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Assessment of Solvents for the Recovery of Nitroglycerine from Hands Using Cotton Swabs

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ABSTRACT: Eight solvents were compared for their relative efficiency in removing nitroglycerine from the hands of persons handling explosives. The amount of interfering material also removed from the hands by the cotton swabs and the stability of the nitroglycerine in the solvent used were also investigated. Aqueous solvents yielded the best recoveries, when the extracts were partially purified by thin-layer chromatography before analysis, but the explosive was degraded rapidly by microorganisms that grew in the solutions. Of the aqueous, organic, and polar solvents tested, ethanol was found to offer the most complete, consistent, and stable recovery.

KEYWORDS: criminalistics, explosives, nitroglycerine, ethanol, gas chromatography

A wide variety of swabbing solvents could be used for the recovery of nitroglycerine (NG) from hands. As the amount of explosive transferred is rather limited in certain handling situations and the decay rather rapid [*1*], it is natural to attempt to optimize the swabbing process. The standardization of procedures in various laboratories is a related and desirable aim.

The recovery of organic explosive residues from hands is usually accomplished with a cotton wool swab soaked in a solvent such as ether or acetone [*1,2*], although the use of aqueous detergent solutions has also been suggested.[4] In previous studies by the authors it has been established that swabs soaked in solvent should not be allowed to dry out if evaporative losses of NG are to be avoided. Therefore, in this work the swabs were (unless otherwise stated) dropped into about 2 mL of solvent and the solution was kept in a sealed container.

Three properties of a swabbing system are of major importance: the system must efficiently remove the residual explosive from the hands, it should extract as little interfering material as possible, and the explosive should be stable in the solution for the few days that might intervene between the swabbing of a suspect and analysis in the laboratory. In this study the above aspects have been investigated for seven different solvents.

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Experimental Procedure

Reagents and Materials

The organic solvents used for swabbing were ether, acetone, cyclohexane, and ethanol. Each solvent (reagent grade) was redistilled in glass apparatus and stored in the dark in a glass vessel. Ferrous sulfate was added to the ether, and this solvent was extracted with three volumes of distilled water prior to use.

The aqueous solutions used for swabbing were distilled water, a buffer solution (pH 7) prepared from a prepacked buffer powder (Pye Unicam, Cambridge, England) according to the manufacturer's instructions, and a detergent solution. The detergent solution contained 0.5% sodium lauryl ether sulfonate (Empicol ® ESB3/S, Albright and Wilson, Marchon Div., Whitehaven, Cumbria, England) in distilled water and was extracted with ether prior to use.

Pieces of coarse, uncombed cotton wool, about the size of a pea and weighing about 30 mg, were used for swabbing. A stock solution of nitroglycerine in toluene (8 μg/μL) was prepared by overnight extraction (16 h) of a standard dynamite (68% NG on kieselguhr; used for transporting NG).

Application of Explosive and Hand Swabbing

A tenfold dilution of the NG stock solution was prepared and 100 μL (80 μg of NG) were applied evenly to each of four palm areas (Fig. 1) with a dispensing pipet. Cotton wool swabs were dipped in about 2 mL of solvent, rubbed over a small area of the hand with tweezers, and rinsed in the solution. This process was then repeated until the appropriate palm area had been covered three times and the swab was dropped into the solvent.

Sample Preparation and Analysis

Explosives were recovered from aqueous solutions by adding 0.5 g of ammonium sulfate followed by threefold extraction with equal volumes of ether or cyclohexane. The combined extracts were then dried over anhydrous sodium sulfate. Usually, the organic solutions were evaporated to a measured volume and analyzed directly, but where necessary solutions were partially purified using thin-layer chromatography (TLC) [*1*]. The amount of explosive in the solutions was determined using gas chromatography with electron capture detection [*1*], by comparing areas under the peaks with those for analytical standards.

