Journal of Chromatography, 234 (1982) 415–425
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,236

TRACE ANALYSIS OF EXPLOSIVES IN HANDSWAB EXTRACTS USING AMBERLITE XAD-7 POROUS POLYMER BEADS, SILICA CAPILLARY COLUMN GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION AND THIN-LAYER CHROMATOGRAPHY

J. M. F. DOUSE
The Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London SE1 7LP (Great Britain)
(Received July 27th, 1981)

SUMMARY

A general method for detecting traces of explosives at the low nanogram level in handswab extracts is described. The method involves a preliminary clean-up using Amberlite XAD-7 porous polymer beads to remove interfering lipid material, followed by detection of explosives in the concentrated extracts by capillary column gas chromatography with electron-capture detection. A method for confirming the presence of explosives in the extracts using thin-layer chromatography is also described.

INTRODUCTION

A previous publication from this laboratory[1] described a sensitive method for the trace analysis of explosives using silica capillary column gas chromatography (GC) with electron-capture detection (ECD). Although pure explosives can be analysed reproducibly at the low picogram level by this method their detection in contaminated samples of forensic interest, such as handswab extracts[2], rapidly leads to deterioration of the column and the detector unless the sample is cleaned up prior to analysis[3–5].

No general clean-up procedure suitable for explosives analysis has yet been described in the literature[6]. This paper describes a clean-up technique suitable for the trace analysis of the important commercial and military explosives at the low nanogram level in heavily contaminated samples, such as handswab extracts. The method uses selective charge-transfer extraction of the explosives from solutions of handswab extracts in pentane on to Amberlite XAD-7 porous polymer beads. The explosives are then removed from the surface of the beads with small volumes of ethyl acetate to give extracts suitable for repeated direct analysis by capillary column GC with ECD.

A method capable of detecting explosives at the low nanogram level in the cleaned up extracts using thin-layer chromatography (TLC) with specific reagent sprays is also described.

0021-9673/82/0000–0000/$02.75

EXPERIMENTAL

Reagents

The explosives studied were ethyleneglycol dinitrate (EGDN), nitrobenzene (NB), nitroglycerine (NG), 2,4-dinitrotoluene (2,4-DNT), 2,4,6-trinitrotoluene (TNT), hexogen (RDX), pentaerythritol tetranitrate (PETN), tetryl, octogen (HMX), and nitrocellullose (NC)[1].

All solvents used were pesticide grade (Fisons, Loughborough, Great Britain).

Amberlite XAD-7 (BDH, Poole, Great Britain) was 20–50 mesh grade, and 9 ml (wet volume) of the beads were cleaned by washing in a column successively with 100 ml portions of distilled water, methanol, ethyl acetate, ether, and pentane. The beads were stored under ether until required for use. Quantitative handling of the beads was achieved in the dry state by carrying out all manipulations in narrow-necked screw-topped glass vials. However, the beads were stored under solvent at all other times.

Cotton wool (Vestric, London, Great Britain) was soxhlet extracted with ether for 4 h.

All materials used in the procedure were checked for interferences by running a control experiment.

Gas chromatography

The following conditions were used: column, flexible-fused silica capillary externally coated with polyimide, 21 m × 0.25 mm I.D. (Phase Separations, Queensferry, Great Britain); stationary phase, OV-101; injection port temperature, 165°C; detector oven temperature, 200°C; temperature programme, 25°C held for 30 sec then programmed at 40°/min to 240°C, cooldown time, 4 min; carrier gas, helium; carrier gas flow-rate 30 ml/min (25 C); make-up gas, methane–argon (1:99); make-up gas flow-rate, 13 ml/min; injection solvent, ethyl acetate. The Varian tritium ECD was operated here in the constant current mode, at a potential of 50 V, and a pulse width of 1 μsec, using a Carlo Erba Model 251 control module. Better baseline stability was achieved using the detector in this mode, and a typical analysis of a mixture of explosives is shown in Fig. 1. The injection port liner was cleaned with swabs soaked in ethyl acetate, and the liner was then rinsed with ether and dried at room temperature. By this technique it was possible to avoid the use of high-temperature baking which, under certain conditions, gave rise to undesirable activity.

