

# Optimisation of solvent desorption conditions for chemical warfare agent and simulant compounds from Porapak Q<sup>TM</sup> using experimental design Part 2: Extraction of sulphur mustard from steel and glass Porapak<sup>TM</sup> tubes

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## Abstract

The vesicant chemical warfare (CW) agent sulphur mustard remains a hazard to personnel involved in demilitarisation activities. Sampling tubes containing Porapak Q<sup>TM</sup> are used to measure personal exposure to sulphur mustard vapour. Presented here is an evaluation of the solvent desorption parameters employed to remove sulphur mustard from steel and glass tubes containing Porapak Q<sup>TM</sup>. Statistical experimental design was used to elucidate the influence of solvent type, tube type, solvent volume and sonication time on sulphur mustard recovery. The order of increasing recovery was established as *iso*-octane < hexane = isopropyl alcohol. The same degree of sulphur mustard is recovered on both steel and glass tubes using hexane or isopropyl alcohol, with hexane exhibiting quantitative recovery. The sorbent mass (50 mg) should be increased when using steel tubes as breakthrough has been demonstrated. Given the inert nature of hexane towards sulphur mustard, its favourable chromatographic properties for splitless injection, and its greater recoveries, this solvent should be used for elution of Porapak Q<sup>TM</sup> tubes for quantitative analysis of sulphur mustard.

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## 1. Introduction and aims of investigation

Sulphur mustard (which has the NATO military designation H), is the common name for the liquid vesicant (blister) agent 1,1-thiobis(2-chloroethane). It causes large painful blisters filled with colourless to yellowish coloured liquid, which are formed at the points of dermal contact [1]. Effects may not be seen until 24 h after exposure.

H was first synthesised by Despretz in 1822 [2] and was first used by the Germans against allied troops near Ypres in Belgium in 1917. Since WW1, H has been stockpiled by many nations and used in further conflicts including the Iran–Iraq war [3–5].

Continual development of effective medical countermeasures, development of protective clothing and assessment

of exposure necessitate trace detection techniques. Several methods have been used to monitor atmospheric concentrations of H, which focus on its collection on a suitable sorbent material, such as Tenax TA<sup>TM</sup> or Porapak Q<sup>TM</sup>, prior to analysis. The H may then be desorbed from the tube using either thermal desorption (TD) [6–7] or solvent desorption (elution). Trace level determination of H is accomplished by thermal desorption at Porton Down [7]. However, the ‘one shot’ nature of this technique is a disadvantage [8]. TD instrumentation is now available to allow the recollection and reanalysis of samples, which will solve this problem [9].

Trials of equipment conducted at Dstl, Porton Down require replicate analysis of samples. To meet this requirement, H is currently analysed by gas chromatography with a flame photometric detector (GC-FPD). Historically H has been extracted from the sorbent tube with 4 ml of propan-2-ol (IPA) and a sonication time of 10 min for a glass tube. Sonication is not employed for steel tubes and eluate is collected

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in a pre-weighed vial. The use of alcohols for extraction of chemical warfare (CW)-related samples have occasionally lead to the formation of esters, which is undesirable [10]. Also, IPA is not ideal when non-polar columns are used for analysis with splitless injection. Therefore, the aim of this investigation was to develop and optimise an extraction method of H from Porapak Q<sup>TM</sup> tubes. Three solvents were investigated, IPA, hexane and 2,4,4-trimethylpentane (*iso*-octane), with different elution volumes on both steel and glass tubes. The sonication time required for the extraction from glass tubes was also investigated.

Examination of these parameters allowed a comparison of the behaviour of H towards its simulant compound, methyl salicylate (MS), and the report was published in a previous paper [11].

IPA was evaluated as it has been traditionally used to elute Porapak Q<sup>TM</sup> tubes at Porton Down. Hexane was investigated as a possible alternative to IPA for reasons mentioned above. *iso*-octane was evaluated due to its excellent chromatographic properties and relatively high boiling point potentially improving sample stability through reduced evaporation.