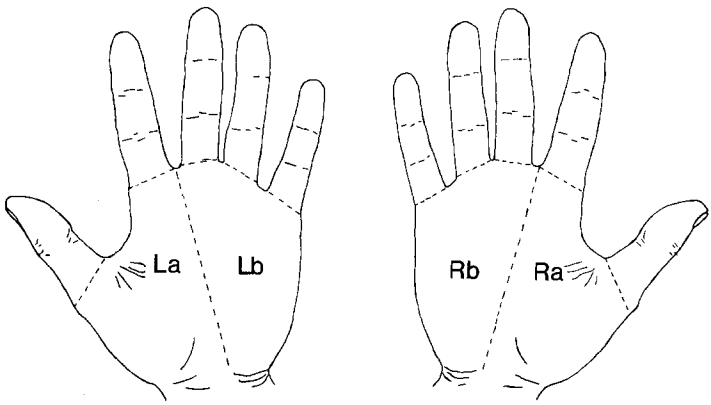

FIG. 1—*Subdivision of hands into four palm areas.*

Experimental Design

In a study of this type, it cannot be assumed that NG will show similar persistence for different individuals, and therefore careful experimental design is essential. Swabs were taken from two separate areas of each palm (Fig. 1), so that each subject contributed four palm areas in each experiment. Subjects and palm areas were allocated to various swabbing systems using a Latin Square design [*3*].

In order to produce a balanced experiment, swabbing with distilled water was carried out twice. One solution was then extracted with ether and the other with cyclohexane. The latter was used in an attempt to produce a cleaner extract for analysis than might be obtained with the former. Two separate experiments were conducted in an attempt to remove systematically the variation arising from the use of different hand areas and individuals (Table 1). Since in Experiment 1 each of the 16 subjects could contribute to only four systems, Experiment 2, in which each subject now contributed to the complementary four systems, was performed on the following day. In contrast to normal Latin Square designs, all the extracts taken with a given solvent were pooled for analysis in each of the two experiments.

Results and Discussion

Efficiency of Various Solvents

Although only seven different solvents were used in these experiments, a total of eight swabbing solvents were compared. The two distilled water swabs differed in their subsequent treatment, one being extracted with ether, the other with cyclohexane. Explosive was applied to the hands of the 16 subjects as described, and the swabs were taken 3 h later. The experimental design shown in Table 1 was used and the amounts of NG found in the eight pooled extracts, obtained from each of the two experiments, are shown in Table 2.

The agreement for the solvents in the two parts of the design is quite good, only cyclohexane showing any substantial discrepancy. The probability of obtaining this degree of correspondence if the two rankings were random is about 0.07 [*4*]. The overall ranking shows that acetone produced the best recovery and water followed by ether extraction produced the next best recovery. Very similar recoveries were obtained for cyclohexane, ethanol, buffer, and detergent. The lowest recoveries were obtained with ether and when water as swabbing solvent was followed by extraction with cyclohexane. The former failed probably because of

TABLE 1—*Experimental design used for the allocation of 16 subjects and four palm areas to eight different solvent systems.*

	Palm Area[a]							
	Experiment 1				Experiment 2			
Subjects	Ra	Rb	La	Lb	Ra	Rb	La	Lb
1 and 9	A	B	C	D	E	F	G	H
2 and 10	B	C	D	E	F	G	H	A
3 and 11	C	D	E	F	G	H	A	B
4 and 12	D	E	F	G	H	A	B	C
5 and 13	E	F	G	H	A	B	C	D
6 and 14	F	G	H	A	B	C	D	E
7 and 15	G	H	A	B	C	D	E	F
8 and 16	H	A	B	C	D	E	F	G

[a] Palm areas as shown in Fig. 1.