Thin-layer chromatography

TLC plates were DC-Alufolien Kieselgel 60F 254 (5 cm × 7.5 cm × 0.2 mm) (Merck, Darmstadt, G.F.R.).

The eluting solvent for EGDN, NG, TNT, PETN, and tetryl (eluent A) was a mixture of toluene and cyclohexane (7:3 by volume). The eluting solvent for RDX and HMX (eluent B) was a mixture of chloroform and acetone (2:1 by volume). The eluting solvent for NC (eluent C) was a mixture of acetone and methanol (3:2) by volume.

The specific reagent spray for EGDN, NG, PETN, RDX, tetryl, HMX, and NC was Griess reagent spray[2]. The plates were eluted, and the solvent was evaporated using a stream of warm air. The plates were sprayed with 1*N* sodium hydrox-

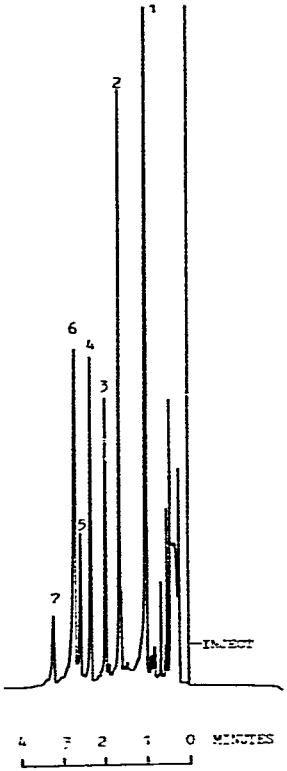

Fig. 1. Mixture of explosives containing 400 pg each of EGDN (1), NG (2), 2,4-DNT (3), TNT (4), PETN (5), RDX (6) and tetryl (7) analysed using the conditions described in the Experimental section.

ide solution and heated to 150°C for 5 min. They were then sprayed with a solution of sulphanilamide (8 g) and N-1-naphthylethylenediamine dihydrochloride (0.4 g) (Sigma, Poole, Great Britain) in 8% orthophosphoric acid (100 ml). The explosives developed a red colouration at room temperature.

The specific reagent spray for TNT and tetryl was a 30% solution of 3,3'-iminobispropylamine[7] (Aldrich, Gillingham, Great Britain) in pyridine. TNT developed a purple colour and tetryl a brown colour at room temperature.

Preparation of handswab extracts

Handswabs were obtained by repeatedly scrubbing the appropriate surface of one hand using a cotton wool swab (40 mg) moistened with ether. The lower surface of the palm and fingers of the hand were swabbed to determine if a subject had handled explosives. The upper surface of the back of the hand was swabbed to determine if a subject had fired a handgun. Spiked extracts of all of the explosives except NC were prepared by distributing standard solutions of explosives throughout the swab using a syringe. Preparation of spiked extracts containing NC was achieved by spiking the ether-insoluble residue of the handswab extract with a standard solution of NC in acetone and allowing the residue to dry.

Extraction of handswabs

The swab was extracted by successive washing with small portions of ether (total volume 12 ml) in a beaker using a glass rod. The combined extracts were centrifuged to remove traces of skin debris, and the clear supernatant was decanted into a silanized conical tube. The ether was evaporated down to near dryness (5–10 μl) using a current of nitrogen, and the last traces of ether were allowed to evaporate at room temperature. Pentane (3 ml) was added to the residue and the resulting solution was thoroughly mixed and transferred to a screw-capped vial. The insoluble residue removed from the solution of the handswab extract in ether by centrifuging was washed with ether, dried, and extracted with acetone in an ultrasound bath. The acetone extract was then concentrated and analysed by TLC using Griess reagent spray to detect NC.

