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Methods for the analysis of thiodiglycol sulphoxide, a metabolite of sulphur mustard, in urine using gas chromatography–mass spectrometry

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ABSTRACT

Two methods have been developed for the analysis of thiodiglycol sulphoxide, a metabolite of sulphur mustard, in urine. The first method recovers thiodiglycol sulphoxide from urine by extraction from a solid absorbent tube and clean up on Florisil. In the second method thiodiglycol sulphoxide is reduced to thiodiglycol with acidic titanium trichloride prior to extraction. This method detects thiodiglycol, thiodiglycol sulphoxide, and their acid-labile esters, as the single analyte thiodiglycol. In both cases the recovered analytes were converted to the bis(pentafluorobenzoyl) derivative of thiodiglycol and detected by gas chromatography–mass spectrometry using negative ion chemical ionisation. The limits of detection were 1 ng per 0.5-ml sample of urine. Urine from five normal human subjects showed low background levels of thiodiglycol sulphoxide in the range 2–8 ng/ml. However, a sixth subject was found to be excreting levels of thiodiglycol sulphoxide as high as 36 ng/ml. The first method has been used in toxicokinetic studies of sulphur mustard and the second method is intended to be used for the retrospective confirmation of mustard poisoning in casualties of chemical warfare.

INTRODUCTION

The use of sulphur mustard, 1,1′-thiobis(2-chloroethane), in the Iraq–Iran conflict [1–3], plus the negotiations towards a verifiable chemical weapons treaty, have lead to renewed interest in analytical methods for the retrospective confirmation of sulphur mustard poisoning [4–9]. There is currently no unambiguous forensic method to support a medical diagnosis of mustard poisoning in casualties believed to have been exposed to the agent. The detection of unchanged sulphur mustard in body fluids, such as blood or urine, is unlikely because of its extensive metabolism, rapid hydrolysis and general reactivity with nucleophiles present in proteins and nucleic acids. It has been assumed that an important excretion product following systemic absorption of sulphur mustard is its simple hydrolysis product thiodiglycol (2,2′-thiobis-ethanol). Early metabolism studies [10,11] suggested that excreted thiodiglycol, plus unidentified acid-labile conjugates which release it on treatment of urine with hydrochloric acid, may account for around 15% of an injected dose of sulphur mustard in the rat. Methods for the analysis of thiodiglycol in urine have been reported by Wils and co-workers [7,8] and by Black and Read [9]. The method of Black and

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Read, which is more specific for thiodiglycol, was used to determine the quantitative elimination of thiodiglycol in rat urine following cutaneous administration of sulphur mustard [12]. Somewhat surprisingly, it was found that excreted thiodiglycol accounted for <0.3% of an applied cutaneous dose of sulphur mustard, and, even after treatment of urine with hydrochloric acid, the amount of thiodiglycol detected in urine over the first nine days following application of the agent accounted for only 1 to 1.5% of the applied dose. This amount was around an order of magnitude lower than had been expected from the reports of early metabolism studies [10,11]. More recent metabolism studies at this establishment [13] have shown that thiodiglycol itself is not the major excretion product derived from the hydrolysis of sulphur mustard. Metabolic oxidation on sulphur converts it to its sulphoxide (2,2′-sulphinylbis-ethanol) which has been consistently observed as a urinary metabolite of sulphur mustard in the rat [13]. Thiodiglycol sulphoxide was also shown to be the major urinary metabolite following intravenous administration of thiodiglycol in the rat [13].

In this present paper we report two analytical methods for the recovery of this very polar, water-soluble metabolite from urine and its quantitative determination. The first method is specific for thiodiglycol sulphoxide and recovers it from urine unchanged. We have applied this method to the quantitative measurement of the urinary excretion of thiodiglycol sulphoxide in the rat following administration of sulphur mustard. However, the method is not ideal for the purpose of retrospective identification of mustard poisoning because it requires the extraction of urine from a solid absorbent using relatively large volumes of polar solvent, a procedure which also recovers large amounts of extraneous materials. This results in faster column degradation and increased chemical noise in the selected ion current chromatograms (see Results and Discussion). The recovery of thiodiglycol on the other hand is easier and gives much cleaner selected ion current chromatograms [9]. We therefore sought an additional method in which the sulphoxide is reduced to thiodiglycol prior to extraction, and which would allow the simultaneous determination of both excretion products as the single analyte thiodiglycol. The second method reported below employs acidic titanium trichloride solution to reduce thiodiglycol sulphoxide to thiodiglycol. This method therefore also detects thiodiglycol, acid labile esters of thiodiglycol and its sulphoxide, and any other metabolites of sulphur mustard which might convert to thiodiglycol under these conditions. It is intended to be used for the retrospective identification of sulphur mustard poisoning rather than for the specific analysis of thiodiglycol sulphoxide. Both methods involve the conversion of the recovered analyte to the bis-pentafluorobenzoyl derivative of thiodiglycol and detection using gas chromatography (GC) combined with negative ion chemical ionisation mass spectrometry (MS), as reported previously by us for the analysis of thiodiglycol [9].