TABLE 2—*The amounts of explosive recovered from hands in pooled solutions 3 h after the application of 80 μg of NG to each hand area.*

Code	Solvent	Experiment 1		Experiment 2		Total NG Recovered, μg	Overall Ranking
		NG Recovered, μg	Ranking of Systems	NG Recovered, μg	Ranking of Systems		
A	acetone	1.20	2	0.99	1	2.19	1
B	distilled water (followed by ether extraction)	1.13	3	0.90	2	2.03	2
C	distilled water (followed by cyclohexane extraction)	0.21	8	0.42	7	0.63	8
D	cyclohexane	1.21	1	0.68	6	1.89	3
E	ether	0.65	7	0.39	8	1.04	7
F	detergent[a] (ether extraction)	0.95	6	0.83	5	1.78	6
G	pH 7 buffer[b] (ether extraction)	0.96	4=	0.84	4	1.80	5
H	ethanol	0.96	4=	0.85	3	1.81	4

[a] Empicol ESB3/S (Albright and Wilson) 0.5% sodium lauryl ether sulfonate in distilled water.
[b] Pye Unicam powder.

the rapid evaporation of ether from the skin, whereas the latter proved ineffective because of poor partition of NG between the cyclohexane and water phases.

As the aqueous solvents performed remarkably well in these experiments, it was decided to examine some practical conditions under which the recovery of explosives by water might be hindered. Similar recoveries were still obtained, however, for subjects who rubbed their hands in engine oil or an oil-based barrier cream, either before or after the addition of the explosive.

Interfering Materials Extracted by Various Solvents

The overall experimental design shown in Table 1 was used to obtain eight pooled solutions from the hands of 16 individuals who had not recently handled explosives. The aqueous solvents were extracted with ether or cyclohexane as described prior to analysis. The volume of each final extract was adjusted to 5 mL, and each extract was examined in two ways. In one experiment each of the extracts was injected into a gas chromatograph, and the resultant traces were examined for peaks that could cause interference during analysis on the column employed. In the second experiment 0.5 mL of each extract was subjected to a partial chromatographic purification [*1*] before gas chromatographic analysis. The amount of NG required to produce a definite peak of about three times background was estimated and then checked experimentally by adding the appropriate quantity of explosive. The results from the two experiments are shown in Table 3, together with the ranking of the solvents in order of increasing detection limits.

Use of the TLC procedure improved the detection limits by a factor of ten for some solvents. Irrespective of whether the extracts were examined directly or after partial purification, acetone and ether showed the poorest detection limits. The much lower efficiency of the procedure for the pooled acetone extracts than reported earlier for single extracts [*1*] suggests that one or more subjects were contributing relatively intractable material to the pool. Although any influences from the variation among subjects was not investigated further, it does illustrate the need for incorporating as many subjects as possible into studies of this type and for careful experimental design. Clearly such comparisons cannot be made in isolation. Solvent extraction after the use of aqueous solvents, such as distilled water and detergent, produced the best detection limits. Ethanol also produced satisfactory results. A general examination of the gas chromatograms also revealed a similar ranking, the chromatogram of the acetone extract, for example, showing several large peaks.

Stability of Nitroglycerine in Stored Solvents

Acetone solutions were initially prepared with NG concentrations of 8, 0.8, and 0.08 ng/μL. The solutions were stored on a laboratory bench for up to 34 days, and the concentration of the explosive was determined at regular intervals (Fig. 2). Fresh dilutions of the stock solution were used as analytical standards on each occasion, since the stock solution was already known to be stable over long periods.

The mean rates of loss for NG ranged from 1%/day in the most concentrated acetone solution to 3%/day in the most dilute solution. The considerable scatter of points observed for the 0.08 ng/μL solution is due to the fact that measurements were being conducted close to the detection limits.

In view of these unexpected results, a larger study was undertaken to examine the stability of the explosives in the eight solvents used for swabbing. Two NG solutions, at a concentration of 0.08 ng/μL, were prepared for each solvent. One was then doped with the pooled hand swab extract, taken with the appropriate solvent from eight subjects using a design similar to that presented in Table 1. All the organic solvents showed similar results (within experimental error) to those already shown for acetone in Fig. 2. The untreated aqueous sol-

TABLE 3—*The detection limits for NG in pooled extracts obtained from blank hands using cotton wool and eight different solvent systems.*

Code	Solvent	Experiment 1 (Direct Injection)		Experiment 2 (with TLC Purification)	
		Detection Limit,[a] ng	Ranking	Detection Limit,[a] ng	Ranking
A	acetone	10	6	5	8
B	water (ether extraction)	2	3	0.2	3
C	water (cyclohexane extraction)	1	1	0.1	1
D	cyclohexane	10	6	0.2	3
E	ether	20	8	0.5	6
F	detergent[b] (ether extraction)	2	3	0.1	1
G	pH 7 buffer[c] (ether extraction)	1	1	2	7
H	ethanol	2	3	0.2	3

[a] Calculated for solution containing composite extract from one pair of hands.
[b] Empicol ESB3/S (Albright and Wilson) 0.5% sodium lauryl sulfonate in distilled water.
[c] Pye Unicam powder.