## 2. Experimental methodology

### 2.1. Mathematical model

Two separate mathematical models were developed in this study; a model for the recovery of H from Porapak Q<sup>TM</sup> using glass tubes, and a model using steel tubes.

The effect of three variables which potentially influence recovery of H from Porapak Q<sup>TM</sup> were considered for the experimental design. Solvent type, solvent volume and sonication time, in the case of glass tubes, were considered for each combination of solvent and tube type. As two of the parameters were categorical variables, a full factorial design (2<sup>3</sup>) could have been constructed. This would have resulted in 96 experiments being necessary to characterise the desorption behaviour of H. Thus, six central composite designs (CCDs) were constructed; a design for each combination of tube type and solvent type. Although this did not reduce the number of experiments performed (102), the CCD provided a clear interpretation of the effects of altering variables on the response (% recovery).

The CCD consists of a star design imposed through the centre of a factorial design. The principles and application of CCD are explained elsewhere [12]. An  $\alpha$ -value of 1.414 was used in the two parameter design and 1.633 in the three parameter design to ensure rotatability and orthogonality as calculated by Eq. (1). The upper and lower limits of each factor were placed on the axial points of the design.

$$\alpha = \pm(N_F)^{1/4} = \pm 1.414 \text{ or } 1.633 \quad (1)$$

where  $N_F$  is the number of experiments in factorial portion of design.

Table 1

Three-factor CCD used to investigate desorption behaviour of H from glass tubes

Factor	Levels				
	−1.631	−1	0	+1	+1.631
$x_1$ solvent volume (ml)	2.0	2.8	4.0	5.2	6.0
$x_2$ applied H mass ( $\mu\text{g}$ )	0.4	1.3	2.7	4.1	5.0
$x_3$ sonication time (min)	2.0	3.6	6.0	8.4	10.0

A CCD was created for glass tubes to consider solvent volume, sonication time and applied H mass (three parameter CCD). A second CCD was used for steel tubes to investigate the influence of applied H mass and solvent volume. Table 1 details the experimental domains used for each CCD. The form of the CCD models can be described in a generalised form by Eq. (2).

$$Y = \beta_0 + \sum \beta_j x_j + \sum \beta_{jj} x_j^2 + \sum \beta_{jk} x_j x_k \quad (2)$$

where  $Y$  is the response of system;  $x_{j,k}$ , variable of system;  $\beta_0$ ,  $\beta_j$ ,  $\beta_{jj}$ ,  $\beta_{jk}$ , regression coefficients for constant, linear, square and interaction terms, respectively.

Regression coefficients are calculated by fitting the values of experimental parameters to the least squares regression line. A quadratic equation or an equation containing only significant terms results. This can then be used to predict the response of the system at given levels of experimental factors.

### 2.2. Reagents

The H stock was prepared in-house with a purity of 99%. IPA and hexane (distol quality) were supplied by Fisher Scientific (UK) and used without further purification. *iso*-octane was purchased from BDH Laboratory Supplies (UK). Steel tubes were packed in house with 50 mg Porapak Q<sup>TM</sup> (50/80 mesh) supplied by Supelco, UK Glass Porapak Q<sup>TM</sup> tubes (100/50 mg) were purchased from SKC, UK and used without further conditioning.

### 2.3. Spiking of Porapak Q<sup>TM</sup> tubes with H

Stocks of 100 and 200  $\mu\text{g ml}^{-1}$  of H in *iso*-octane and hexane, respectively, were used to spike the tubes. Regarding IPA, the tubes were spiked with the hexane stock first and then eluted with IPA to minimise the chance of reaction between H and IPA. Each tube was connected to a personal sampling pump with a flow rate of 750 ml min<sup>−1</sup>. The pump was initialised and stock injected onto the packing to give the desired H mass on-tube. The pump was left running for 30 s, then the tube removed and the caps replaced. Once the tubes were spiked, they were eluted following the design quantities and analysed by GC-FPD. Note: although adequate for this experiment, a more realistic spiking method has been adopted by our laboratory now. Spiking tubes with a vapour loading rig is used as reported previously [11,12].