EXPERIMENTAL

Materials

Thiodiglycol (99%) was purchased from Aldrich (Gillingham, UK). [1,1,1′,1′-$^{2}H_4$]thiodiglycol was prepared by reacting sodium sulphide with ethyl bromoacetate to give diethyl thiodiglycollate, followed by reduction with lithium alumin-

ium deuteride [14]. Thiodiglycol sulphoxide, m.p. 113–4°C, was obtained by oxidation of thiodiglycol with 30% hydrogen peroxide in water; [1,1,1′,1′-$^2H_4$]thiodiglycol sulphoxide was prepared similarly from [$^2H_4$]thiodiglycol [14]. Standard solutions were made up in methanol at concentrations of 0.1–100 μg/ml.

Titanium trichloride (15% solution in 20–30% hydrochloric acid) was purchased from Aldrich. Pentafluorobenzoyl chloride (puriss) was purchased from Fluka (Glossop, UK) and pyridine (Regis derivatisation grade) from Phase Separations (Deeside, Clwyd, UK). Fisons (Loughborough, UK) Distol grade solvents were used, with the exception of toluene which was Aldrich HPLC grade.

Florisil Sep-Pak cartridges were purchased from Fisons and were conditioned with ethyl acetate before use. Chem Elut tubes (Analytichem International, Harbor City, CA, USA) were washed with methanol (3 × 5 ml) and dried in a vacuum oven at 60°C prior to use. All glassware was pretreated with Aquasil siliconising fluid (Pierce & Warriner, Chester, UK).

Samples of human urine were collected from laboratory volunteers. Samples of rat urine were collected from male Porton strain rats in the Biology Division, CDE.

Extraction and clean-up

Method A. Urine (0.5 ml), to which [1,1,1′,1′-$^2H_4$]thiodiglycol sulphoxide (50 ng) (or 25 ng) was added as internal standard, was absorbed onto a 3-ml Chem Elut tube. Some less polar extraneous materials were eluted with ethyl acetate–methanol 100:2 (3 × 5 ml) (from which thiodiglycol can be recovered if required) and the eluate discarded. The sulphoxide was then extracted by elution with ethyl acetate–methanol 100:7 (5 × 5 ml) into a 50-ml round-bottomed flask. After concentration of the eluate to dryness at 40°C using a rotary evaporator, the residue was loaded onto a Florisil Sep-Pak cartridge in acetone (2 × 2 ml). The cartridge was washed with chloroform–methanol (100:20) (2 × 5 ml), which was discarded, and then eluted with chloroform–methanol (50:50) (2 × 4 ml) into a 25-ml round-bottomed flask. The eluate was concentrated to dryness at 40°C on a rotary evaporator, the residue transferred to a 1-ml vial with methanol (2 × 0.5 ml) and the solution finally concentrated to dryness under a stream of nitrogen at 60°C prior to derivatisation.

Method B. To urine (0.5 ml) in a micro-centrifuge tube was added titanium trichloride solution (0.5 ml) and the mixture incubated at 40°C overnight (16 h) (or 75°C for 1 h). [1,1,1′,1′-$^2H_4$]thiodiglycol (50 ng) was then added as internal standard and the mixture transferred to a 3-ml Chem Elut tube connected in series to a Florisil Sep-Pak cartridge. The tube plus cartridge were eluted with ethyl acetate (5 × 5 ml) into a 50-ml round-bottomed flask. The solution was concentrated to *ca.* 1 ml at 30°C on a rotary evaporator, transferred to a 1-ml vial with methanol (2 × 0.5 ml) and concentrated to dryness under nitrogen at 40°C.

Derivatisation

To the dried residue was added pyridine (80 μl method A or 50 μl method B) and pentafluorobenzoyl chloride (20 μl method A or 10 μl method B). The mixture was vortexed and then stood at ambient temperature for 2 min. The solution was made up to 500 μl with toluene, vortexed and centrifuged. This procedure converts both thiodiglycol sulphoxide and thiodiglycol to the bis-(pentafluorobenzoyl) derivative of thiodiglycol.

