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MICROCOLUMN CLEAN-UP AND RECOVERY TECHNIQUES FOR ORGANIC EXPLOSIVES COMPOUNDS AND FOR PROPELLANTS TRACES IN FIREARMS DISCHARGE RESIDUES

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SUMMARY

Microcolumn recovery and clean-up procedures suitable for use with a variety of adsorbents of explosives compounds are described. The procedures employ 1 mm I.D. columns that are charged and eluted by the use of the sample loop of a valve injector as a solvent reservoir. Some examples are given of the recovery of nitroglycerin from firearms discharge residues in clothing extracts and from explosion debris.

INTRODUCTION

Despite the small size of many items submitted to the analysis of their trace components, *e.g.* in forensic science work, for a given analytical system the practical detection limits often are restricted by the quality of the analysed sample rather than by the performance characteristics of the system, just as in larger scale work. Hence minituarized clean-up procedures are required. In organic explosives traces work several techniques employing adsorbents for the removal of interfering materials from liquid extracts have been described. These techniques, conditioned by the selectivity demands both of the analytical system and of the questions asked of the samples, include the use of silica[1,2], Amberlite XAD 7[3,4], alumina and octadecylsilyl silica[5], and cyclohexyl and cyanopropyl silicas[6]. The adsorption characteristics of these or related adsorbents and of others used in explosives work generally are the subject of a recent paper[7]. The results given there are the basis of the procedures described below, which are especially designed to facilitate the manipulation of the restricted amounts of eluents and adsorbents needed in the trace analysis of heavily soiled small samples. Although the emphasis here is on explosives work, particularly on the use of the negative selectivity of charcoal for the nitrate esters[7] in the detection of nitroglycerin traces, the procedures are broadly applicable.

EXPERIMENTAL

Adsorbents
    Porapak T, 75–100 μm, (Waters) and Chromosorb 104, 125–150 μm, (Johns-

Manville) are Soxhlet-extracted with acetonitrile for 16 h, dried in air at ambient temperatures and finally at 120°C for 2 h. Charcoal ("for gas adsorption", BDH Chemicals) is crushed and sieved to give a 70–150 μm fraction, which is Soxhlet-extracted with 10% (v/v) acetic acid in methanol, washed with methanol, and dried in air at ambient temperatures and finally at 250°C for 8 h.

Apparatus

The reusable columns (procedure 1, below) are made from nominally 22-mm lengths of 1 mm I.D., 1.6 mm O.D., stainless-steel tubes with end-fittings containing steel meshes (2 μm) held in place by 70-mm lengths of 0.2 mm I.D., 1.6 mm O.D., tube at the inlet and outlet. The columns are slurry-packed with a suspension of the Porapak T in acetonitrile delivered from a hypodermic syringe. A finished column with an actual length of 22.4 mm and an actual internal volume of 21.2 μl contained 8.0 mg (6.6 μl) of adsorbent and a void of 14.6 μl.

The disposable column tubes (procedure 2) are made from 50-mm lengths of 1 mm I.D., 1.6 mm O.D., PTFE tube. A 1–2 mm plug of cotton wool (methanol-extracted) is pushed into the outlet end of the tube to a depth of 5 mm, and held in place by a crimp made between the plug and the outlet. These columns are packed as described in procedure 2.

The inlet tube of a reusable column is attached to a Rheodyne 7125 injection valve directly. For a PTFE column the attachment is a piece of PTFE sleeve holding the column inlet butted to a stainless-steel outlet tube from the injector. The valve is fitted with a 1-ml sample loop, and connected to a Constametric III pump (Laboratory Data Control) driven with a one-third speed motor set to give a flow-rate of 1 μl sec^{-1} (to purge the column this is increased by a factor of 2–3). Usually the pump is charged with methanol–water (1:3, v/v), but any convenient fluid may be used because this need never pass further than the sample loop.

Procedure 1 (reusable columns)

This is applied to solutions which remain homogeneous on the addition of water.