Clean-up method

First clean-up. Amberlite XAD-7 (10 mg dry weight) was added to the solution of the handswab extract in pentane, and the mixture was gently shaken for 15 min so that the beads circulated throughout the solution. The supernatant was then decanted using a Pasteur pipette, and retained for further processing if required. The beads were thoroughly rinsed with pentane. The residual traces of pentane were evaporated using a current of nitrogen, and the beads transferred to a clean vial. Ethyl acetate (80 μl) was added and after 2 min equilibration the solvent was decanted, and the beads washed with a further two portions of 40 μl of solvent. The extracts were combined and stored in a sealed sample vial.

Second clean-up. The combined ethyl acetate extract was evaporated to near dryness using a current of nitrogen, the residue dissolved in pentane (1 ml) and the Amberlite XAD-7 extraction repeated using 3 mg of fresh beads. (The supernatant pentane solution from this extraction was retained for confirmation of the presence of explosives in the extract by TLC). The beads were then extracted with 30 μl of ethyl acetate, and 1 μl of this extract was analysed by GC with ECD.

Stability of solutions of explosives

Standard solutions of explosives in organic solvents can be unstable under certain conditions and should therefore be regularly monitored for evidence of decomposition. Fresh solutions should always be prepared at regular intervals. The explosives tetryl, TNT and HMX were found to be the most prone to decomposition in solution. For this reason, handswab extracts containing traces of explosives should be processed and analysed without delay.

RESULTS AND DISCUSSION

Attempts to detect traces of explosives in handswabs by analysis of uncleaned-up extracts using capillary column GC with ECD were unsuccessful. Interfering compounds present in the extracts obscured the response for the explosives and heavily contaminated the GC system[3–6]. This contamination prevented the analysis of explosives at low levels without the use of priming[1], and caused rapid deterioration of

the column and the detector. A clean-up method was therefore developed which would allow the routine trace analysis of explosives at the low nanogram level in heavily contaminated samples such as handswab extracts.

Initial experiments using silica gel column chromatography as a clean-up method failed because the wide range of polarity of the explosives prevented their selective elution and gave extracts still heavily contaminated with involatile materials. Experiments using selective charge-transfer extraction[8] of explosives from pentane solutions of handswab extracts with polar non-ionic Amberlite XAD porous polymer beads[9] gave clean samples suitable for GC analysis, and this method of clean-up was further investigated.

Handswab extracts were prepared by the method described in the Experimental section using ether[2], because this solvent could be readily removed by evaporation without the loss of the important volatile explosive EGDN. After evaporation of the ether the extracts were dissolved in pentane, since in these experiments explosives could only be efficiently recovered from saturated hydrocarbon solvents by the porous polymer beads. Three types of bead were investigated (Amberlite XAD-2, -7, and -12)[9] but only Amberlite XAD-7 and XAD-12 showed any potential for the recovery of explosives. Initially, Amberlite XAD-12 (amine oxide polystyrene) was used in these experiments, and useful recoveries for the nitrate ester explosives and RDX were demonstrated. However, these beads were unstable on storage and gave only very poor recoveries of the nitroaromatic explosives at low levels and their use was discontinued. Amberlite XAD-7 [poly(methylmethacrylate)] was found to give practical recoveries of all of the explosives shown in Table I, and optimum conditions were therefore determined using this bead by varying the mass of the beads, the volume of pentane and time required for the extraction. After the extraction, the beads were thoroughly rinsed with pentane to remove unadsorbed handswab material and the beads dried using a current of nitrogen. Ethyl acetate was used to extract the explosives from the surface of the beads since this solvent gave good recoveries of the

TABLE I

CHARACTERISTICS OF THE AMBERLITE XAD-7 EXTRACTION METHOD AND THE ANALYSIS OF EXPLOSIVES BY GAS CHROMATOGRAPHY

Explosive	*Recovery of 200 ng of explosives from a handswab (mean of 3 determinations)*		*Minimum detectable level of explosive in a handswab (ng/swab)*
	First clean-up (%)	*Second clean-up (%)*	
EGDN	19	5	10
NG	77	47	10
2,4-DNT	27	6	50 (25*)
TNT	50	33	20
PETN	80	45	50
RDX	73	47	50
Tetryl	38	25	50

* Using the primary clean-up only.