GC–MS analysis

Analyses were performed using a Finnigan 4600 quadrupole GC–MS system with only minor modifications to the instrumental conditions reported previously [9]. The gas chromatograph was fitted with a 25 m × 0.22 mm OV-1701 bonded phase column (Thames Chromatography, Maidenhead, UK) or a 25 m × 0.22 mm BP 10 column (SGE, Milton Keynes, UK), film thickness 0.25 μm; carrier gas helium at 15 p.s.i. The oven was held at 90°C for 0.5 min then programmed from 90 to 230°C at 20°C/min, from 230 to 260°C at 3°C/min and finally held at 260°C for 1 min. Splitless injections (0.5 μl) were made with toluene needle flush; split delay 0.5 min; 2 mm I.D., injector liner; injector temperature 250°C. The transfer line and interface were at 260°C. The mass spectrometer was operated in the selected ion monitoring mode using negative ion chemical ionisation with methane as reagent gas; source pressure 0.8 Torr; source temperature 120°C; electron energy 150 eV; emission current 0.3 mA; electron multiplier 1300 V. Molecular ions m/z 510 and 514 (internal standard) were monitored, dwell time 0.2 s, total scan time 0.5 s. For additional confirmation the isotope ion at m/z 511 could also be monitored with a relative intensity (peak area) of 10–15% compared to m/z 510. The retention time for thiodiglycol bis-(pentafluorobenzoate) was *ca.* 12 min.

Quantitation was performed by comparing the computer integrated peak area for the ion m/z 510 at the appropriate retention time for thiodiglycol bis-(pentafluorobenzoate) with that for the analogous peak for m/z 514 derived from the tetradeuterated internal standard. Calibration curves were established for thiodiglycol sulphoxide and thiodiglycol standards, and for samples of human and rat urine spiked with thiodiglycol sulphoxide at concentrations of 0, 2, 5, 10, 25, 50, 75, 100, 150 and 200 ng/ml. These calibration curves were superimposable on curves constructed from standards after allowing for any background levels of the sulphoxide present in urine (see below) and the molecular weight difference between the sulphoxide and thiodiglycol. Quantitation for method A was therefore performed against a calibration curve constructed form thiodiglycol sulphoxide standards and for method B against a calibration curve constructed from thiodiglycol standards. For method B, spiking experiments were also performed using thiodiglycol.

RESULTS AND DISCUSSION

Recovery and clean-up

Method A. Thiodiglycol sulphoxide is considerably more polar than thiodiglycol, as is evident from its strong retention on normal-phase silica gel [15]. Conversely, it is not retained from aqueous solution by non-polar reversed phases such as C_{18}, nor by polymeric matrices such as the various XAD resins. Two possible alternative approaches to recovery were, (i) removal of anionic and cationic materials from urine using ion-exchange resins followed by concentration of the remaining solution to dryness, or, (ii) extraction with a polar solvent after absorption of urine onto a solid phase, as was used previously by us for the recovery of thiodiglycol [9] and other water soluble materials such as nivalenol [16], followed by clean-up on normal-phase chromatographic material. Attempts at recovery by concentration of deionised urine gave poor and very variable recoveries, even with the use of silanised glassware. Extraction from a solid absorbent was therefore adopted as the most fruitful approach.

Preliminary experiments indicated that the use of ethyl acetate as extracting solvent, as used for thiodiglycol [9], gave no recovery of the sulphoxide. The polarity of the eluting solvent for Chem Elut tubes can be increased up to a maximum of ethyl acetate-methanol (10:1) before breakthrough of the aqueous phase occurs, but the problem with highly polar elution is that as the polarity increases so does the amount of extraneous material extracted. A compromise therefore has to be made between recovery of the analyte and clean-up. Experiments indicated that for a solution of the sulphoxide in water (50 ng/ml) absorbed onto a 3-ml Chem Elut tube, 4 or 5 × 5 ml elutions with ethyl acetate–methanol (10:1) were required for near quantitative extraction. However, not surprisingly, these elution conditions when applied to urine recovered large amounts of extraneous materials, which may cause problems in the derivatisation stage and introduce significant chemical background into the selected ion current traces. Experiments using gradually decreasing amounts of methanol in the eluting solvent indicated that ethyl acetate–methanol (100:7) was the least polar mixture which gave more than 75% recovery. This extract when applied to urine then required additional clean-up. Silica and Florisil cartridges were compared for retention of the sulphoxide. Florisil was more retentive than silica and was selected to allow some separation of the sulphoxide from extraneous materials. The sulphoxide was not eluted from Florisil with chloroform–methanol (100:20), allowing some of the extraneous material to be removed. The sulphoxide was then recovered by elution with chloroform-methanol (50:50). Using this procedure recoveries of thiodiglycol sulphoxide, from 5 replicate samples of urine spiked at levels of 50 ng/ml, were 52, 52, 44, 47 and 50% respectively, average recovery 49% (coefficient of variation of 6.8%).

Although thiodiglycol is essentially excluded by this procedure, spiking experiments indicated that small amounts (*ca.* 7% from a 0.5-ml aliquot spiked with 50 ng) were recovered in the final eluate. This presumably arises from a small amount of thiodiglycol being bound more strongly to a few very polar sites in the Chem Elut tube and Florisil cartridge, which was eluted only with the more polar eluting solvent. However, since thiodiglycol is normally present in much lower amounts than the sulphoxide in urine from rats treated with mustard, the small amount recovered using this procedure was considered insignificant for the purpose of determining elimination profiles of the sulphoxide.

