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Investigating TNT loss between sample collection and analysis

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Abstract

Explosives residues are often collected from explosion scenes, and from surfaces suspected of being in contact with explosives, by swabbing with solvent-wetted cotton swabs. It is vital that any explosives traces present on the swabs are successfully extracted and detected when received in a laboratory. However, a 2007 proficiency test initiated by the European Network of Forensic Science Institutes (ENFSI) Expert Working Group on Explosives involving TNT-spiked cotton swabs highlighted that explosives may not always be detected from such samples. This paper outlines work performed to determine potential reasons for this finding. Cotton swabs were spiked using a solution of TNT and stored in nylon bags and glass vials for periods of 1, 2 and 4 weeks. Simulated swab extracts were also prepared and investigated. The samples were stored in a freezer, or at room temperature either in the dark or exposed to daylight. Overall, the cotton swabs stored at room temperature and exposed to daylight showed a very rapid loss of TNT over time, whereas cotton swabs stored in the freezer, and all simulated swab extracts, gave high recoveries over time. These results will be of benefit for practicing forensic explosives laboratories and for persons undertaking cold-case reviews involving explosive-based samples.

Keywords

Explosives; forensic analysis; TNT; post-blast; proficiency test

1. Introduction

The detection of trace (i.e. very low levels of) explosives during the investigation of an explosives-related incident can be of high forensic significance. It is therefore vital to be able to detect any traces of explosives present in samples submitted to a forensic laboratory. High explosives, such as TNT (2,4,6-trinitrotoluene), have limited non-explosives uses and a

relatively low prevalence in the environment [1-3]. The detection of TNT can, as a consequence, be indicative of contact between a surface and an explosive, with a potentially unlawful origin [4, 5], or can provide evidence of secondary transfer of such materials. On the other hand, a lower forensic significance may be assigned if low explosives constituents are detected, such as ammonium nitrate, as these may have an innocent origin (such as originating from fertilisers).

Following an explosion, intact explosives molecules and any degradation products will be widely scattered around the scene as explosives residues. These often have a low persistence in the environment and must be recovered rapidly [6]. Explosives residues are typically collected from post-explosion debris, as well as from other possible explosives sources, such as the hands or clothing of a suspect [4], by swabbing using cotton swabs wetted with an organic solvent [1]. Swab extracts may then be analysed using a variety of techniques, such as gas chromatography (GC)- and liquid chromatography (LC)-based techniques [4]. Currently there is no internationally agreed 'best practice' for exactly how to collect explosive residues by swabbing [7]. Due to the limited amount of literature investigating the stability of explosive materials on swabs and in solutions, forensic laboratories differ in their approach to storing samples from explosion scenes, and from surfaces suspected of being in contact with explosives.

When considering the potential forensic significance of detecting trace high explosives, it is of paramount importance that the detection and identification of explosives is reliable. If explosives residues are detected, knowledge of the type of explosives present may be sufficient to provide a link between a suspect and an explosion scene. Such evidence may subsequently be used in court, to describe the type of device used in a bombing [8]. For these reasons it is essential that high quality procedures are used during the detection and identification of trace high explosives [4].

One such example of an organic high explosive is TNT, used for both military and industrial applications (such as mining) [5]. TNT is cheap and safe to manufacture, safe to handle, has a high explosive power, good chemical stability and a low sensitivity to impact and friction [8, 9]. For these reasons, TNT is a very commonly-used explosive.

In 2007, the European Network of Forensic Science Institutes (ENFSI) Expert Working Group on Explosives instigated an inter-laboratory proficiency test, where cotton swabs were spiked with a known quantity of TNT using a methanolic TNT solution to act as simulated

explosion scene samples. The swabs were packaged in nylon bags and sent to member laboratories worldwide for the analysis and quantitation of any analytes present. Surprisingly, TNT was only detected by 5 out of 19 laboratories, at significantly lower levels than the initial spiked quantities [10]. This result was of concern, as it suggested that even if TNT traces are collected from a real-life explosion scene using a swab, they may not be detected during subsequent laboratory analyses, resulting in the potential to lose crucial investigative links.