#### 2.4. Tube elution and GC-FPD analysis of eluates

Steel tubes were eluted by passing the solvent into a pre-weighed vial through a pipette tip attached to the tube with a clip. The vial was then weighed again after the elution was complete and the volume of solvent collected determined. Glass tubes were opened and all packing, including the slip, was poured into a vial. The solvent was added from an auto-dispenser and then sonicated for the specified time.

Analysis was carried out on a Hewlett Packard 6890 with an FPD. A DB-5 (15 m × 0.32 mm × 1 μm) capillary column was used with an initial GC oven temperature of 50 °C (IPA and hexane), which was maintained for 1 min, then increased by 50 °C min<sup>-1</sup> to 260 °C. This final temperature was held for 1 min giving a total run time of 6.20 min. For *iso*-octane-eluted tubes, the initial oven temperature was 70 °C. A filter with a wavelength of 394 nm was used to detect the sulphur atom of sulphur mustard. The FPD was operated at 250 °C with gas flows of H<sub>2</sub> at 75.0 ml min<sup>-1</sup>, air at 100 ml min<sup>-1</sup> and N<sub>2</sub> at 15.0 ml min<sup>-1</sup>.

#### 2.5. Peak identification

The only significant peak in the chromatograms obtained corresponded to H. The peak was identified by comparison with an authentic, single component H standard by the coinciding retention time of 3.7 min.

#### 2.6. Calibration of GC

Standards were prepared by diluting the H stock to produce concentrations of 0.032, 0.048, 0.08, 0.2, 0.4, 0.8, 1.2, 2.0 and 4.0 μg ml<sup>-1</sup>. H standards were prepared in *iso*-octane, IPA and hexane. Three replicates of each concentration were analysed in the same sequence as samples and the peak areas plotted against concentration to produce a quadratic calibration plot.

### 3. Results and discussion

#### 3.1. Optimisation of extraction conditions for steel and glass Porapak Q<sup>TM</sup> tubes

Mathematical models based on Eq. (1) were developed for each tube type and solvent. Full quadratic models were initially fitted to the experimental results then insignificant terms eliminated to derive reduced equations. All CCDs were performed at the 95% confidence limit. All terms in the reduced equations had regression coefficients, which were significant at this confidence level ( $p < 0.05$ ) and all reduced equations showed no evidence of lack of fit ( $p > 0.05$ ).

##### 3.1.1. Glass tubes

The reduced models for H recovery from glass tubes eluted with hexane, *iso*-octane and IPA are shown in Eqs. (3)–(5),

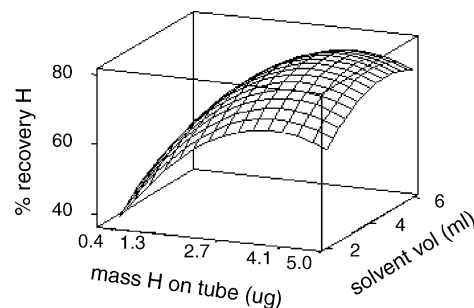


Fig. 1. Surface plot of H recovery from glass Porapak Q<sup>TM</sup> tubes using IPA.

respectively.

$$PA_H = 14.1 + 46.6x_1 - 6.0x_1^2 \quad (3)$$

$$PA_H = 8.8 + 14.4x_1 - 1.54x_1^2 + 19.5x_2 - 2.5x_2^2 \quad (4)$$

$$PA_H = -3.8 + 0.9x_1 + 3.2x_2 \quad (5)$$

where  $x_1$  is the solvent volume (ml);  $x_2$ , applied H mass (μg);  $x_3$ , sonication time (min).

The positive coefficients for  $x_2$  in Eqs. (4) and (5) indicate that recovery increases with applied H mass. The reduced recoveries at low concentration may be due to the adherence of H to the packing or walls of the glass tubes. Fig. 1 illustrates the recovery of H from glass tubes using IPA.