Method B. There are few reagents which will efficiently reduce sulphoxides to sulphides in aqueous solution. One possible reagent is titanium trichloride, as was used by Nishimura *et al.* [17] to reduce dimethyl sulphoxide to dimethyl sulphide prior to analysis using GC–MS. Preliminary experiments with thiodiglycol sulphoxide indicated that reduction with titanium trichloride in hydrochloric acid proceeded in high yield and was tolerant to a wide variation in reaction conditions. Reduction to thiodiglycol occurred in essentially quantitative yield (>98%) in less than 1 h at 75°C or within 16 h at 40°C. Reduction at 75°C had the slight disadvantage of some leakage of the acidic solution around the caps of the plastic micro-centrifuge tubes. For our purposes an overnight (16 h) reduction at 40°C was convenient and was adopted as the standard procedure. Optimum yields were obtained using an equal volume of reagent to 0.5 ml of urine. Since the reagent is highly acidic (pH <1) it should also hydrolyse any acid-labile esters of thiodiglycol, or of the sulphoxide, which may be present in urine. This was supported by the analysis of urine from rats which had been treated with sulphur mustard. Treatment of 0–24 h urine with acidic titanium trichlo-

ride released amounts of thiodiglycol which were up to 1.3 to 1.4 times greater than the total amounts of thiodiglycol and the sulphoxide determined separately after treatment of urine with hydrochloric acid. These experiments in fact suggested that there may be other metabolites which convert to thiodiglycol under these conditions: further details will be reported elsewhere. The titanium trichloride reagent did not affect the extraction of thiodiglycol from Chem Elut tubes or clean up on Florisil, although extraction on Chem Elut tubes was noticeably slower due to clogging of the top of the tube or the frit, presumeably with colloidal titanium salts.

Recoveries determined for replicate 0.5-ml samples of urine spiked with 50 ng of deuterated thiodiglycol sulphoxide were 68, 67, 61, 69 and 70% respectively, average recovery 67%, coefficient of variation 5.3%. These were similar to the recoveries (60–80%) determined previously for thiodiglycol [9].

Derivatisation

As reported previously [9] the derivatisation procedure results in reduction of the sulphoxide to the bis-(pentafluorobenzoyl) derivative of thiodiglycol. The conversion proceeds in essentially the same yield as from thiodiglycol itself. Reductions of sulphoxides to sulphides by electrophilic reagents have been reported previously and proceed by initial O-acylation of the sulphoxide group to form a sulphonium species which then eliminates an acyloxy moiety [18]. Due to the greater amount of extraneous material in the residue using method A, a larger amount of derivatising agent was required to ensure reliable derivatisation.

Quantitation

Method A. The procedure gave a linear calibration over the range 10–200 ng/ml for spiked urine with a correlation coefficient of 0.9991, slope 0.252. Slight curvature was observed at concentrations below 10 ng/ml: quantitation in this lower range was performed using calibration points at 0, 2, 5 and 10 ng/ml. Calibration curves for pure standards and for spiked urine were superimposable after adjusting for any low background levels of thiodiglycol sulphoxide present in the urine (see below). The limit of detection for urine was estimated as 2 ng/ml (1 ng per 0.5-ml sample) based on a signal-to-noise ratio of 3:1. However, as discussed below, background levels in normal control urine were usually higher than this. Fig. 1 shows the selected ion current trace for a normal sample of human urine containing a small amount of the sulphoxide, quantitated at 4 ng/ml. Fig. 2. shows selected ion current traces for the same urine spiked with the sulphoxide at 50 ng/ml. These selected ion current trace show considerably greater chemical noise than was observed for the analysis of thiodiglycol [9], reflecting the greater amounts of extraneous materials recovered from urine by the polar extracting/eluting solvents required to recover the sulphoxide. Urine sample volumes were accordingly limited to 0.5 ml to minimise any interference of extraneous materials in the derivatisation procedure. The method showed good precision. Six replicate determinations for a sample of urine, containing a background level of 7 ng/ml and spiked with 50 ng/ml of the sulphoxide, gave values of 58, 58, 58, 56, 62 and 60 ng/ml, average 59 ng/ml, with a coefficient of variation of 3.5% (standard deviation, $\sigma_{n-1} = 2.07$).