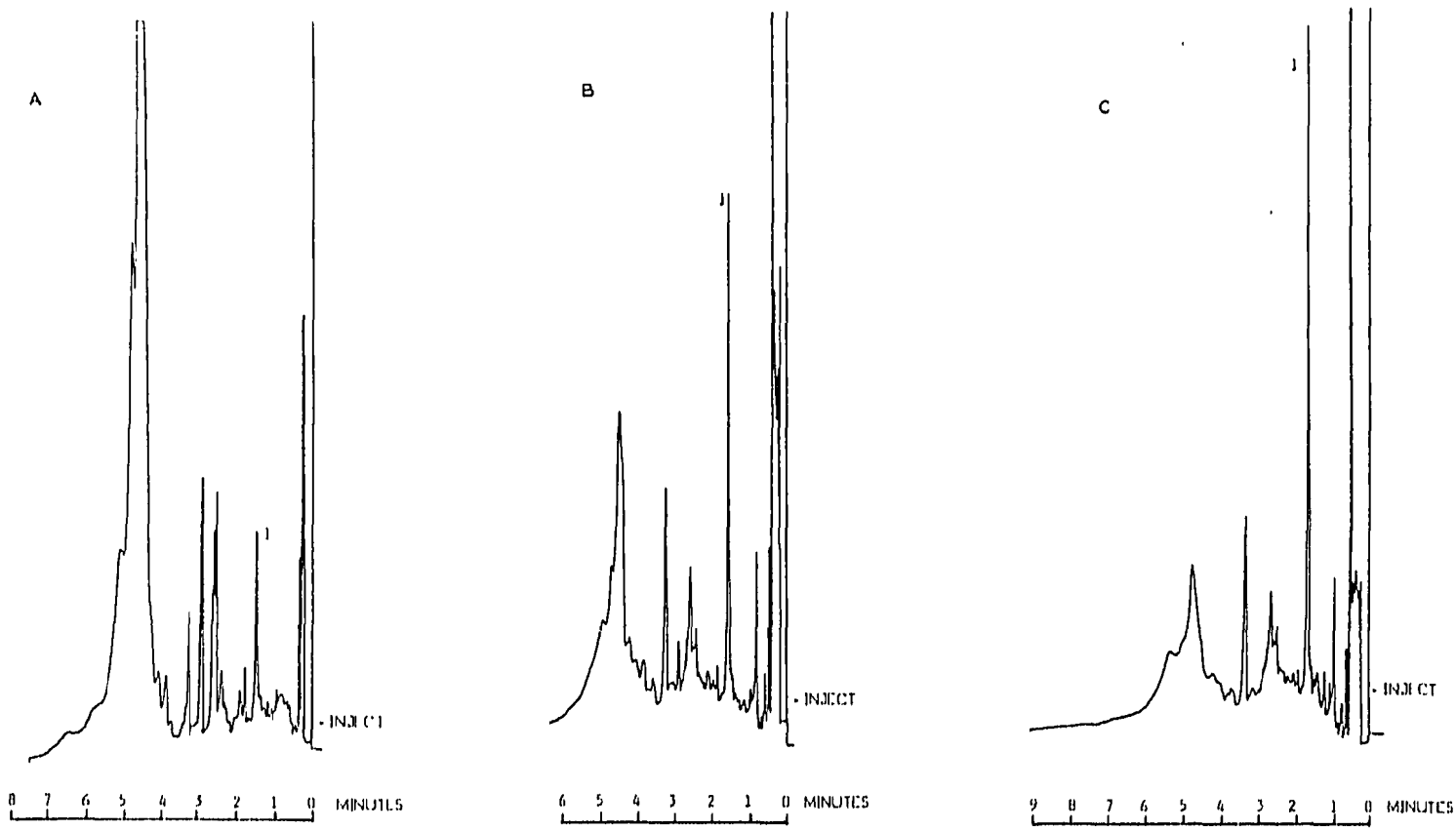

Fig. 2. (A) Unpurified handswab extract taken from a laboratory worker and spiked with 50 ng of NG (1). 0.1% of the sample was analysed, using the standard conditions as described in the Experimental section. (B) The same handswab extract spiked with 50 ng of NG (1) cleaned up once by the Amberlite XAD-7 clean-up method. 0.6% of the sample was analysed, using the standard conditions described in the Experimental section. (C) The same handswab extract spiked with 50 ng of NG (1) cleaned up twice by the Amberlite XAD-7 clean-up method. 2% of the sample was analysed, using the standard conditions described in the Experimental section.

explosives and was also a suitable injection solvent. Only small volumes (15–80 μl) of ethyl acetate were required for the extraction, thus avoiding the need to concentrate the extract prior to analysis by GC. The primary clean-up of the extract by this method gave a sample almost free from lipid material that was clean enough to be analysed by GC.

Because of the sensitivity of the ECD only a few per cent of the sample at most was required for analysis, but because of the relatively poor selectivity of this detector the minimum detectable levels of the explosives in handswabs were limited by the background. Thus it was not worthwhile analysing a larger fraction of the sample since no improvement in the signal-to-background ratio was obtained. However, the level of background could be successfully reduced and the response for explosives present at the low nanogram level enhanced by repeating the clean-up, as demonstrated in Fig. 2, and by this method the minimum detectable levels (MDL) shown in Table I were obtained. Thus using the dual clean-up method it was possible in the case of NG and EGDN routinely to detect levels of explosives in the range 15–20 ng/swab. It should be noted that NB cannot be recovered from handswabs using either Amberlite