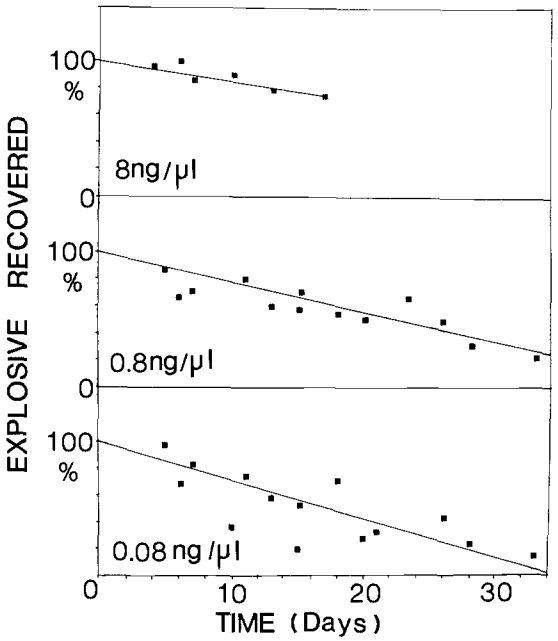

FIG. 2—*The stability of NG in acetone solution.*

vents also showed comparable results, but, when the aqueous solvents containing the hand swab extract were analyzed, all the NG was found to have been lost within 24 h. These solutions appeared cloudy and the growth of microorganisms was suspected. The use of inhibitors for aqueous solutions was therefore investigated.

Nitroglycerine was added at a concentration of 0.8 ng/μL to distilled water containing the pooled hand extract. Aliquots of the solution were stored with and without various inhibitors. The solutions were then extracted at regular intervals and the levels of NG determined. The results were compared directly with a NG standard in acetone (0.8 ng/μL) stored under the same conditions. The unpreserved control sample contained no detectable NG after eight days, whereas samples preserved with 0.1% w/v of sodium azide or 0.1% sodium metabisulfite (Table 4) were as stable over a 14-day period as acetone solutions (Fig. 2). Addition of 0.1% cupric chloride or 1% centrimonium bromide produced solutions that were stable for a few days. Sodium fluoride was found not to be effective. Although stabilization was demonstrated in these limited experiments, it remains possible that some types of microorganisms could continue to grow and destroy the explosive in aqueous media.

Storage of Nitroglycerine on Solvent-Moist Swabs

An alternate method for storing the swab and recovered explosive was also investigated. This method, employed in earlier hand test kits, used larger (0.5-g) swabs moistened with solvent, which were stored in nylon 11 bags (150 by 100 mm) sealed with adhesive tape. Table 5 shows the results from analysis of such swabs moistened with 2 mL of appropriate solvent and doped with 5 μg of NG prior to storage. The poor recovery of NG when ether was used could have resulted from the high volatility of the solvent. The very low recoveries with ethanol were almost certainly due to the ability of the solvent to permeate nylon. This effect

TABLE 4—*Levels of NG in stored aqueous hand swab solutions.*

Time, days	Relative Concentration of NG in Solutions[a]		
	Unpreserved	0.1% Sodium Azide	0.1% Sodium Metabisulfite
0	100	100	100
2	50	110	102
5	8	100	110
8	0	97	98
14	0	101	96

[a] Concentrations expressed as percentages relative to an acetone standard (0.08 ng NG/μL) stored under similar conditions.