Method B. A calibration curve constructed from urine spiked with thiodiglycol sulphoxide was linear over the range 10–200 ng/ml with slight curvature in the 0–10

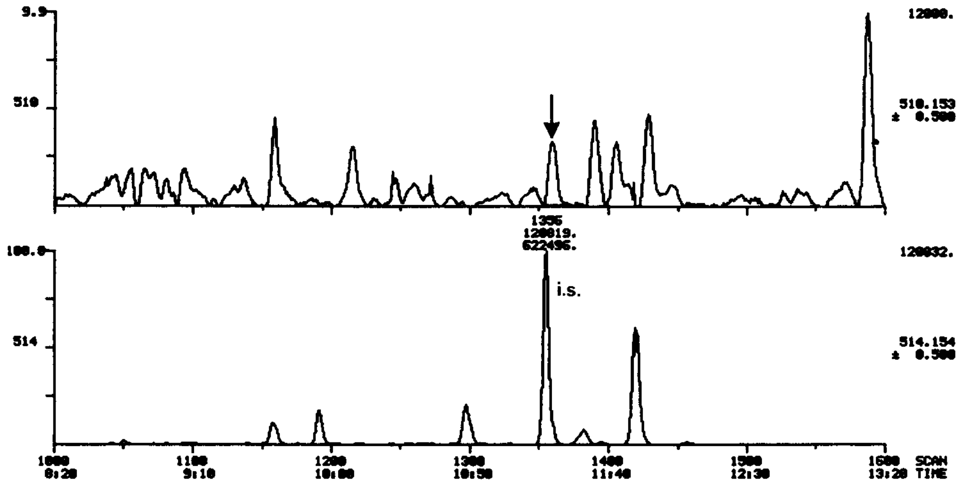

Fig. 1. Selected ion current chromatogram (method A) showing the presence of low levels (4 ng/ml) of thiodiglycol sulphoxide in normal human urine monitoring m/z 510 (upper) and the response to the internal standard (50 ng/ml) monitoring m/z 514 (lower). Time in min:s.

ng/ml range. Quantitation in this lower range was performed using calibration points at 0, 2, 5 and 10 ng/ml. Calibration points constructed from thiodiglycol standards lay on the same curve, shown in Fig. 3, after adjusting for any background level of the sulphoxide present in the urine and the molecular weight difference between thiodiglycol and the sulphoxide. Quantitation of unknowns was performed against a calibration curve constructed from thiodiglycol standards. The limit of detection was

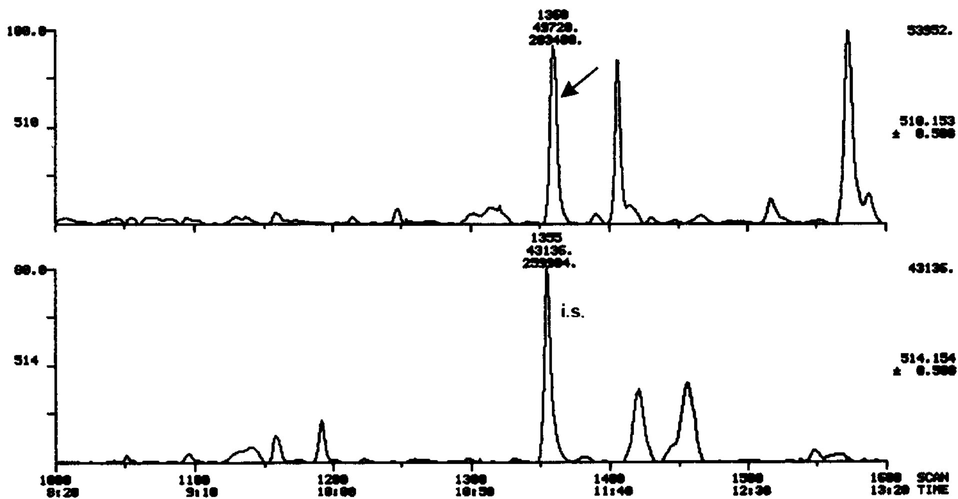

Fig. 2. Selected ion current chromatograms (method A) showing the detection of thiodiglycol sulphoxide (50 ng/ml), spiked into human urine, monitoring m/z 510 (upper) and the response to the internal standard (50 ng/ml) monitoring m/z 514 (lower). Time in min:s.

