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Determination of *N,N′,N″*-triethylenthiophosphoramide in biological samples using capillary gas chromatography

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Abstract

A sensitive assay for the determination of *N,N′,N″*-triethylenthiophosphoramide (thioTEPA) in microvolumes of human plasma and urine has been developed. ThioTEPA was analysed using gas chromatography with selective nitrogen–phosphorus detection, after extraction with ethyl acetate from the biological matrix. Diphenylamine is the internal standard. The limit of quantitation was 0.1 ng/ml, using only 100 μl of sample; recoveries ranged between 85 and 100% and both accuracy and precision were less than 10%. Using a flame ionisation nitrogen–phosphorus detector, the assay was not linear over the concentration range of 2–1000 ng/ml for plasma and 10–1000 ng/ml for urine. Linearity was accomplished in the range of 1–1000 ng/ml for plasma and urine when a thermionic nitrogen/phosphorous detector was used. The stability of thioTEPA in plasma proved to be satisfactory over a period of 3 months, when kept at −20°C, whereas it was stable in urine for at least 1 month at −80°C. ThioTEPA plasma concentrations of two patients treated with thioTEPA are presented demonstrating the applicability of the assay. © 1997 Elsevier Science B.V.

Keywords: *N,N′,N″*-Triethylenthiophosphoramide

1. Introduction

The cytotoxic drug *N,N′,N″*-triethylenthiophosphoramide (ThioTEPA, Fig. 1) has been applied in cancer therapy now for more than 40 years [1]. Interest in thioTEPA has been renewed by the finding that its dose can be increased dramatically when bone marrow toxicity is not dose-limiting, such as in the bone marrow transplantation setting. The highest dose of thioTEPA that can be given safely intravenously without hematopoietic stem cell support is 65 mg/m^2 [2]. Phase I studies of high-dose thioTEPA in the transplantation setting have demonstrated a maximal tolerated dose of 900–1125 mg/m^2 given in divided doses in 2-h infusions over 3 days [3]. Because of its broad spectrum of anti-tumor activity, thioTEPA is currently being employed in many high-

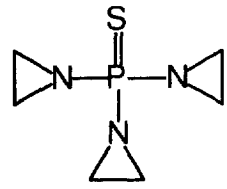

Fig. 1. Chemical structure of thioTEPA.

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dose chemotherapy regimens for breast cancer, ovarian cancer and other solid tumors [4]. In combination with cyclophosphamide and carboplatin, it may share responsibility for severe renal and hepatic toxicity in multi-cycle high-dose regimens [5], and pharmacokinetic monitoring in order to individualize dosing has been suggested as a strategy to prevent this. Despite the extensive use of thioTEPA, only little is known about the pharmacokinetics particular in high-dose regimens. To determine optimal dosing regimens in these high-dose settings, comprehensive pharmacokinetic studies are necessary. Analytical assays used in pharmacokinetic studies of thioTEPA in conventional dose regimens, are mostly based on methods described by Egorin et al. [6], Hagen et al. [7] or McDermott et al. [8]. Briefly, thioTEPA is extracted from the biological sample using either liquid–liquid extraction (LLE) [9] or solid-phase extraction (SPE) [8] followed by gas chromatography (GC) utilizing a selective flame ionisation nitrogen/phosphorous detector (FI-NPD). Detection limits of these assays range from 1 to 5 ng/ml, but need plasma or urine volumes of 400 μl or more. The intention of this study is to develop a more sensitive assay with a large dynamic, linear range for micro volumes (100 μl) of biological materials. This will reduce the volume of blood necessary, while samples could even be taken by a simple puncture in the tip of the finger. To gain the same detection limit a more sensitive method is necessary. Sensitivity can be increased by the use of a selective thermionic nitrogen/phosphorous detector (TI-NPD). The standard FI-NPD has been compared with the TI-NPD with regard to linearity and sensitivity.

2. Experimental

2.1. Chemicals

ThioTEPA was obtained from Cyanamid Benelux (Etten-Leur, The Netherlands). Diphenylamine, used as internal standard, was purchased from Baker Analysed® Reagent (Deventer, The Netherlands). All other chemicals used were of analytical grade unless otherwise specified.