A follow-up study was conducted by the UK Defence Science and Technology Laboratory (Dstl) in 2009 [11]. This involved spiking swabs with TNT and either analysing them immediately, or leaving them for a period of 5, 10 or 30 days before analysis. The study found significantly lower quantities of TNT were detected from the spiked samples compared to the initial spiked quantity. The loss of TNT appeared to be compounded with increased storage time between swab spiking and analysis, though no definitive conclusions were drawn for the low quantities of TNT detected. A further follow-up study was performed in 2009 [12]. This aimed to determine the effect of different solvents and packaging materials, and the effect of UV light, on samples containing TNT. The study tentatively identified three UV light-induced degradation products of TNT. Similarly, work by Song-im et al. investigated the stability of TNT extracts in a methanol:water mixture, finding that storage at low temperatures, and in a dark environment, was best [13]. However, less work has been done to determine the stability of TNT extracts in purely organic solvents, so we sought to explore this shortfall in the current work.

This paper presents work performed to follow up on the ENFSI results, to provide possible reasons for why the proficiency test gave such poor TNT detection. Several aims were identified for this work: to assess how TNT recovery from samples changes with increased storage time, to assess the effect of storage temperature on TNT recovery, to assess the effect of UV light exposure on TNT recovery, and to compare nylon bags and glass vials as storage containers for cotton swabs containing TNT residue. Cotton swabs were spiked with TNT using a solution of TNT in ethyl acetate. The swabs were packaged into nylon bags or glass vials and stored at room temperature in daylight, at room temperature in the dark, or in a freezer in the dark. Samples were prepared and stored for periods of 0 days (i.e. immediate analysis), 1 week, 2 weeks and 4 weeks. Additionally, simulated swab extracts containing TNT were produced in ethyl acetate and stored under the same conditions. The results showed that TNT on cotton swabs stored at room temperature and exposed to daylight

underwent a very rapid loss over time, whereas cotton swabs stored in the freezer, and all simulated swab extracts, gave higher TNT recoveries even after 4 weeks. The study's results may be of use for those engaging in both the present-day analysis of explosives, and potentially for cold-case reviewers.

2. Experimental

2.1 Reagents and consumables

2,4-Dinitrotoluene (2,4-DNT) was obtained from Sigma Aldrich. Ampoules of 1000 µg/mL TNT in methanol were obtained from SPEX CertiPrep. Musk Tibetene (1-tert-butyl-3,4,5-trimethyl-2,6-dinitrobenzene) was obtained from Givaudan. GC-grade ethyl acetate was obtained from Sigma Aldrich. Nylon bags were supplied by Rilsan. Cotton ball swabs were Happy Shopper brand. 7 mL glass snap-cap vials were Samco brand. Clear glass GC vials were obtained from Chromacol.

2.2 GC-MS instrument and conditions

The GC-MS instrument consisted of an Agilent 6850 GC oven coupled to an Agilent 5795C VL MSD quadrupole with triple-axis detector. A 14 m silica BP5 column was used with a 0.25 mm i.d., coated with bonded 5% diphenyl-dimethylsiloxane at a 0.25 µm film thickness. The injector temperature was held at 175 °C and the oven temperature programme was 60 °C for 1 minute, then increased at 20 °C/minute to 200 °C for 2 minutes. The source used was electron impact, with 70 eV energy and a temperature of 230 °C. Masses were monitored between *m/z* 50-550.

2.3 Experimental conditions and parameters

Samples were stored as spiked cotton swabs in nylon bags or clear glass snap-cap vials, or as ethyl acetate extracts in clear glass vials. The swabs and extracts were either analysed immediately, or stored for periods of 1, 2 or 4 weeks. Once prepared, samples were stored in three different storage conditions: 1) In a cardboard box in a freezer, 2) At room temperature, on a bench top (exposed to ambient daylight), 3) At room temperature, stored in a dark cupboard. Room temperature samples were stored at temperatures ranging between 20-26 °C. Three replicates were prepared for each combination of conditions. A negative control was also prepared for each combination of conditions.

2.4 Experimental system development using 2,4-DNT

Initially, the use of a sonication step during swab extraction was considered. Using 2,4-dinitrotoluene (2,4-DNT) as an inexpensive TNT analogue, swab extractions were performed both with and without the use of a sonication step, to assess whether inclusion of a sonication step would be beneficial. For this, a 0.4 mg/mL 2,4-DNT spiking solution was prepared in ethyl acetate. Three sets of samples were prepared and analysed, with each set consisting of three replicates and a negative control. Two sets involved spiking cotton swabs using a solution of 2,4-DNT in ethyl acetate, and then extracting the swabs with or without a sonication step. The third set acted as a positive control, and involved extracting blank swabs, then spiking the 2,4-DNT solution into the blank extract.