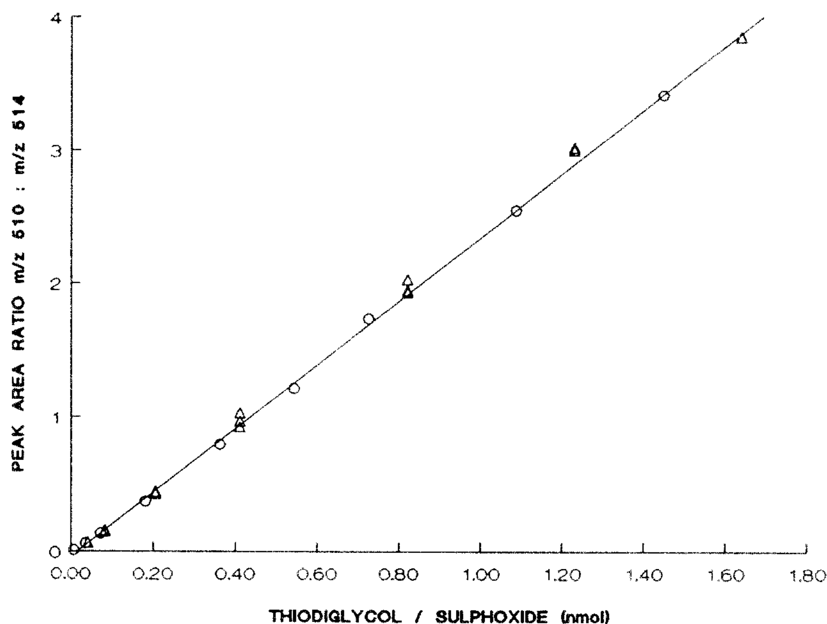

Fig. 3. Calibration curve for thiodiglycol sulphoxide in urine (adjusted for background level) using method B, and thiodiglycol standard. ○ = Thiodiglycol sulphoxide; △ = thiodiglycol.

1 ng per 0.5-ml sample. However, consistent with our observations with thiodiglycol sulphoxide using method A, normal control urine (rat and human), after reduction with titanium trichloride, contained a small background level of thiodiglycol, normal-

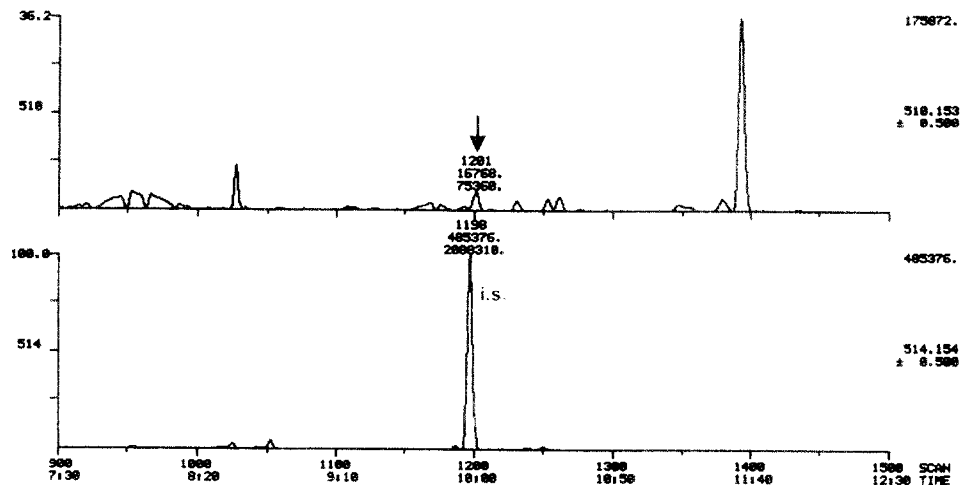

Fig. 4. Selected ion current chromatograms (method B) showing the presence of low levels (7 ng/ml) of analyte in normal human urine monitoring m/z 510 (upper) and the response to the internal standard (100 ng/ml) monitoring m/z 514 (lower). Time in min:s.

ly in the range 2–10 ng/ml (see further discussion below). Fig. 4 shows selected ion current chromatograms from a control sample of human urine with a background level of analyte determined as 7 ng/ml. Fig. 5 shows the same sample spiked with 20 ng/ml of thiodiglycol sulphoxide. Additional experiments indicated that the reducing agent did not interfere with the analysis of free thiodiglycol. The precision of the method was good. Six replicate determinations performed on rat urine, containing a background level of 6 ng/ml of thiodiglycol sulphoxide and spiked with 100 ng/ml of sulphoxide ($\equiv$93.7 ng/ml of thiodiglycol), gave values of 90, 92, 98, 98, 96 and 90 ng/ml, average 94 ng/ml, with a coefficient of variation of 4.0% (σ_{n-1} = 3.8). Because the method is intended to be used for the combined detection of thiodiglycol, thiodiglycol sulphoxide and their acid-labile esters, as evidence of mustard poisoning, [$^2H_4$]thiodiglycol was employed as internal standard and added after treatment with titanium trichloride. Experiments indicated no significant difference when [$^2H_4$]thiodiglycol sulphoxide was added as internal standard before treatment with titanium trichloride.