The column (Porapak T) is purged with 200 μl of acetonitrile, delivered from the sample loop, followed by 200 μl of methanol–water (1:3, v/v). The sample, *e.g.* 10–500 μl, in the same aqueous methanol or similarly aqueous solvent, is transferred (1 μl sec^{-1}) to the column by way of the sample loop, in which the remaining volume is occupied by the aqueous methanol, and washed through with 200 μl of the aqueous methanol. Inorganic components, picric and styphnic acids, and other highly polar compounds including nitroguanidine, appear in the effluent at this stage. The elution is continued with solvents, transferred through the sample loop, in a sequence determined by the compounds sought. A typical sequence is: (a) isopentane (100 μl), which displaces the aqueous methanol occupying the column void and substantially desorbs lipophilic material as well as nitrobenzene and the nitrotoluenes; (b) diethyl ether (50 μl), which removes the remainder of the latter compounds together with most other explosives compounds except tetryl and HMX (the full name is in the footnote to Table I); (c) acetonitrile (50 μl), which completes the desorption.

Procedure 2 (disposable columns)

This is applied to heavily contaminated samples, *e.g.* extracts in water-miscible solvents of hand swabs, explosion debris, or debris vacuumed from clothing.

To the extract is added water to give a 1:3 (v/v) solvent–water dilution. Often a large amount of material is thrown out of solution at this stage to form a relatively stable suspension. To this is added the adsorbent (*e.g.* Porapak T, Chromosorb 104, charcoal; 5 mg). This is conveniently dispensed from a piece of 1 mm I.D. glass tube attached to a bulb pipette and plugged with cotton wool at a point from its outlet corresponding to the required amount of adsorbent, which is sucked into and blown out of the glass tube. The mixture is shaken intermittently over a period of 10 min, the adsorbent allowed to sediment, and the supernatant removed along with any suspended material. The adsorbent is washed with 100-μl amounts of methanol–water (1:3, v/v) until the supernatant is free from suspended material, then the suspended adsorbent is drawn into a prepared empty microcolumn, the outlet of which is attached to a filter pump. The column is eluted according to procedure 1, with eluents chosen according to the compounds of interest and the characteristics of the adsorbent[7]. In a commonly used application, the detection of nitroglycerin, the charcoal column is eluted immediately with methanol (50–100 μl), which may be evaporated down subsequently.

Extracts of clothing

These are prepared by microvacuuming the area of interest through a fibreglass filter essentially according to Jane *et al.*[8]. Typically, 50–100 mg of debris are collected, and extracted with 100-μl amounts of acetonitrile–water (100:5, v/v).

Explosives detection and determination

High-performance liquid chromatography (HPLC) is carried out essentially as described previously[9] on 150 mm × 4.5 mm I.D. columns of ODS-Hypersil, 3 μm, (Shandon) at 40°C, eluted (1 ml min⁻¹) with deoxygenated methanol–aqueous phosphate (0.035 *M*, pH 3). The aqueous phosphate content is varied in the range 38–47% (v/v) according to the characteristics of the particular column packing and to the requirements of any other usage of the system. The detector is a pendent mercury drop electrode (3 mg) maintained at a potential of −0.9 V *vs.* Ag/AgCl. The recovery values in Tables I and II are from determinations internally standardized with *m*-dinitrobenzene.