For the 'sonicated' sample set, a clean cotton swab was placed into four glass vials. 0.5 mL of the 2,4-DNT spiking solution was spiked onto the surface of three of the swabs. The fourth swab was a negative control, and was not spiked. 4 mL ethyl acetate was added to each vial, and the vials were capped and sonicated for 10 minutes. After cooling to room temperature, each swab was pounded for 2 minutes using the tip of a Pasteur pipette, and the extract drawn up through the swab using a Pasteur pipette and transferred into a 5mL volumetric flask. The swab was rinsed with a further 1 mL of ethyl acetate, and the washings added into the volumetric flask, before making up to 5 mL (some solvent was lost due to evaporation whilst pounding the swab). 100 μL of a 2 mg/mL solution of Musk Tibetine in ethyl acetate was added as an internal standard prior to GC-MS analysis.

The second set of samples was prepared in an analogous manner, but did not involve the 10-minute sonication step. The third set of samples were simulated swab extracts, and acted as a positive control with which to assess the recovery rate of the respective swab extractions: clean cotton swabs were placed into four glass vials. 4 mL ethyl acetate was added to each vial, and the vials were capped and sonicated for 10 minutes. After cooling to room temperature, each swab was pounded for 2 minutes using the tip of a Pasteur pipette, and the extract drawn through the swab using the pipette and transferred into a 5mL volumetric flask. 0.5 mL of the 2,4-DNT spiking solution was added directly into three of the flasks (with one left blank as a negative control). Each flask was made up to 5 mL using ethyl acetate. 100 μL of a 2 mg/mL solution of Musk Tibetine in ethyl acetate was added as an internal standard prior to GC-MS analysis. Samples were injected in triplicate and the results averaged.

2.5 Validation of the developed experimental systems using TNT

After optimising the swab extraction procedure using 2,4-DNT, analogous extractions were performed with TNT. Due to the expense of the TNT standard compared to 2,4-DNT, the procedure was scaled-down: cotton swabs were divided into 5 pieces before use, with all cotton swab pieces weighing between 0.13 g and 0.16 g. The swabs were spiked using 0.1 mL of a 0.4 mg/mL solution of TNT in ethyl acetate, following the sequences described above for 2,4-DNT. Three sets of swabs were prepared and extracted – one with sonication, one without sonication, and one as a positive control. The swabs were then extracted using 1 mL ethyl acetate. If necessary (due to solvent evaporation during the pounding stage) the solvent level was made up to 1 mL. 20 μL of a 2 mg/mL solution of Musk Tibetine in ethyl acetate was added as an internal standard prior to GC-MS analysis.

2.6 Sample preparation and extraction of TNT-spiked swabs in glass vials

A cotton swab piece was placed into a clean vial. 100 μL of a 0.4 mg/mL solution of TNT in ethyl acetate was added to the swab surface. The vial was sealed with a plastic snap cap and placed into its allocated storage environment. Three replicates were performed for each condition, along with a negative control. After the desired ageing time, the vial was opened and 1 mL of ethyl acetate was added to the swab. The vial was capped and sonicated for 10 minutes, before removing and cooling to room temperature. The cap was removed and the swab pounded with the tip of a glass Pasteur pipette for 2 minutes, before drawing the extract through the swab with the pipette and transferring into a GC vial. The solvent was extracted from the swab using a glass Pasteur pipette until the swab appeared to be visibly dry and no regions of the swab adhered to the glass vial. If necessary (due to solvent evaporation during the pounding stage) the solvent level was made up to 1 mL. 20 μL of a 2 mg/mL solution of Musk Tibetine in ethyl acetate was added as an internal standard prior to GC-MS analysis. Samples were injected in duplicate and the results averaged.

2.7 Sample preparation and extraction of TNT-spiked swabs in nylon bags

A cotton swab piece was placed into a nylon bag. 100 μL of a 0.4 mg/mL solution of TNT in ethyl acetate was added to the swab surface. The nylon bag was sealed using a double swan-neck and Sellotape, and placed into its allocated storage environment. Three replicates were performed for each condition, along with a negative control. After the desired ageing time, an incision was made into the bag near to the swab, and the swab transferred into a 7 mL glass vial. The area in the nylon bag from where the swab was taken was rinsed with 1 mL of ethyl acetate, and the ethyl acetate extract transferred into the glass vial. The vial was capped and

sonicated for 10 minutes, before removing and cooling to room temperature. All subsequent steps mirrored those used to extract swabs in glass vials.