As referred to above, control samples of human urine contained a small background level of analyte using both of the methods described. Levels in urine from five different subjects were in the range 2–8 ng/ml, one subject being sampled on eight different occasions. Negative control samples of distilled water run prior to these samples were negative, thereby eliminating any possibility that the background levels reflected contamination of laboratory equipment. However, a sixth subject was found to be excreting levels of analyte determined as 32 and 24 ng/ml respectively using method B, when sampled on two different occasions. Analysis of the first of these samples separately for thiodiglycol [9] and for the sulphoxide indicated that this background level was due almost entirely to the sulphoxide. Levels of thiodiglycol

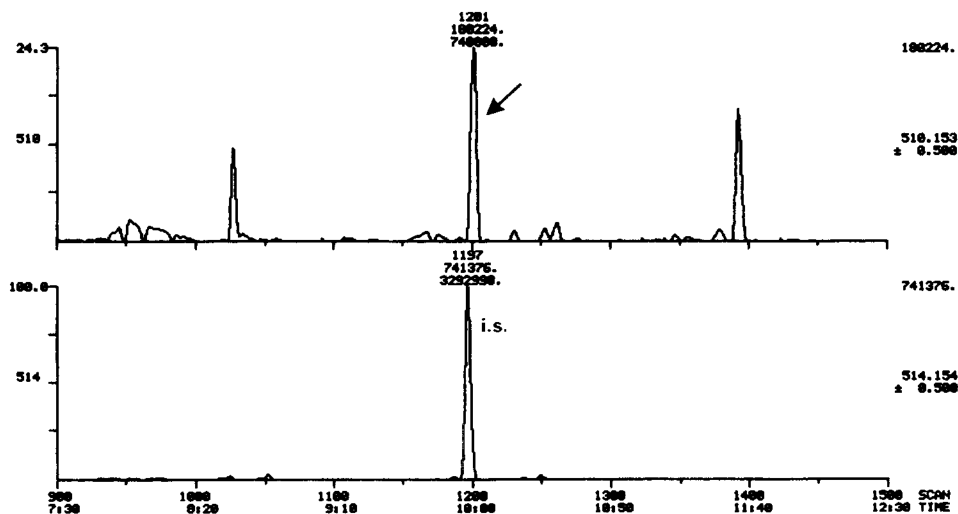

Fig. 5. Selected ion current chromatograms (method B) showing the detection of thiodiglycol sulphoxide (20 ng/ml), spiked into human urine, monitoring m/z 510 (upper) and the response to the internal standard (100 ng/ml) monitoring m/z 514 (lower). Time in min:s.

and the sulphoxide were determined as < 1 ng/ml and 36 ng/ml (= 32 ng/ml thiodiglycol), respectively. The detection of this background using both of the methods reported in this paper lends additional support to its identification as the sulphoxide rather than an unknown interferent. The chances of an interferent being present which has similar GC–MS characteristics to thiodiglycol after derivatisation, and which behaves like the sulphoxide before and after reduction, must be extremely remote. As we have discussed previously [9], the concentration of the ion current in the molecular ion with minimal fragmentation gives the analytical method great sensitivity but does present problems where additional confirmation of identity is required. The isotopic ion at m/z 511 can be monitored and shown to be within the ratio 10–15% (peak area) of the parent ion. We have also employed GC columns with a different selectivity to lend additional support to the identification [9]. Low levels of the sulphoxide (< 2–10 ng/ml) were also found in control rat urine.

In our previous work [9] we reported that normal urinary levels of thiodiglycol were < 1 ng/ml but that levels up to *ca.* 16 ng/ml were found in blood. These earlier results with urine contrasted with those of Wils and co-workers [7,8] who, using a method less specific for thiodiglycol, found background levels in control subjects mostly in the range equivalent to 1–10 ng/ml of thiodiglycol but in one case as high as 55 ng/ml [7] and in another 21 ng/ml [8]. Our findings with background levels of the sulphoxide are more consistent with those reported [7,8] for thiodiglycol. The procedure used by Wils and co-workers [7,8] converts thiodiglycol back to mustard by heating with concentrated hydrochloric acid. We thought that thiodiglycol sulphoxide might be reduced to thiodiglycol via a sulphonium species under these conditions, since reductions of sulphoxides with hydrochloric acid have been reported previously [18]. However, refluxing a solution of the sulphoxide with concentrated hydrochloric