Eluates from each experiment were examined for the presence of thiodiglycol (TDG), the main hydrolysis product of H, which may account for decreased H recovery. Eluates were reduced to dryness and treated with trimethylsilyl chloride [13]. The bis-trimethylsilyl derivative of TDG was also prepared and its retention time established using GC-FPD. No TDG or associated hydrolysis products were detected supporting the theory of adsorption of intact H on Porapak Q<sup>TM</sup> or glass walls of the tube at low concentrations.

The desirability function [12,14] was applied to optimise desorption conditions. Maximum recoveries of H were obtained when using 4 ml of hexane or IPA with an applied H mass of 5 μg. Interestingly sonication time ( $x_3$ ), over the range investigated, did not have a statistically significant effect on the recovery of H using any of the solvents with glass tubes. Therefore, this parameter was set to its centre point value (6 min).

##### 3.1.2. Steel tubes

The reduced models for steel tubes are shown in Eqs. (6) and (7) for hexane and IPA, respectively. No full quadratic or reduced model could be obtained for *iso*-octane and hence, that is excluded from further discussion.

$$PA_H = 129 - 9.8x_2 + 1.0x_2^2 \quad (6)$$

$$PA_H = 124 - 14.5x_2 - 13.2x_2^2 + 3.6x_1x_2 \quad (7)$$

In contrast to glass tubes, increasing the applied H mass on-tube leads to a decrease in recovery. Given that steel tubes were packed with 50 mg of Porapak Q<sup>TM</sup>, and the higher

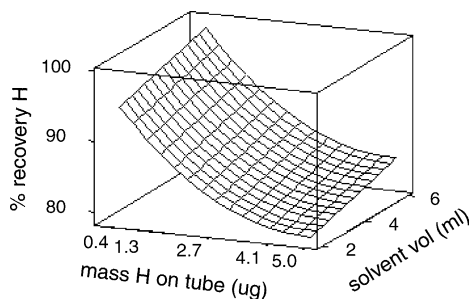


Fig. 2. Surface plot of H recovery from steel Porapak Q<sup>TM</sup> tubes using hexane.

mass of H present on tubes required injection of 25  $\mu\text{l}$  of the 200  $\mu\text{g ml}^{-1}$  standard, the decrease in recovery at high H masses may be caused by breakthrough of H using large injection volumes. Fig. 2 illustrates a decrease in recovery with increasing hexane volume. This suggests that small injection volumes of highly concentrated standards should be used while preparing standards or the sorbent mass in steel tubes should be increased for sampling and standard preparation. The incomplete retention of H on steel tubes appears reproducible as excellent calibration plots were obtained. Clearly for trace analysis all analytes should be retained on the tube during standard preparation and sampling, therefore, an increased sorbent mass should be used when steel tubes are employed for sampling H.

The interaction of solvent volume and applied H mass ( $x_1, x_2$ ) in Eq. (7) suggests that the elution-volume of IPA is important. The absence of this term in Eq. (6) suggests hexane is a more efficient solvent for the removal of H from steel tubes. This suggests non-polar interactions are of importance while removing H from Porapak Q<sup>TM</sup>.

The optimum conditions for recovery of H from steel tube vary with solvent used. The 0.4  $\mu\text{g}$  eluted using between 2 and 6 ml hexane gives quantitative recoveries. A solvent volume of 6 ml IPA gives quantitative recovery of 0.4  $\mu\text{g}$  H on-tube.

We have previously reported [11] that the H simulant compound methyl salicylate was also more efficiently eluted from Porapak Q<sup>TM</sup> tubes using hexane. The optimised desorption condition for MS was 4 ml of hexane for both glass and steel tubes, with a maximum of 86% of MS being recovered. Clearly, H is recovered quantitatively in this study, which illustrates a difference in the efficiency of recovery between the two compounds. A statistically significant difference was observed for recoveries of MS from glass and steel tubes with steel tubes giving the highest recoveries. However, as both compounds are eluted using the same optimum conditions with the same solvent, the adsorption/desorption mechanism is similar. This supports the use of MS as a simulant for H from a sampling perspective.