XAD-2, -7, or -12; however, this explosive can still be detected at high levels in handswabs by analysis of very dilute solutions of uncleaned-up extracts. Although HMX can be analysed by GC under carefully optimised conditions[1], it is an explosive of only relatively minor forensic importance and was not studied in this work.

The general effectiveness of the analytical method was evaluated by analysing a range of handswabs each representing different degrees of contamination. The method was shown to be succesful both in the case of extracts from clean hands (Fig. 3) and heavily contaminated hands. A garage mechanic's hands, covered in dirt and grease, were chosen to approximate the worst possible case of contamination likely to be encountered, and analysis of the cleaned-up extracts showed profiles similar to those of clean hands (Fig. 3) but with an increased level of background. It should be noted that some hands give extracts showing coextractive peaks eluting in the region of the chromatogram after tetryl (Fig. 2) but these do not interfere with the analysis of the explosives described in this paper.

In the Metropolitan Police laboratory the analytical method is being experimentally applied to the detection of firearms residues on hands, and the results of an experiment using a handgun and a double base propellant ammunition demonstrating the presence of NG on the upper surface of the back of the firer's hand are shown in Fig. 4.

The reproducibility of recovery of 100 ng of NG and TNT from ten different handswabs using the primary clean-up were measured and found to be satisfactory: 73%, R.S.D. 10% ($n = 10$), and 49%, R.S.D. 21%, ($n = 10$), respectively. The level of recovery of the most important explosive, NG, was shown to be constant within the experimental error over the range 50–300 ng/swab. Recoveries for the explosives obtained using 10 mg of beads at the 200 ng/swab level are shown in Table I.