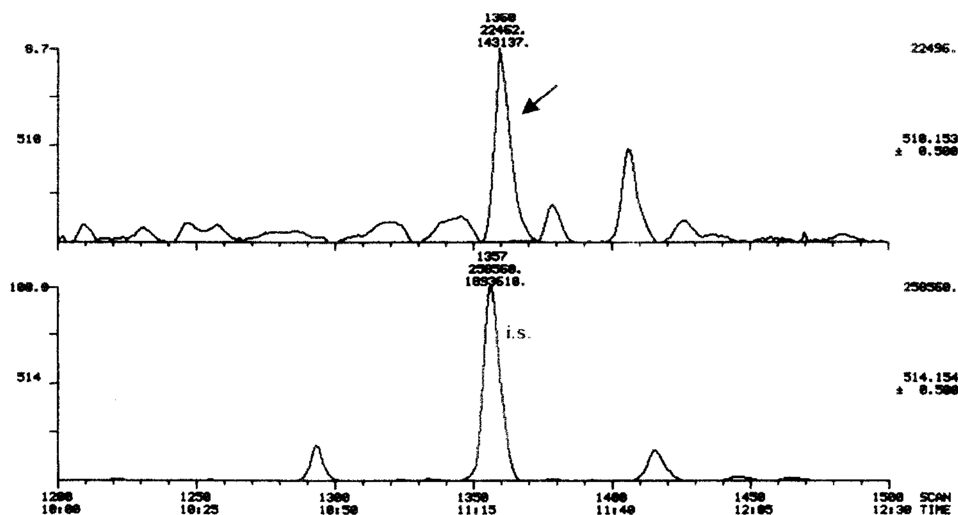

Fig. 6. Selected ion current chromatograms (method A) showing the detection of thiodiglycol sulphoxide (16 ng/ml) in rat urine, collected 8 days after a cutaneous application of 2 μmol of sulphur mustard, monitoring m/z 510 (upper) and the response to the internal standard (100 ng/ml) monitoring m/z 514 (lower). Time in min:s.

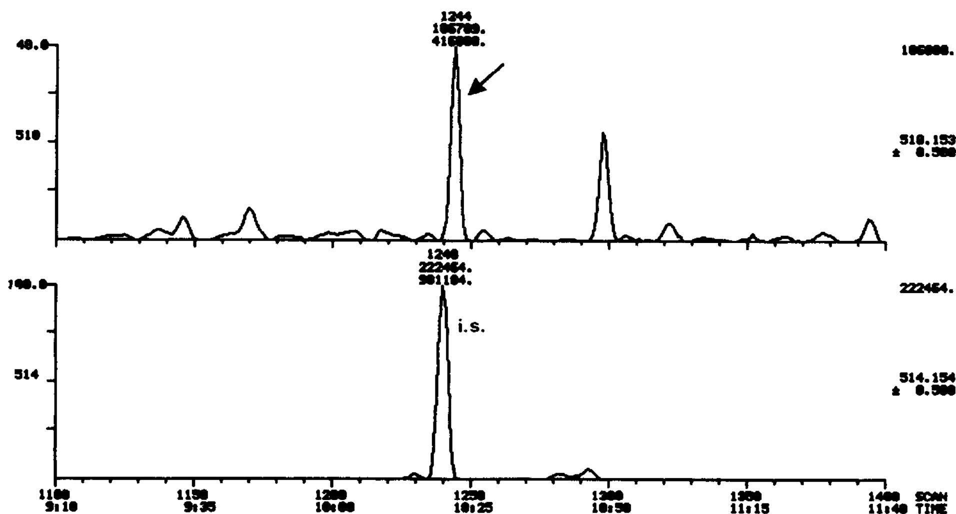

Fig. 7. Selected ion current chromatograms (method B) showing the detection of thiodiglycol (60 ng/ml) in rat urine after reduction with titanium trichloride, collected 8 days after a cutaneous application of 2 μmol of sulphur mustard, monitoring *m/z* 510 (upper) and the response to the internal standard (100 ng/ml) monitoring *m/z* (lower). Time in min:s.

acid for 1 h, and neutralisation with sodium hydroxide, did not produce any thiodiglycol.

The source of this low background level is unknown, but sulphur–rich foods in the diet is one possibility. A compound which was identified by Reichstein and Goldschmidt [19,20] in 1936 as thiodiglycol sulphoxide, on the basis of elemental analysis, melting point and comparison with a synthetic sample, was isolated from the saponified lipid fraction of the adrenal glands (1000 kg) of cattle. The source of the compound was unknown but the authors postulated that the sulphoxide was associated with lipids by esterification in the same way as glycerol. This explanation seems unlikely on the basis of known naturally occurring lipids, but there appears to be no evidence to refute it.

The applicability of the two methods to the detection of excretion products derived from sulphur mustard is shown in Figs. 6 and 7. Fig. 6 shows the detection of 16 ng/ml of thiodiglycol sulphoxide in rat urine, eight days after a cutaneous application of 2 μmol of sulphur mustard. Fig. 7 shows the detection of 60 ng/ml of thiodiglycol in rat urine, after treatment of urine with titanium trichloride using method B, also eight days after a cutaneous application of 2 μmol of sulphur mustard.

CONCLUSIONS

Sensitive methods have been developed for the detection and quantitation of thiodiglycol sulphoxide in urine. The limits of detection were 2 ng/ml of urine. The methods were validated by the analysis of spiked human urine samples and by the analysis of urine from rats which had been exposed to sulphur mustard. For the purpose of retrospective confirmation of mustard poisoning the second method is

preferred, which detects thiodiglycol sulphoxide, thiodiglycol, and their acid-labile esters, as the single analyte thiodiglycol. A complicating factor is the presence of low levels of analyte in samples of normal human urine. These were in the range 2–8 ng/ml in five subjects but were as high as 36 ng/ml in one subject.

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