RESULTS AND DISCUSSION

Operating conditions and performance

Table I lists the recoveries given by procedure 1 (Porapak T), with various eluents, of explosives compounds dissolved in 400-μl amounts of aqueous methanol. The results are as expected from the distribution coefficient data previously reported[7]. Styphnic and picric acids are only weakly retained in the initial transfer to the microcolumn, which is reflected in their subsequent recoveries. To a lesser degree the mononitroaromatic compounds and ethyleneglycol dinitrate are similarly affected. In general, with 50 μl of eluent, the elution is largely complete for compounds with distribution coefficients[7] of less than 3, which, with allowance for the effects of band

TABLE I

RECOVERIES OF ORGANIC EXPLOSIVES COMPONENTS (50 ng) IN VARIOUS SOLVENTS FROM A PORAPAK T MICROCOLUMN (PROCEDURE 1)

Component	Recovery (%)*				
	Methanol–water (1:3, v/v), 500 μl	Methanol, 50 μl	Diethyl ether, 50 μl	Acetonitrile, 50 μl	Isopentane, 100 μl
HMX**	–***	9.1	4.4	104.9	–
Styphnic acid	49.8	18.0	8.7	21.6	–
Picric acid	29.4	31.0	41.2	35.4	–
RDX§	–	46.4	63.8	85.7	–
Ethyleneglycol dinitrate	11.6	74.7	73.8	79.7	5.4
Isosorbide dinitrate	–	64.8	79.9	93.5	–
Tetryl	–	26.3	37.9	90.2	–
Nitrobenzene	5.8	92.3	78.9	93.6	46.7
Nitroglycerin	–	64.3	80.4	96.0	–
2,4,6-Trinitrotoluene	–	23.2	57.0	93.3	–
2,6-Dinitrotoluene	–	69.8	80.7	95.5	6.8
2,4-Dinitrotoluene	–	66.5	80.8	95.0	3.9
2-Nitrotoluene	0.8	102.8	88.1	102.0	31.7
4-Nitrotoluene	3.6	99.3	86.0	99.5	29.5
3-Nitrotoluene	3.2	99.1	87.2	102.9	28.9
Pentaerythritol tetranitrate	–	31.9	69.6	96.8	–

* Means of replicated determinations; standard error, 2.6 (excluding picric and styphnic acids, part of which were not retained in the initial loading of the column; and the isopentane results, which are single determinations).

** 1,3,5,7-Tetranitro-1,3,5,7-tetrazacyclooctane.

*** Less than 0.5%.

§ 1,3,5-Trinitro-1,3,5-triazacyclohexane.

dispersion and a reduction in solvent strength during the early stages of an elution by traces of water in the column, is in satisfactory correspondence to the value of 5.4 calculated in the usual way from the column parameters quoted under Experimental. For the desorption of most of the compounds from Porapak T, diethyl ether is a sufficiently strong eluent. All of the compounds are readily desorbed in acetonitrile. A valuable result is the desorption of some compounds by isopentane despite the hydrated state of the column owing to the previously used aqueous methanol. The compounds desorbed are predictable from their distribution coefficients in n-hexane[7], to which their recoveries are inversely related. The recoveries increase with larger volumes of isopoentane, the high volatility of which facilitates its removal with only slight loss of these relatively volatile compounds. This elution also serves to displace the aqueous solvent from the column void when the absence of water in the following eluates is of importance. Lipophilic materials are removed too.

In the adsorption of explosives Porapak T compares closely with Amberlite XAD 7, which has been applied in related work[3,4], but the Porapak is much the less fragile material. Single columns have functioned satisfactorily for up to 50 determinations, when a rise in the generally negligible back-pressure, which could not be

remedied by a reversal of the eluent flow, has necessitated the column's replacement. Although the use of a metering pump is convenient, and enables highly volatile eluents to be delivered accurately from a sample loop, the column could be charged and eluted manually with a syringe, or with a motor-driven syringe as described in some early microcolumn liquid chromatography work[10]. Because of the high mass transfer terms due to the relatively large particle sizes of the various adsorbents used in this work, low flow-rates are essential if the volumes of the eluates are to be minimized.