2.8 Sample preparation and extraction of TNT-spiked simulated swab extracts

A cotton swab piece was placed into a clean vial. 1 mL of ethyl acetate was added to the swab. The vial was capped and sonicated for 10 minutes, before removing and cooling to room temperature. 100 μL of a 0.4 mg/mL solution of TNT in ethyl acetate was added to a GC vial. The swab was pounded with the tip of a glass Pasteur pipette for 2 minutes, before drawing the extract through the swab with the pipette and transferring it into the GC vial. The solvent was extracted from the swab using a glass Pasteur pipette until the swab appeared to be visibly dry and no regions of the swab adhered to the glass vial. If necessary (due to solvent evaporation during the pounding stage), the solvent level in the GC vial was topped up to 1 mL. The GC vial was capped and placed into its allocated storage environment. Three replicates were performed for each condition, along with a negative control. After the desired ageing time, the vial was opened and 20 μL of a 2 mg/mL solution of Musk Tibetine in ethyl acetate was added as an internal standard prior to GC-MS analysis. Samples were injected in duplicate and the results averaged.

3. Results and discussion

3.1 Assessing the use of a sonication step using 2,4-DNT

Initial experiments were performed to assess whether a sonication step would be beneficial for extraction from the swabs. At Dstl, a sonication step is not included during swab extractions – swabs are pounded with the tip of a Pasteur pipette to loosen any bound residues. However, Song-im reports using a 10 minute sonication step during swab extractions [14]. Similarly, DeTata suggests that the use of a sonication step will reduce any possible variation which may occur from the action of manual agitation with a Pasteur pipette [1]. From the sonication trials performed within the current work, the inclusion of a sonication step was deemed beneficial for 2,4-DNT recovery, as shown in Figure 1.

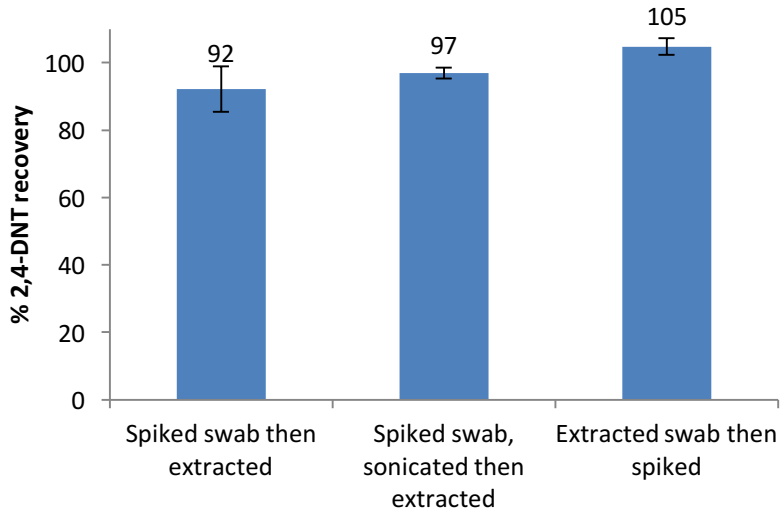

Figure 1: Assessing the use of sonication for 2,4-DNT extraction from a swab, using ethyl acetate. Error bars show the calculated RSD between the three replicate experiments for each condition.

It can also be seen from Figure 1 that in addition to higher recoveries, the inclusion of a sonication step led to less variability in the recovery of 2,4-DNT from the swabs.

3.2 Validation of the developed experimental systems using TNT

Following this, the developed method was then assessed with regards to the recovery of TNT from the swabs. As observed with 2,4-DNT, higher recoveries were obtained when a sonication step was included (Figure 2).

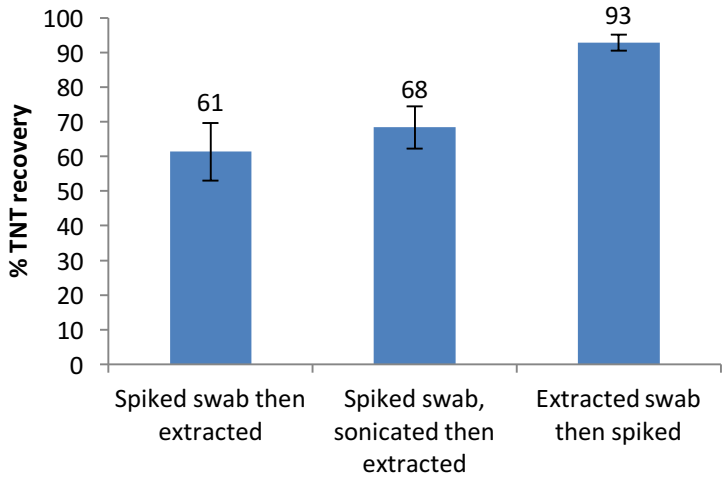

Figure 2: Assessing different extraction methods for TNT recovery from swabs. Error bars show the calculated RSD between the three replicate experiments for each condition.