The extracts obtained by the Amberlite XAD-7 clean-up method are clean enough to allow the routine detection of the explosives shown in Table I at the low nanogram per swab level without degrading the performance of the column or the detector. Regular cleaning of the injection port liner is required to remove septum cores and any traces of involatile residue which can accumulate and give rise to

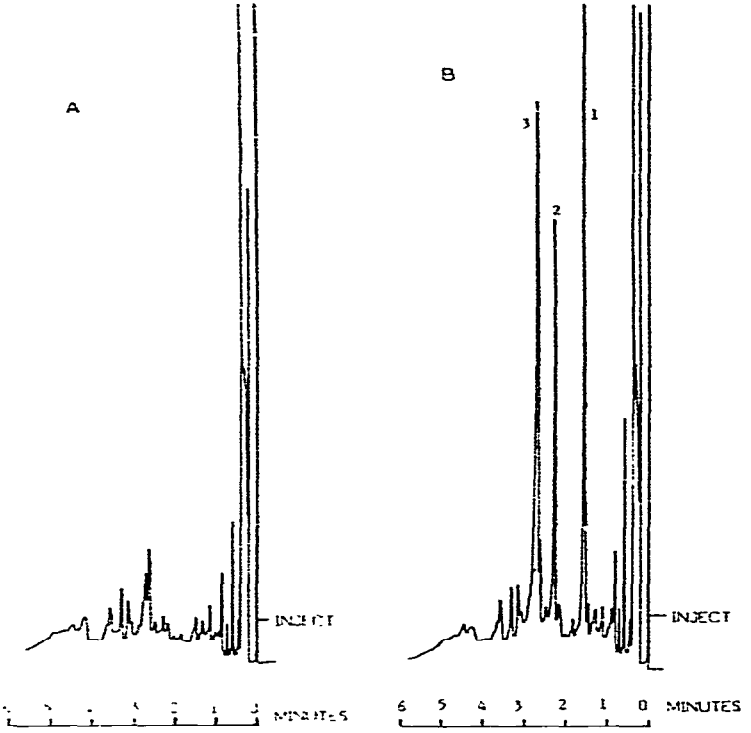

Fig. 3. (A) Unspiked extract of a laboratory worker's left hand, cleaned up twice using the Amberlite XAD-7 clean-up method. Standard conditions as described in the Experimental section. (B) Spiked extract of a laboratory worker's right hand containing 50 ng of NG (1), and 100 ng each of TNT (2) and RDX (3), cleaned up twice by the Amberlite XAD-7 clean-up method. Standard conditions as described in the Experimental section.

undesirable activity. This is especially important when analysing the very readily adsorbed explosive PETN, and it is advisable to test the system regularly for the response of this explosive and to clean the injection port liner regularly to obtain maximum response for this compound. However, for the other explosives described in this paper, the injection port liner need be cleaned only at the end of each working day. Removal of the short length of column protruding into the injector was very effective in restoring the peak shape of the longer-retained explosive RDX should this deteriorate in the long term.

The long-term performance of the system was satisfactory, and in this laboratory one OV-101 silica capillary column has been used over a period of 9 months to analyse over 400 handswab extracts. Regular cleaning of the injection port liner effectively prevents contamination of the detector, and one tritium foil has been in use for over 6 months without deterioration. The analytical traces shown in this paper were obtained at the end of this time using this system. It should be noted that the long useful working life of the fused silica capillary columns in this situation effectively offsets the disadvantage of their high cost.

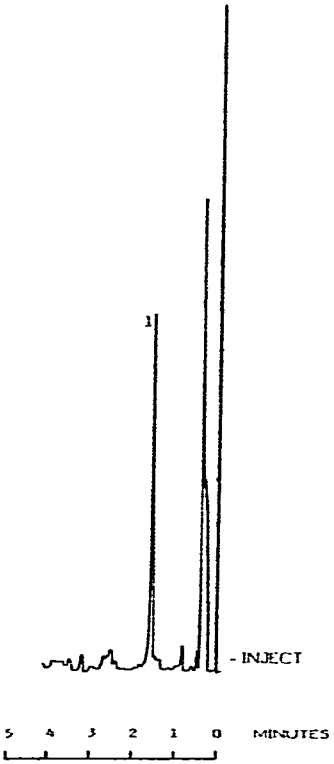

Fig. 4. Extract of the upper surface of the right hand of a laboratory worker who had just fired three rounds of Smith and Wesson Super X double base propellant ammunition, using a Smith and Wesson Model 27, 357 Magnum, showing NG (1). The extract was cleaned up once using the Amberlite XAD-7 method, and analysed using the standard conditions described in the Experimental section.