2.2. Instrumentation

A gas chromatograph (HRGC 5300, Carlo Erba Strumentazion, Milan Italy) equipped with a split/splitless injector and flame ionization or thermionic N/P-selective detector (FI-NPD or TI-NPD, respectively) was used. Separation was performed using a 25 m×0.32 mm CP Sil-8 CB capillary column (Chrompack, Middelburg, The Netherlands) with a film thickness of 0.25 μm. The oven temperature was programmed as follows: starting temperature 140°C, ramped to 180°C with 5°C/min. Temperature was kept at 180°C for 2 min and ramped to 200°C with 20°C/min. The injector was kept at 250°C using a split ratio of 1:10 and the detector temperatures were 250 and 300°C for the FI-NPD and TI-NPD, respectively. Helium was used as carrier gas at a flow-rate of 3 ml/min. The gas flow-rates for the FI-NPD were: flame gases, hydrogen, 35 ml/min and air, 350 ml/min; make-up, 35 ml/min. Gas flow-rates used for the TI-NPD were: hydrogen, 4 ml/min; air, 160 ml/min; and make up, 13 ml/min. The current was set at 2.70 A and the voltage at 3.5 V. Mass spectrometry (MS) measurements were performed on a Varian GC–MS combination (Wallnut Creek, CA, USA). GC separation was performed on a 10 m×0.25 mm DB5-MS capillary column (J&W Scientific, California CA, USA) with a film thickness of 0.25 μm, using helium as carrier gas at a flow-rate of 1 ml/min. The oven temperature was programmed as follows: 1 min at 85°C, ramped to 280°C at 15°C/min. The temperature was kept at 280°C for 6 min. A temperature programmed injector was used, with the following program: 45 s at 80°C, ramped to 280°C at 100°C/min. The temperature was kept at 280°C for 6 min. MS measurements were performed on a Saturn-2 operating in positive chemical ionization (CI) mode, with isobutane as reaction gas and the multiplier was set at 2 kV.

2.3. Sample pre-treatment

Samples of 100 μl plasma or urine were transferred to polypropylene microcentrifuge tubes (7805 00, Brand, Wertheim, Germany). The same amount of water, 20 μl of a 20 μg/ml diphenylamine solution in methanol, 10 μl of a 1 *M* NaOH solution

and 500 μl ethyl acetate were added. The mixture was vortexed for 1 min and centrifuged for 5 min at 1100 *g*. The ethyl acetate layer was transferred into a 0.7-ml amber glass vial (Chromacol, Trumbull, USA) containing 40 μl 1-propanol and then evaporated under a nitrogen stream at room temperature to a final volume of approximately 20 μl; 1–2 μl were injected into the chromatograph.

2.4. Calibration

Calibration samples contain 100 μl drug-free human plasma or urine and were spiked with 30–100 μl of an appropriate amount of a thioTEPA solution in water. If necessary this amount was supplemented with water to a final volume of 200 μl. An aliquot of 20 μl of a 20 μg/ml diphenylamine solution in methanol, 10 μl of a 1 *M* NaOH solution and 500 μl ethyl acetate were added. The mixture was further treated as described in Section 2.3.

2.5. Validation

A three-run validation was completed for the determination of thioTEPA in both human plasma and urine. The following parameters were evaluated: linearity, within-run and between-run precision, accuracy, recovery and stability.

2.6. Linearity

Calibration curves ranging from 2 to 10 ng/ml in plasma, 10 to 100 ng/ml and 100 to 1000 ng/ml in plasma and urine, were each prepared from seven thioTEPA calibration standards. Samples were analysed in singular and the goodness-of-fit test [10] was used to evaluate the linearity of the calibration curve. The squared scaled residuals were calculated by Eq. (1):

$$Q = \sum_i [(y_i - a - bx_i)/s_i]^2 \quad (1)$$

in which $i = 1,n$; y_i is the detector response; x_i is the corresponding thioTEPA concentration; s_i is the standard error of y and n is the number of replicates. Q is distributed as χ^2 with $n-2$ degrees of freedom.

2.7. Accuracy and precision

Quality control samples containing 5, 50 and 500 ng/ml thioTEPA in plasma, and 50 and 500 ng/ml thioTEPA in urine, were prepared to determine the accuracy and precision. Five replicates of each quality control sample were analysed together with a standard curve in each run. The accuracy was calculated as the mean deviation (in percent) from the nominal concentration. The within- and between-run precisions were calculated by one-way analysis of variance (ANOVA) using the run day as the classification variable. The day mean square (DayMS), error mean square (ErrMS) and grand mean square (GM) were obtained from the ANOVA analysis. Within- and between-run precision were calculated from Eq. (2) and Eq. (3), respectively, where n is the number of replicates.

$$\text{Between-run precision} = 100 \times ((\text{DayMS} - \text{ErrMS})/n)^{0.5}/\text{GM} \quad (2)$$

$$\text{Within-run precision} = 100 \times (\text{ErrMS})^{0.5}/\text{GM} \quad (3)$$

2.8. Specificity

Plasma and urine samples of six different individuals were analysed to see if endogenous components co-eluted with thioTEPA or diphenylamine.