A sonication step was therefore included during all subsequent experiments. It is interesting to note that even at this stage, a decrease in TNT recovery was observed relative to the positive control (the spiked extracted swab), which showed a recovery of 93 %. Similarly, lower TNT recoveries were obtained than the analogous 2,4-DNT recoveries (Figure 1). This appears to suggest that TNT undergoes tighter binding to a cotton swab than 2,4-DNT, even when extraction is performed soon after spiking the swab. This finding would be worth exploring further using a higher number of replicates for each sample set.

3.3 Analysis of aged samples: Nylon bags

Figure 3 shows the TNT recoveries from spiked swabs packaged in nylon bags after immediate analysis, as well as after 1, 2 and 4 weeks, extracting using ethyl acetate. The immediate analysis results show identical TNT recoveries as a single set of three swabs was spiked and extracted straight away in each case.

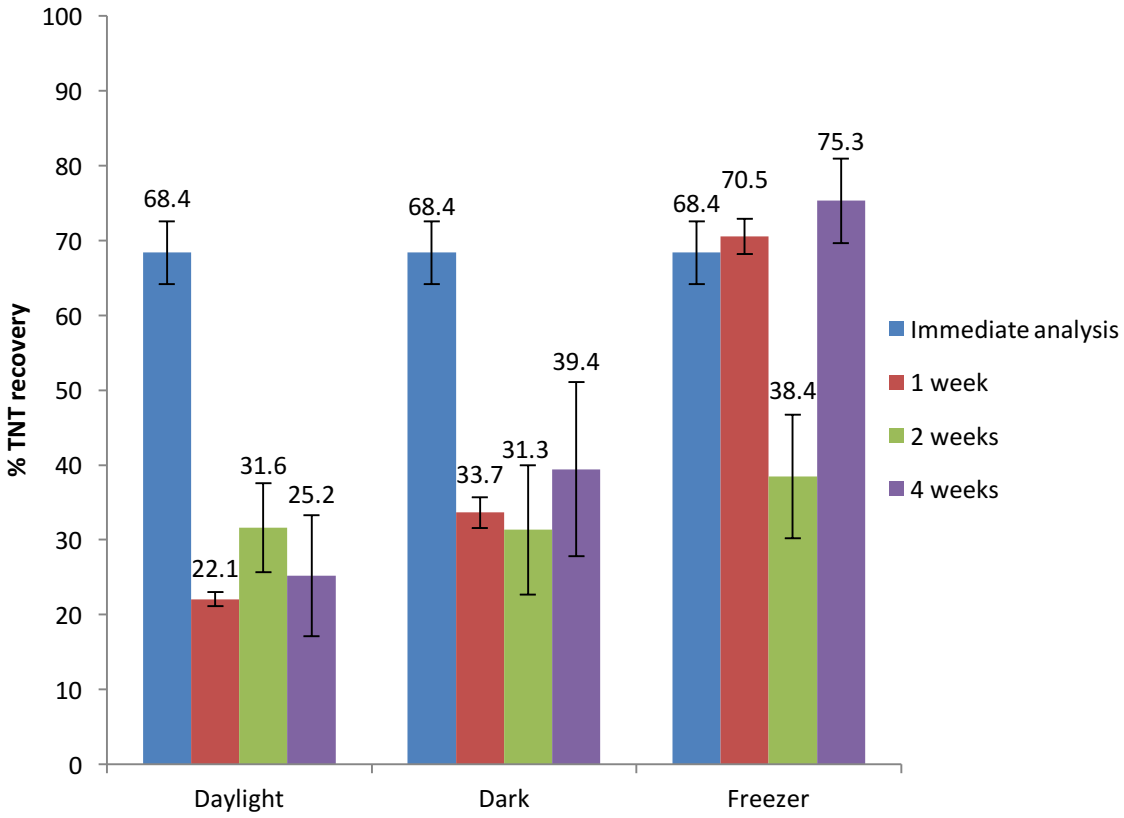

Figure 3: TNT recoveries from aged samples stored in nylon bags, extracting with ethyl acetate. Error bars show RSDs between the three replicate samples for each condition.

It can be seen from Figure 3 that the spiked swabs exposed to daylight at room temperature gave slightly lower recoveries than the samples stored in the dark at room temperature. The swabs stored in the freezer gave the highest recoveries at every time point, suggesting that the swab storage temperature has a large effect on TNT recoveries, with lower temperatures minimising TNT loss. This may be due to TNT volatilisation and redeposition on the inside of the nylon bag at higher temperatures. Efforts were therefore made to rinse the area of the bag immediately surrounding the swab with ethyl acetate, to improve recoveries in spite of any such volatilisation.