The restrictions of procedure 1 to robust adsorbents and to sample solutions free from particulate matter do not apply to the generally applicable procedure 2, although the latter gives slightly reduced recoveries for a given adsorbent because both the initial washing and the desorption steps are extractions rather than chromatographic elutions. This is shown in Table II for three adsorbents and a selection of compounds. The low recovery values in Table II can be increased appreciably if more than the indicated volumes of eluent are used, at the expense of reduced concentrations in the eluate. However, when heavily contaminated extracts are processed the reduced efficiency is offset in that any explosives compounds entrained in the lipophilic material often thrown out of solution when such samples are made aqueous may be saved by transfer to the adsorbent. Typically, in the case of nitroglycerin a 10% loss is averted in this way.

Applications and selectivity considerations

Because of its negative selectivity for nitrate esters[7], charcoal is the preferred adsorbent when only this class of explosives is of interest, as in the detection of firearms discharge residues from double-based (nitroglycerin-containing) propellants. Such instances represent most of the explosives-related cases received in forensic science work in this country.

Fig. 1 shows the results of some recovery experiments in which the samples, containing 1–10 ng of nitroglycerin, were aliquots of a pooled clothing extract each

TABLE II

RECOVERIES OF SELECTED EXPLOSIVES COMPONENTS (50 ng) FROM PORAPAK T, CHROMOSORB 104, AND CHARCOAL MICROCOLUMN CLEAN-UP TECHNIQUES (PROCEDURE 2)

Compound	Recovery (%)*		
	Porapak T (acetonitrile, 50 µl)	Chromosorb 104 (acetonitrile, 50 µl)	Charcoal (methanol, 100 µl)
RDX**	77.1	69.8	15.0
Ethyleneglycol dinitrate	52.7	70.2	72.6
Nitroglycerin	87.1	79.5	68.5
2,4-Dinitrotoluene	90.6	82.1	4.0
2-Nitrotoluene	90.9	85.7	11.2
Pentaerythritol tetranitrate	91.6	90.6	56.3

* Means of replicated determinations; standard error, 1.9.
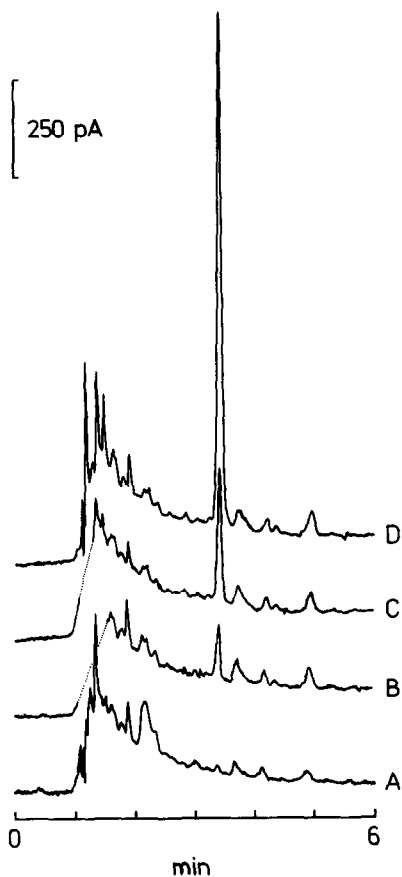** 1,3,5-Trinitro-1,3,5-triazacyclohexane.

Fig. 1. Chromatograms showing the recovery of nitroglycerin added to 100-μl aliquots of a clothing extract prior to clean-up with charcoal by procedure 2: no addition (A); 1 ng (B); 3 ng (C); 10 ng (D). Each aliquot was equivalent to 0.2 m² of the clothing surface. Further details are given in the text.

corresponding to debris collected from 0.2 m² of a moderately soiled outer garment. Procedure 2 was applied, with adsorption on charcoal and elution with methanol (50 μl). The clearly distinguishable peaks (Fig. 1) correspond to 0.102, 0.252, and 0.970 ng of nitroglycerin, and to recoveries of 51, 42, and 48%, respectively (one-fifth of each eluate was chromatographed). The recoveries are low relative to those in Table II because of the reduced volume of the elutions here.