### 3.2. Model validation

Subsequent to the central composite data being generated, validation data were collected to test the models calculated

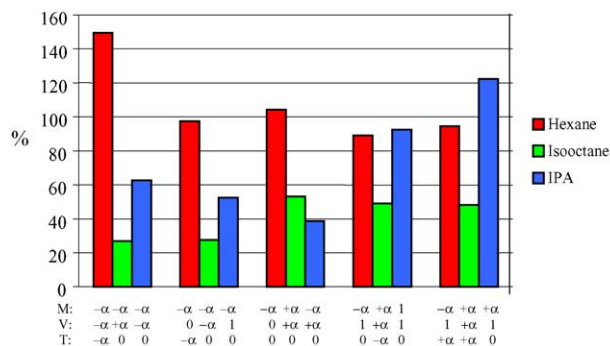


Fig. 3. Percent agreement between model predicted and observed values for H recovery from glass tubes with solvent type. M: mass H on-tube; V: solvent elution volume and T: sonication time.

by the optimisation function. Various combinations of concentration and solvent volume were selected and then three replicates of each combination were performed. The average of the three replicates was then taken to be the true observed recovery value and in this way, the model was tested for accuracy. Validation data were produced for each solvent and for both glass and steel tubes.

The regression coefficients for each model were used to predict the recovery given a by concentration and solvent volume value. In the case of glass tubes, the sonication time was set at its centre point value of 6 min. The predicted value was divided by the observed value to calculate the percentage recovery. This allowed the comparison of agreement between the true observed value and the predicted value. There were five sets of test values for each combination of tube type and solvent type.

#### 3.2.1. Glass tubes

Fig. 3 shows a bar plot of the percentage agreement for each of the five variable combinations used in the validation data and for each of the three solvents. The vector of numbers underneath each bar indicates the level (from  $-\alpha$  to  $+\alpha$  as indicated in Table 1) of each factor used to obtain the recovery for each solvent in each of the validation experiments.

As can be seen from Fig. 3 the model for hexane gives the best agreement between predicted and observed values. The hexane model appears to fit best for the higher solvent volumes. The model for *iso*-octane appears to have the least predictive power of the three.

#### 3.2.2. Steel tubes

Fig. 4 shows a bar plot of the percentage agreement for each of the five cases used in the validation data and for each of the three solvents for the steel tubes. The vector of numbers beneath each bar indicates the level (from  $-\alpha$  to  $+\alpha$  as indicated in Table 2) of each factor used to obtain the recovery.

As can be seen in Fig. 4, the models for both hexane and *iso*-octane appear to have a reasonable amount of predictive power. The model for isopropyl alcohol however appears to

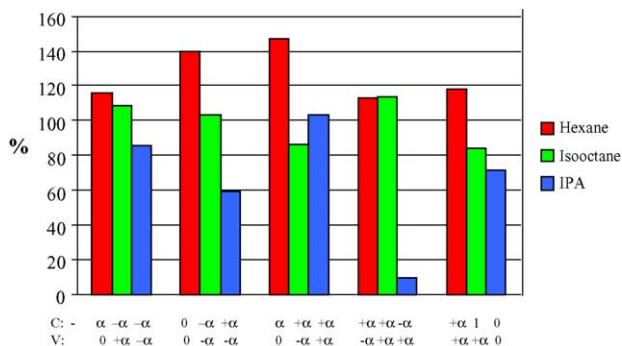


Fig. 4. Percent agreement between model predicted and observed values for H recovery from steel tubes with solvent type. M: mass H on-tube and V: solvent elution volume.

Table 2  
Two-factor CCD used to investigate desorption behaviour of H from steel tubes

Factor	Levels				
	-1.414	-1	0	+1	+1.414
$x_1$ solvent volume (ml)	2.0	2.6	4.0	5.4	6.0
$x_2$ H mass(μg)	0.4	1.1	2.7	4.3	5.0

have less predictive power, particularly for those cases when concentration and solvent volume is either at the lowest level or at the highest level.