The results seem to imply that the effects of UV light are less significant than the effects of increased temperatures, as even the samples kept in the dark at room temperature have demonstrated the loss of a significant proportion of their TNT, whereas those stored in the dark in the freezer have retained the majority of their TNT. Overall, the results suggest that UV light has degraded a small proportion of the TNT, but loss due to storage at room temperature, possibly due to volatilisation, plays a larger role in TNT loss.

3.4 Analysis of aged samples: Glass vials

Figure 4 shows the analogous TNT recoveries from spiked swabs packaged in glass vials after immediate analysis, and then after 1, 2 and 4 weeks.

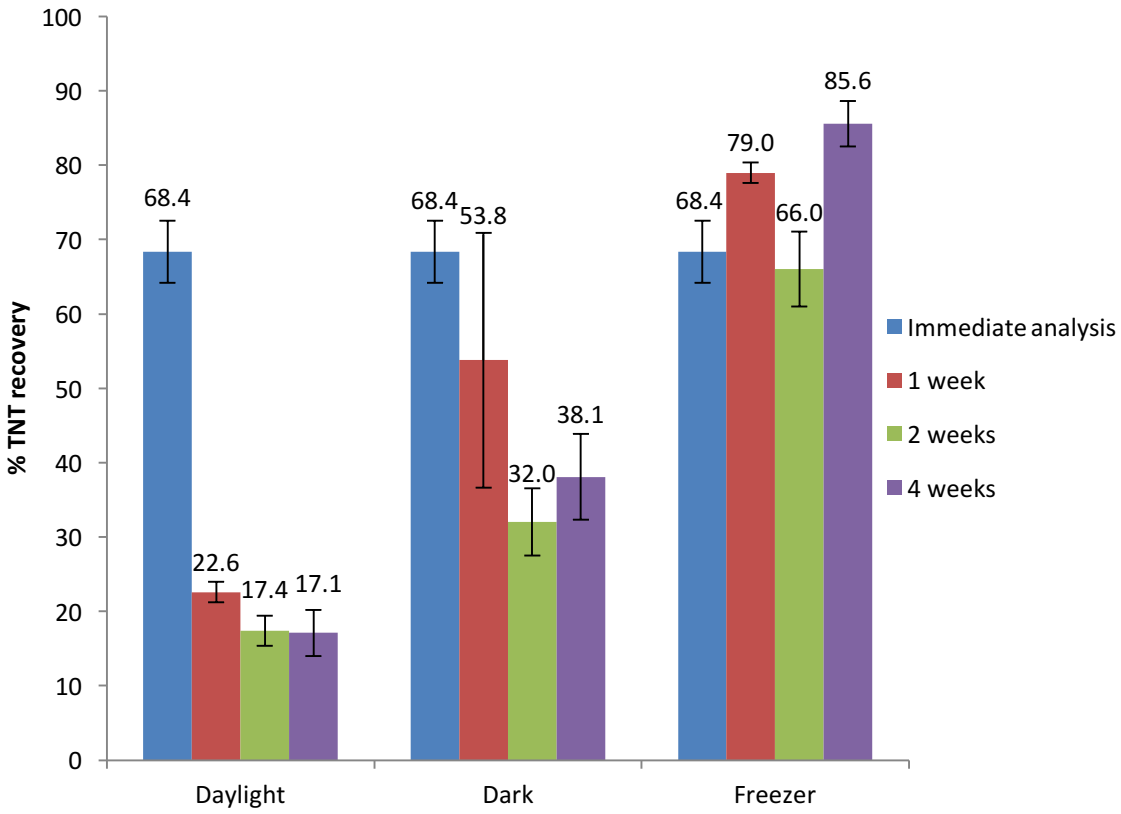

Figure 4: TNT recoveries from aged samples stored in glass vials, extracting with ethyl acetate. Error bars show RSDs between the three replicate samples for each condition.

These results show a similar overall pattern to the results in Figure 3 from samples stored in nylon bags - it appears UV light does have an effect on TNT loss, but temperature also seems to be an important factor contributing to TNT loss. A general decrease in TNT recovery can be seen over time in the samples stored in daylight and the dark at room temperature, whereas the levels of TNT recovered from the samples stored in the freezer seem to remain stable over time. Importantly, a large drop in the levels of recovered TNT can be seen after just one week of the swabs being stored at room temperature, particularly for the samples exposed to daylight. This is very concerning, suggesting that a large proportion of TNT loss can occur in the very early stages after collection of a swab. This suggests that it is very important to extract and analyse a swab as soon as possible after recovery, or, at the very minimum, to store it in a dark place at sub-zero temperatures. It may not be possible to store collected swabs in a freezer during transportation to a lab, but it would be sensible to at least transport them in a cool-box.