　　　Fig. 2 refers to an outer garment worn during a 2.5-h car journey after the wearer (the driver) had discharged four rounds from a Smith and Wesson magnum revolver. An extract collected from part of the right sleeve (firing hand) was processed by procedure 2 with charcoal to give the chromatogram shown (B, Fig. 2) alongside the chromatogram of the original extract (A). Clearly, almost all of the detectable compounds in the original extract are removed by the treatment apart from nitro-glycerin, the peak of which in chromatogram B represents a recovery of 5 ng from the sampled area. Similar results are obtained several days after a firearm has been used[11].
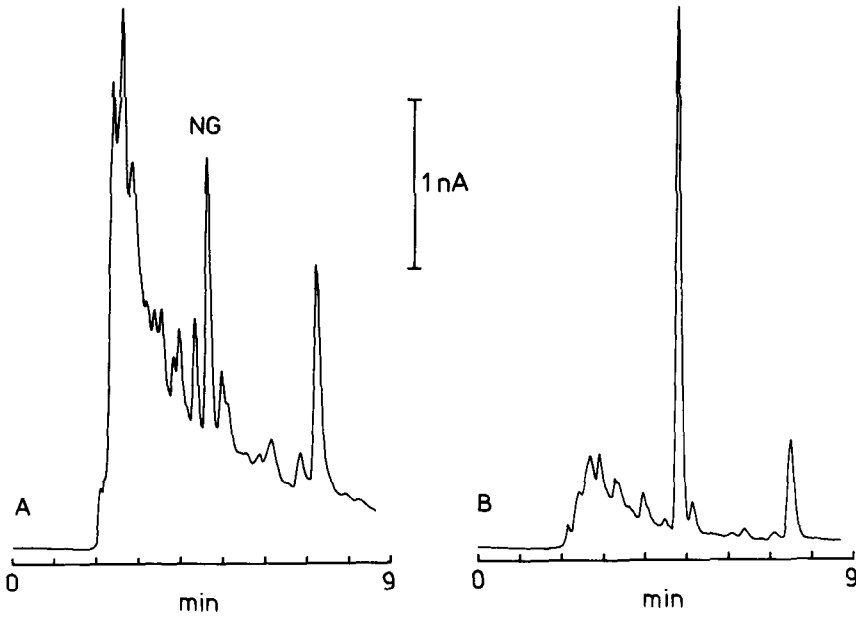
Fig. 2. Chromatograms of a clothing extract before (A) and after (B) clean-up with charcoal by procedure 2. The clothing had been worn whilst a Smith and Wesson magnum revolver was discharged (four rounds), and for 2.5 h afterwards. The peak coincident with nitroglycerin (NG) under the same chromatographic conditions is indicated on chromatogram A. Further details are given in the text.
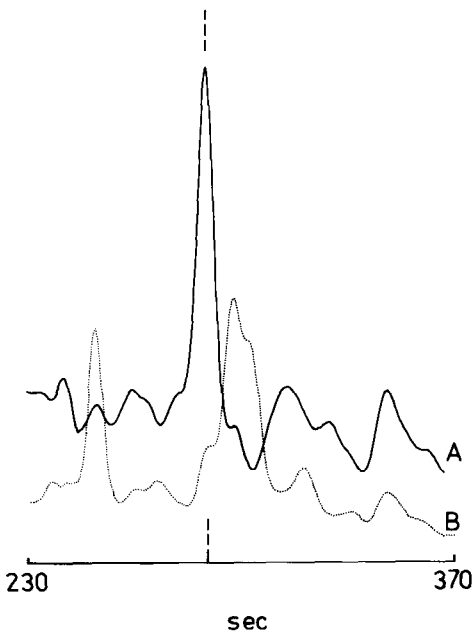

Fig. 3. Sections of the nitroglycerin region of chromatograms given by an extract of gelignite explosion debris before (B) and after (A) charcoal clean-up (procedure 2). The nitroglycerin position under the same chromatographic conditions is indicated by the broken lines. Further details are given in the text.