TABLE 5—*The recovery of NG from cotton swabs stored under different conditions.*

Solvent	Mean Recovery of NG After Storage Time (Days),[a] μg				
	1	2	3	7	8
			EXPERIMENT 1[b]		
Cyclohexane	4.9 (98%)	...	4.2 (84%)	3.8 (76%)	...
Acetone	4.6 (92%)	...	4.7 (94%)	3.0 (60%)	...
Ether	4.3 (86%)	...	1.5 (30%)	0.6 (12%)	...
Ethanol	3.3 (65%)	...	0.8 (16%)	0.2 (4%)	...
			EXPERIMENT 2[c]		
Ethanol	4.9 (98%)	4.8 (96%)	4.8 (97%)	...	4.7 (94%)

[a] The mean of several determinations on extracts from two or three swabs.
[b] Storage in nylon bags.
[c] Storage in glass vials.

is well recognized in these and other laboratories.[5] When glass containers (10 mL with snap-on polyethylene lids) were used instead of nylon bags (Table 5, Experiment 2), the storage of NG on ethanol soaked swabs gave good results.

General Discussion

This study dealt mainly with the effects of several factors (namely, the use of cotton wool, the size of swabs, and the swabbing procedure) on three important aspects (efficiency, detection limits, and sample stability) of the recovery of NG by seven different solvents. Although the use of dry cotton swabs or of different types of swabbing material were briefly investigated, cotton wool soaked in solvent was found to produce the best recoveries. Originally it had been hoped to attach the cotton swab to a polypropylene stick for easy handling, but no swab manufacturer could be found who would wind a swab to the necessary specifications. This mandated the selection of a small swab weighing about 30 mg. The swabbing procedure was based on studies that indicated that NG was more stable in solvent than on swabs soaked in solvent, if the latter subsequently dried out. In any event, significant variations in the size of swab or in the swabbing procedure could affect the results.

[5] S. M. Fleet, Metropolitan Police Laboratory, London, personal communication.

Both aqueous solvents examined showed surprisingly high recoveries of NG, even from oily hands, and very good detection limits in the resultant solvent extracts. Extraction of the aqueous media with ether produced better recoveries than did extraction with cyclohexane. To what extent the extraction of the aqueous solvents prior to analysis purified the solutions was not investigated. The aqueous buffer and the detergent solution, which were chosen to achieve neutral conditions, showed no advantages over distilled water.

The use of distilled water as the swabbing solvent is simple, safe, and in many ways attractive. Organic and inorganic materials can be simultaneously removed from the hands and then separated by solvent extraction. Unfortunately, microorganisms can grow in aqueous media and degrade the explosive. Although aqueous solutions of suitable stability were produced in this work, a more extensive study of the growth of microorganisms is required before distilled water can be used in practice.

The optimum choice for an organic solvent can be obtained by considering Tables 2 and 3. Although the losses of NG during storage were similar for acetone, cyclohexane, ether, and ethanol, consideration of both relative recovery and detection limits (after partial TLC purification), indicates that cyclohexane and ethanol are preferable to ether and acetone. Without TLC purification, ethanol is preferred. In practice, factors other than those considered here might be deemed important, for example, the slower rate with which ethanol solutions can be evaporated compared to acetone or the risk of ignition of ether vapor.

Summary

Seven different solvents (eight solvent systems) have been assessed for use in the removal of NG from hands by 30-mg cotton swabs. The specific factors investigated were the efficiency of the solvent in removing NG, the amount of interfering material concurrently removed, and the stability of the explosive in the resultant solution.

Acetone and distilled water were only slightly more efficient, while ether was much less efficient, than the other solvents. Both ether and acetone extracted high levels of interfering materials from uncontaminated hands. Aqueous swabs that were extracted with ether or cyclohexane prior to analysis produced the cleanest extracts and hence the lowest detection limits for NG (about 0.2 ng in the total extract). Concentrations of NG decreased slowly in organic solvents and very rapidly in aqueous media, all the explosive disappearing within a few days. Although aqueous solutions could be stabilized against the growth of microorganisms by the use of certain preservatives, extensive testing is required before aqueous swabs should be used in practice. On the basis of the factors investigated in this study ethanol appears to be the best organic solvent for removing nitroglycerine from hands using cotton swabs.

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