The swabs stored in nylon bags and glass vials show broadly the same result for each condition, with the least TNT recovered from swabs exposed to daylight, and the most TNT recovered from swabs stored in the freezer. For the nylon bags, more TNT was recovered from the swabs exposed to daylight than for those in glass vials. This suggests that UV light penetration to induce TNT degradation is greater through glass than nylon. The results of a statistical comparison between the TNT recoveries from swabs stored in nylon bags and glass vials are provided in Table 1.

Table 1. Results of a statistical comparison between TNT recoveries from swabs stored in nylon bags and glass vials under identical storage conditions. Results were obtained using a paired T-Test at $p < 0.05$.

Statistically significant at $p < 0.05$?		Swab stored in glass vial under analogous storage conditions		
		1 week	2 weeks	4 weeks
Nylon bag	Daylight (rt)	No	Yes	No
	Dark (rt)	No	No	No
	Freezer	No	Yes	No

From this it can be seen that in the majority of cases, the TNT recoveries were not significantly different from the two different storage containers used.

3.5 Analysis of aged samples: Simulated swab extracts

Figure 5 shows the TNT recoveries from simulated swab extracts in ethyl acetate after immediate analysis, as well as after 1, 2 and 4 weeks.

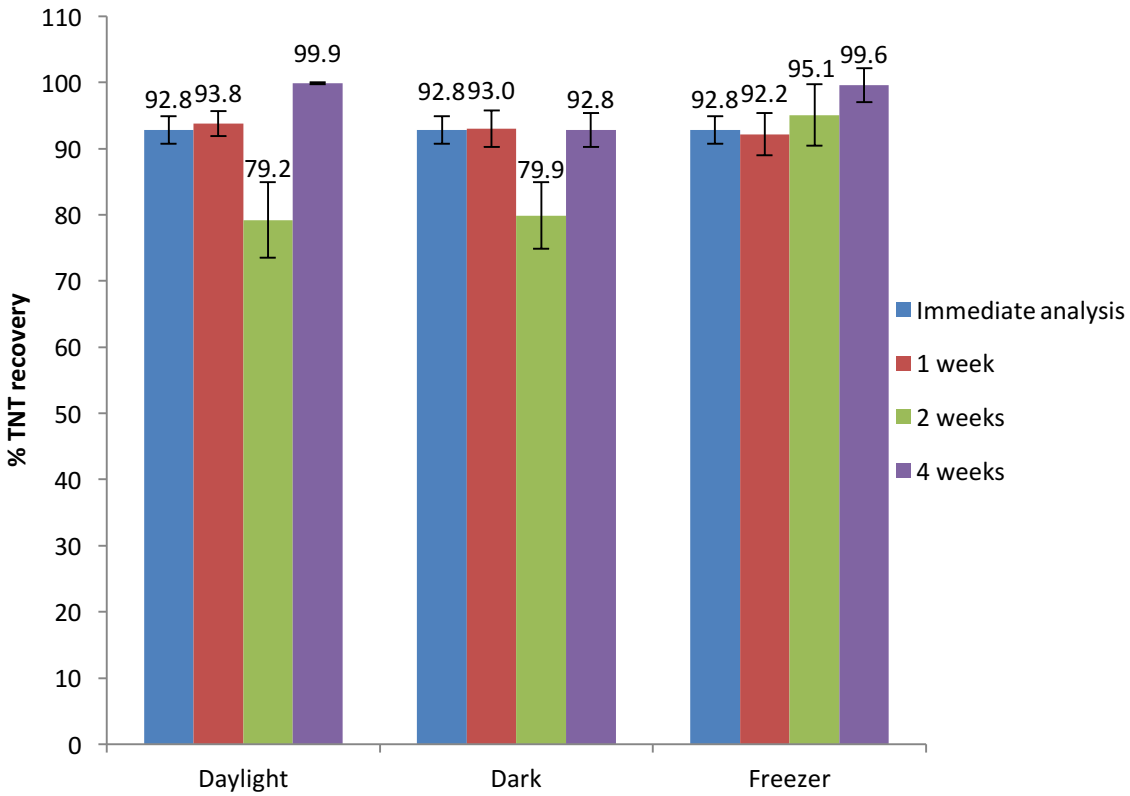

Figure 5: TNT recoveries from aged simulated swab extracts in ethyl acetate. Error bars show RSDs between the three replicate samples for each condition.