An example of the application of the same technique to extracts of gelignite explosions debris is shown in Fig. 3. In the expanded section of chromatogram B, from a non-selective clean-up procedure[5], any nitroglycerin present is almost completely obscured by adjacent peaks. However, a further clean-up (procedure 2, charcoal/methanol) gave chromatogram A with a well-resolved peak corresponding to 2.6 ng of nitroglycerin from 0.5 ml of the original extract.

Procedure 1 is useful mainly in the repetitive general-purpose recovery and concentration of explosives traces into small volumes of non-aqueous solvents from relatively uncontaminated solutions, e.g. the products of an initial clean-up or from HPLC eluates. The procedure enables any selectivity that may be available from the variation of the eluents to be exploited readily, as indicated for Porapak T in Table I. Although any mechanically robust adsorbent may be used, the selectivity of the suitable adsorbents is severely restricted, as is the selectivity of all of the generally
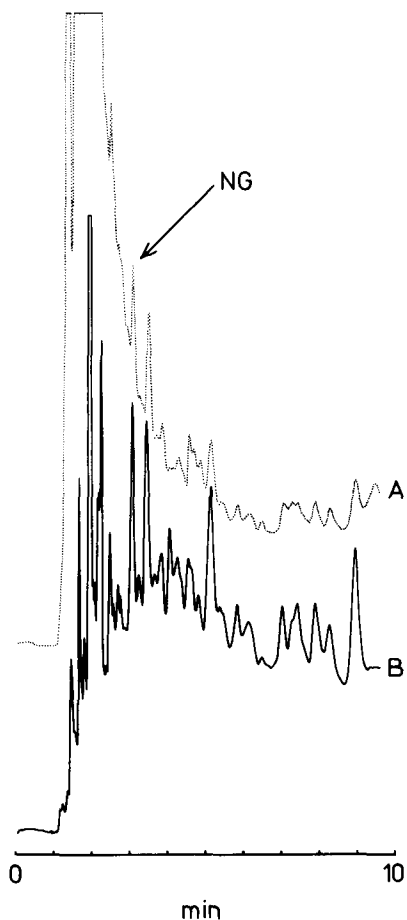
Fig. 4. Chromatograms of a clothing extract before (A) and after (B) clean-up with Porapak T by procedure 1. The clothing had been worn whilst a Smith and Wesson .22 revolver was discharged (five rounds) and for 1 h afterwards. The peak coincident with nitroglycerin (NG) under the same chromatographic conditions is indicated on chromatogram A. Further details are given in the text.

used types of polar adsorbent, the characteristics of which are usually correlated[7]. An example of the low selectivity from the practical use of Porapak T is given in Fig. 4, which shows chromatograms of an extract from 0.2 m² of an outer garment worn for 1 h after a firearm had been discharged (Smith and Wesson .22 revolver, 5 rounds). Although the extract after processing optimally for the majority of explosives compounds including nitroglycerin (diethyl ether elution, following isopentane) gave a chromatogram (B) in which was apparent at the nitroglycerin position a peak representing an approximately tenfold increase in concentration over that in the initial extract (A), very many non-explosives compounds were concentrated also. Even though the low selectivity of such adsorbents may be mitigated by the selectivity of other detection techniques, *e.g.* the thermal energy analyser[4], it remains that the adsorption is inherently unselective.

CONCLUSION

The described procedures enable the selectivity and sensitivity of detection in some important areas of explosives work to be considerably advanced. In particular, it is becoming increasingly apparent that contact with double-based firearms propellants residues may be detected more efficiently and very much more rapidly[11] than by the frequently applied scanning electron microscopy/energy dispersive X-ray analysis technique for inorganic primer residues[12]. Although one of the original objects was to develop readily applied HPLC screening techniques for propellants traces, analogous to the gas chromatography work of Jane *et al.*[8], given the incorporation into the described procedures of suitable confirmatory techniques, the characterization of organic explosives traces will become a mainstay of firearms residues detection.

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