Analysis of extracts by TLC

Standard mixtures of many of the important commercial and military explosives may be analysed by TLC with sensitivities at the low nanogram level[2,10] (Table II). However, attempts to analyse uncleaned-up extracts by this method were unsuccessful because the lipid material present prevented elution of the extract from the origin because of overloading of the adsorbent.

Analysis of the extracts cleaned-up once by the Amberlite XAD-7 method was successful, with minimum detectable levels in the low nanogram range (Table II). It should be noted that these levels were achieved only by analysing most of the sample in one analytical run. However, this method of analysis could be successfully used to confirm the presence of explosives detected in the extracts by the GC method.

Nitrocellullose (NC) was successfully analysed by TLC using an eluting solvent containing acetone, and Greiss reagent spray. This method of analysis was successfully used to detect NC on the upper surface of the hand of a subject who had fired a handgun (Smith and Wesson Model 27 357 Magnum) using double base propellant ammunition. The NC was readily detected by TLC analysis of the acetone-soluble fraction of the residue obtained by centrifuging the ether extract of the handswab. The feasibility of routinely detecting traces of firearms residue (NG and NC) on

TABLE II

CHARACTERISTICS OF THE TLC ANALYSIS OF EXPLOSIVES

Where an R_F value is not quoted in this table, the explosive concerned eluted close to the solvent front.

Explosive	R_F *eluent A*	R_F *eluent B*	R_F *eluent C*	*MDL of explosives (ng)*		*MDL of explosives in a handswab (ng/swab). Cleaned up once using Amberlite XAD-7*
				Griess spray	*3,3'Iminobis propylamine spray*	
EGDN	0.53	–	–	25	–	300
NG	0.42	–	–	5	–	20
TNT	0.57	–	–	–	10	50
PETN	0.45	–	–	5	–	30
RDX	0.03	0.72	–	5	–	20
Tetryl	0.25	–	–	20	30	60
HMX	0	0.35	–	10	–	500
NC	0	0	0.64	50	–	300*

* This value represents the amount of NC present in the ether-insoluble residue of the handswab.

hands is currently under investigation, and the results will be reported in a future publication.

It should be noted that the majority of the above results (both GC and TLC) were obtained using handswabs spiked with explosives, whereas in reality further losses can be expected to occur in the recovery of explosives from the surface of hands. This will result in higher achievable minimum detectable levels and it will probably be necessary, for some applications, to detect explosives at levels lower than those described in this paper. Two possible approaches to improving the sensitivity of the method are to develop a more selective clean-up method, or to use a more specific detector such as the thermal energy analyser[1] or the mass spectrometer in the chemical ionisation negative specific ion monitoring mode[1]. However, a preliminary clean-up of the samples will still be required to protect the GC system from contamination when using these detectors.

CONCLUSION

This paper describes a general method for analysing traces of important commercial and military explosives at the low nanogram level in handswabs. The clean nature of the extracts obtained by the Amberlite XAD-7 clean-up technique is believed to be responsible for the success of the method, enabling routine reproducible analysis of subnanogram amounts of explosives in the presence of a complex organic background.

Other possible applications of the technique are the detection of explosives in body fluids, environmental samples and post explosion residues, and the detection of firearms residues on hands.

ACKNOWLEDGEMENT

I thank I. Jane and B. B. Wheals for their guidance and advice.

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