It can be seen from Figure 5 that all simulated swab extracts gave very high TNT recoveries, regardless of their storage temperature and degree of light exposure. This strongly suggests that volatilisation is playing a part in the TNT loss from the swabs. Additionally, as there is little difference between the extracts stored in daylight and the dark at room temperature, this suggests that the effects of UV light are minimised if the TNT is dissolved in a solvent.

As high TNT recoveries were observed from each extract, regardless of the length of time between extract preparation and analysis or the storage conditions to which they were exposed, this suggests that it is far preferable to extract a swab immediately once received in a laboratory, and store it as an extract until analysis is possible. The results of a statistical comparison between the TNT recoveries from simulated swab extracts against TNT recoveries from swabs stored in nylon bags and glass vials under identical storage conditions are provided in Table 2.

Table 2. Results of a statistical comparison between TNT recoveries from simulated swab extracts against swabs stored in nylon bags and glass vials under identical storage conditions. Results were obtained using a paired T-Test at $p < 0.05$.

Statistically significant at $p < 0.05$?		Simulated swab extract under analogous storage conditions		
		1 week	2 weeks	4 weeks
Nylon bag	Daylight (rt)	Yes	Yes	Yes
	Dark (rt)	Yes	Yes	Yes
	Freezer	Yes	Yes	Yes
Glass vial	Daylight (rt)	Yes	Yes	Yes
	Dark (rt)	No	Yes	Yes
	Freezer	Yes	Yes	No

From this it can clearly be seen that in the majority of cases, significantly different (and higher) TNT recoveries were obtained from the simulated swab extracts, compared to those from swabs stored in nylon bags or glass vials under identical storage conditions.

It does not appear that any extracted chemicals from the swabs are contributing towards the lower recoveries from the swabs in nylon bags and glass vials – the simulated swab extracts involved extracting a blank swab, then adding a spike of TNT solution, yet they still gave high TNT recoveries across all variables.

The decreased TNT recoveries from the spiked swabs are therefore likely to be due to a combination of TNT loss due to volatilisation, and UV light-induced degradation of the TNT. It appears that the presence of a solvent can prevent the TNT from volatilising. These results suggest that it would be sensible to ensure that collected swabs are completely saturated with solvent prior to transportation and storage, to minimise any volatilisation and UV degradation which may occur prior to analysis.

Regarding the proficiency test results, based on this study's results it is likely that significant TNT loss occurred during the transit time to each of the respective laboratories involved in the original proficiency test, particularly if the samples were transported at room temperature. Additionally, if the swabs were stored in the laboratories for some time prior to analysis, then

TNT loss would again be likely to occur, likely *via* volatilisation or photodegradation, especially if the swabs were stored at room temperature and exposed to daylight.

4. Conclusions

This work has shown that significant TNT degradation or loss can occur from spiked cotton swabs. The proportion of TNT lost appears to depend on the environmental factors to which samples are exposed. TNT loss is greatest when samples are exposed to UV light and stored at room temperature, is slightly reduced when samples are stored at room temperature in the dark, and is lowest when samples are stored in a freezer in the dark. The results suggest that UV light induces photodegradation of TNT on a swab, and that volatilisation of TNT from swabs stored at room temperature also occurs. The greatest recoveries of TNT from swabs came from samples stored in a freezer. The simulated swab extracts tended to give significantly higher TNT recoveries compared to TNT recoveries from swabs stored in nylon bags or in glass vials, suggesting that swabs of explosives residues should either be immediately stored in a freezer upon receipt into a laboratory, or extracted into a solvent and stored as an extract when received.

Comparing nylon bags and glass vials as storage containers for TNT-spiked swabs showed little difference in the levels of TNT recovered over time, suggesting that either container is suitable for packaging swabs. However, it would be sensible to ensure that packaged swabs are saturated with solvent prior to transportation, to minimise any potential loss due to volatilisation. During transport, packaged swabs should also be shielded from UV light, and ideally they should be transported in a cool-box. As the results demonstrate that the greatest proportion of TNT is lost within one week, it is essential that swabs are processed as quickly as possible following their collection.

In this work, TNT was spiked onto clean cotton swabs. However, in real-life samples it is likely that other compounds will be present, and these may interact with TNT to have an effect on the rate of TNT degradation. It would therefore be forensically useful to investigate the rate of TNT degradation in ‘dirtier’ spiked samples.

5. Acknowledgements

(Submitted as a separate document to exclude author details from blinded manuscript).

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