent 1.

One major purpose of the work was to establish how long visualization of the spots takes from the time of spraying. A 1978 RARDE document described the color of mononitrotoluene spots as "green-blue (after 24 h)." This suggested that it was normal practice to view plates a day after they had been sprayed, probably in addition to an earlier inspection. From the observations described above it is clear that visualization according to the instructions given by Jenkins and Yallop [3] is a slow process for both the dinitro- and mononitrotoluenes. Drying of the plate in air after spraying with titanium(III) chloride takes about 2 h, and the mauve Ti(III) background color persists longer than this. After spraying with DMAB/HCl faint spots appear, but do not become intense even after 24 h. Spots of good intensity were only obtained by heating the plate. A brown color was produced by TNT some time after heating, but at no stage (even several days later) did the MNT spots appear green-blue.

Although Jenkins and Yallop [3] do not make reference to heating of the plate this is, of course, a very common practice in TLC visualization. In view of the enhancement of spot intensity observed on heating it is difficult to believe that this was not employed at least on some occasions. A 1974 report by Bilson [6], who was working in laboratories adjacent to those of RARDE, does suggest heating at  $105^{\circ}$ C for 15 min. If heat is applied the process of visualization can be accelerated and the spot intensity increased.

Jenkins and Yallop [3] give the detection limited for DNT as 0.001 mg (1  $\mu$ g), but they also give the detection limit for NG as 0.019 mg (19  $\mu$ g), which is certainly incorrect. A RARDE report by the same authors, upon which the paper was based, gives the limit for DNT as 0.001  $\mu$ g (1 ng) and that for NG as 0.019  $\mu$ g. (It seems most likely that in the *Explosivstoffe* article the letter m was incorrectly substituted for the greek letter  $\mu$ in both cases.) The 0.001  $\mu$ g (1 ng) detection limit for DNT given by Jenkins and Yallop is very low, being exceptionally good for a TLC method using conventional plates. The limit observed here was 10 ng, for the two most important DNT isomers, and this was achieved only after heating the plate. Before heating the limit was 50 ng.

# Rate of PETN Spot Color Development

Figures 2, 3, and 4 are photographs taken of the plates in the third experiment described above, using Griess reagent 2. Both 1  $\mu$ g and 2  $\mu$ g PETN spots of moderate intensity appeared immediately upon spraying the plate which had been heated at 100°C, as can be seen in Fig. 2, which was taken within 5 min of the Griess spraying and without deliberate exposure to daylight. The PETN spots on the plate that had been heated at

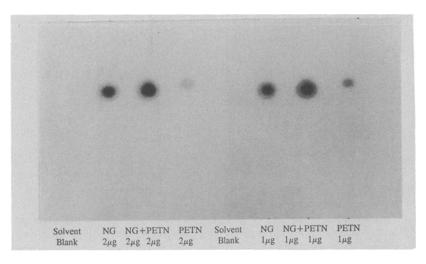


FIG. 2—Photograph of PETN and NG spots on plate that had been heated at 100°C taken immediately after spraying with Griess reagent 1.

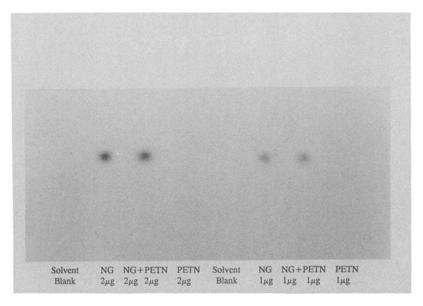


FIG. 3—Photograph of PETN and NG spots on plate that had heated at 82°C taken immediately after spraying with Griess reagent 1.

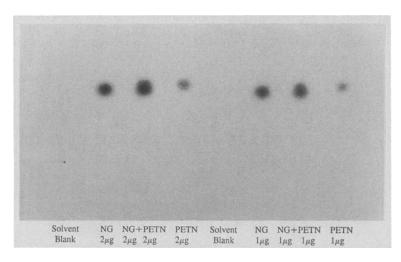


FIG. 4—Photograph of PETN and NG spots on plate that had been heated at 82°C taken after spraying with Griess reagent 1 and exposure to daylight for 15 minutes.

82°C were barely visible immediately after Griess spraying and have not registered on the photograph taken at that stage, Fig. 3. However after exposure to exterior daylight the PETN spots increased greatly in intensity, as seen in Fig. 4. Essentially similar results were observed when this experiment was repeated using Griess reagent 3, but in this case the PETN spots which appeared immediately on spraying the plate which had been heated at 100°C were rather weak and were observed to become more intense on exposure to daylight.

Jenkins and Yallop [3] stated that TLC plates should be heated at 100°C for 10 min after spraying with sodium hydroxide and before Griess reagent spraying. Thorburn-Burns [4] used a temperature of 110°C. In this work it was observed that at the lesser temperatures of 80°C and 90°C visualization of NG is unaffected, but that of PETN becomes much slower. The effect has been observed with Griess reagents of three different compositions. Griess reagent 3 is a formulation that was given in a RARDE Woolwich draft laboratory manual probably dating from 1976 to 1977. Exposure to daylight also plays a part in the slow visualization of PETN when the plate has been heated at less than 100°C, but the causes of this effect have not been studied. Thus it could be that, at least during the experiments that gave rise to the RARDE view that PETN spots develop more slowly, the heating temperature employed was less than 100°C and the plates were exposed to daylight of significant intensity.

Because PETN was not among the standard spots applied to the TLC plates used in the Maguire case it would not have been possible for the observer to confirm that conditions under which PETN spots would develop more slowly than those of NG had definitely been achieved. In these circumstances rate of color development would not have been a reliable means to distinguish between NG and PETN.

#### Conclusions

From these studies the following conclusions can be drawn: When toluene is used as eluent NG and PETN are not clearly distinguished, but that 200 ng TLC spots of NG and PETN visualized using the sodium hydroxide/Griess reagent system can practically be recovered from such an initial analysis and reanalyzed using a different eluent system.

TLC visualization of nitrotoluenes using the titanium(III) chloride/DMAB reagent system is a slow process unless heat is applied, and that the application of heat both speeds the development of spots and enhances the sensitivity. Mononitrotoluene spots are the slowest to visualize.

The detection limit for dinitrotoluene using titanium(III) chloride/DMAB is about 10 ng when the plate has been heated.

Under certain conditions there is a difference between the rates of spot color development for NG and PETN, but that in the absence of PETN standard spots on the test plate this would not be a reliable way to distinguish the two compounds. The recovery of spots and their reanalysis using a different eluent system would be a far superior way to distinguish NG from PETN.

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Address requests for reprints or additional information to R. W. Hiley, Ph.D. Forensic Explosives Dept. Defence Research Agency Fort Halstead, Sevenoaks Kent TN14 7BP England