### 3.3. Recovery of H from steel and glass Porapak Q<sup>TM</sup> tubes

Fig. 5 summarises the recoveries of H from steel and glass packed Porapak Q<sup>TM</sup> tubes using each solvent. It can be seen that hexane and isopropyl alcohol give similar recoveries on both tube types, while *iso*-octane gives poor recovery. An analysis of variance was performed for the effect of tube type and solvent type on recovery, adjusting the concentration and solvent volume. Ninety-five percent confidence intervals were derived for the difference between glass and steel tubes for all three solvents and for the pair-wise differences between the solvents for each tube type. These confidence intervals were calculated using Bonferroni adjustments for multiple comparisons.

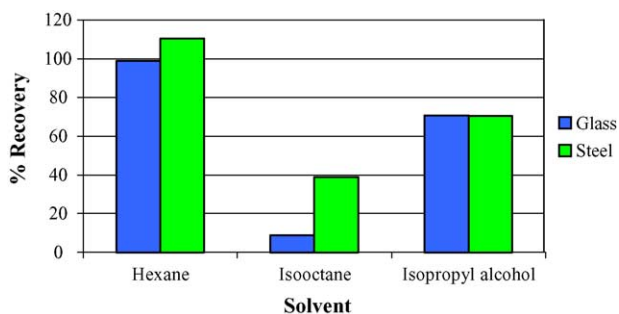


Fig. 5. Percent recovery of H from glass and steel tubes with solvent type.

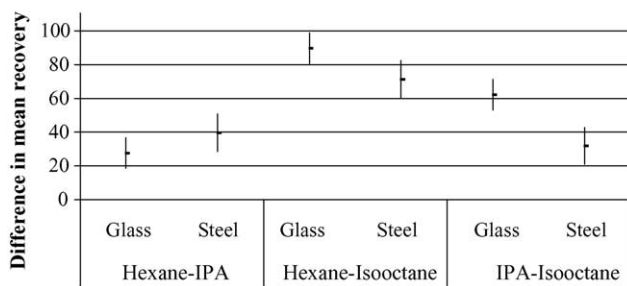
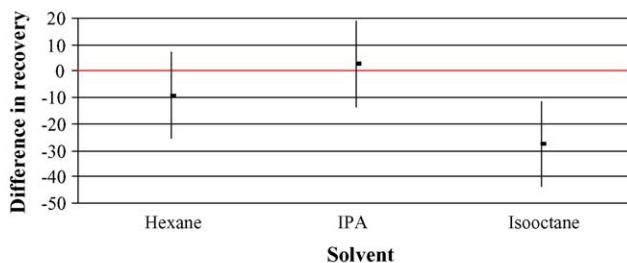


Fig. 6. Plots of 95% confidence intervals for the difference between solvents, and for the pair-wise differences (ANOVA) between the three solvents for each tube type.

Fig. 6 shows plots of these confidence intervals. It can be seen from Fig. 6 that there is only a statistically significant difference at the 5% level between glass and steel tubes when *iso*-octane is used. All pair-wise differences between solvents were not statistically significant for both glass and steel tubes. Therefore, IPA and hexane give statistically similar recoveries. However, this is due to the relatively high degree of variance in the results using both solvents. In practice, hexane gives recoveries, which are around 100% whereas IPA gives recoveries of around 75%, and therefore, hexane should be used as the elution solvent.

## 4. Conclusions and recommendations

Statistical experimental design has allowed the development of mathematical models to explain the influence of key parameters in desorption of sulphur mustard from glass and steel tubes packed with Porapak Q<sup>TM</sup>. *iso*-octane shows poor recovery on both tube types, but the highest (40%) value is obtained when using steel tubes. Hexane and IPA show similar recoveries on both tube types with hexane exhibiting quantitative recovery of H from both types of tube with greater precision. The sorbent mass should be increased when using steel tubes as breakthrough is suspected when 50 mg Porapak Q<sup>TM</sup> is used.

The experimental results of this study confirm common sense. The inert nature of hexane towards sulphur mustard, its favourable chromatographic properties for splitless injection, and its greater recoveries imply that, this solvent should be used for elution of Porapak Q<sup>TM</sup> tubes for quantitative analysis. This recommendation is also consistent with the optimum conditions required for the desorption

of the sulphur mustard simulant, methyl salicylate, from Porapak Q<sup>TM</sup>.

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