2.9. Recovery

The recovery of the thioTEPA extraction from plasma and urine was calculated by dividing the slope of the processed and non-processed calibration curves. The extraction efficiency of diphenylamine was determined by comparing the response of three processed samples to the response of three unprocessed samples at a concentration of 4 μg/ml.

2.10. Stability

The stability of thioTEPA has been studied at concentrations of 5, 50 and 500 ng/ml in plasma and urine during a period of 3 months at −20°C and 1

month at −80°C, respectively. Three replicates were analysed for each concentration level.

The stability of thioTEPA in 1-propanol during storage in the autosampler over a time period of 24 h has been studied at a concentration of 50 ng/ml.

2.11. Pharmacokinetics

The method described above was used to study the thioTEPA plasma concentration of two patients treated with 80 and 120 mg/m^2 thioTEPA, respectively. Immediately after thioTEPA administration the patients received cyclophosphamide as part of the chemotherapy regimen. Blood samples were obtained before the start of the treatment, at the end of a 30 min infusion, 1, 3–4 and 24 h after thioTEPA administration. Samples were centrifuged and plasmas were stored at −20°C and were analysed within 2 weeks.

2.12. GC–MS

GC–MS measurements were performed on the plasma and urine samples of the patients to verify the identity of the putative thioTEPA peaks in the chromatograms. Therefore an amount of 1.5 ml of a plasma sample containing 5 μg/ml thioTEPA and 6 ml of an urine sample containing 0.25 μg/ml thioTEPA were extracted with ethyl acetate. The organic layer was removed and evaporated to a final volume of 100 μl with a concentration of 75 and 15 μg/ml for plasma and urine, respectively; 1 μl was injected.

3. Results and discussion

3.1. Extraction

The extraction procedure with ethyl acetate has proved to be satisfactory in pharmacokinetic studies of thioTEPA [6,9,11]. Aliquots of 400 μl plasma or more were used for these extractions. Also solid-phase extraction of thioTEPA requires approximately 1 ml of biological specimens [8,12,13]. In this study an ethyl acetate extraction was applied using 100 μl of the biological matrix.

3.2. Chromatography

Fig. 2 shows chromatograms of plasma and urine samples of a patient before and after treatment with thioTEPA. The concentration of thioTEPA was 5741 ng/ml in plasma and 256 ng/ml in urine. The chromatographic conditions provide good separation between thioTEPA and diphenylamine. Retention times of thioTEPA and diphenylamine were 4.4 and 5.7 min, respectively. Endogenous materials of plasma or urine gave no interferences with the analysis (Fig. 2A and Fig. 2C, respectively). Cyclophosphamide, a cytotoxic drug co-medicated in the high-dose regimen of thioTEPA, was also extracted from the biological matrix and gave two peaks with retention times of 11.4 and 12.8 min, due to the thermal instability of the drug [14,15]. The two peaks were identified by GC–MS as dehydrohalogenated cyclophosphamide (11.4 min), formed by intramolecular alkylation and cyclophosphamide (12.8 min). Thus far, one peak eluting prior to thioTEPA (Fig. 2D, 3.3 min) could not be identified by GC–MS.

3.3. Validation

For the validation of the described assay the GC system was equipped with the FI-NPD. The TI-NPD and FI-NPD detectors were compared with regard to linearity and sensitivity.

3.4. Linearity

Calibration curves are measured in decades: 2–10 ng/ml, 10–100 ng/ml and 100–1000 ng/ml, obtaining correlation coefficients (r^2) of 0.995 or better, using the FI-NPD. The linear relationships were evaluated by least-squares regression analysis and by the goodness-of-fit test. Performing the goodness-of-fit test, P values <0.05 were obtained for calibration curves in the range of 2–1000 ng/ml, stating a non-linear relationship. Linearity of the assay can be achieved by dividing the calibration curve in decades, resulting in P values >0.05. For every calibration curve, concentrations were back-calculated from the peak areas and the average values are presented in Table 1 for plasma and Table 2 for urine. The overall calibration curve gave a linear relationship with correlation coefficients of 0.9999 or

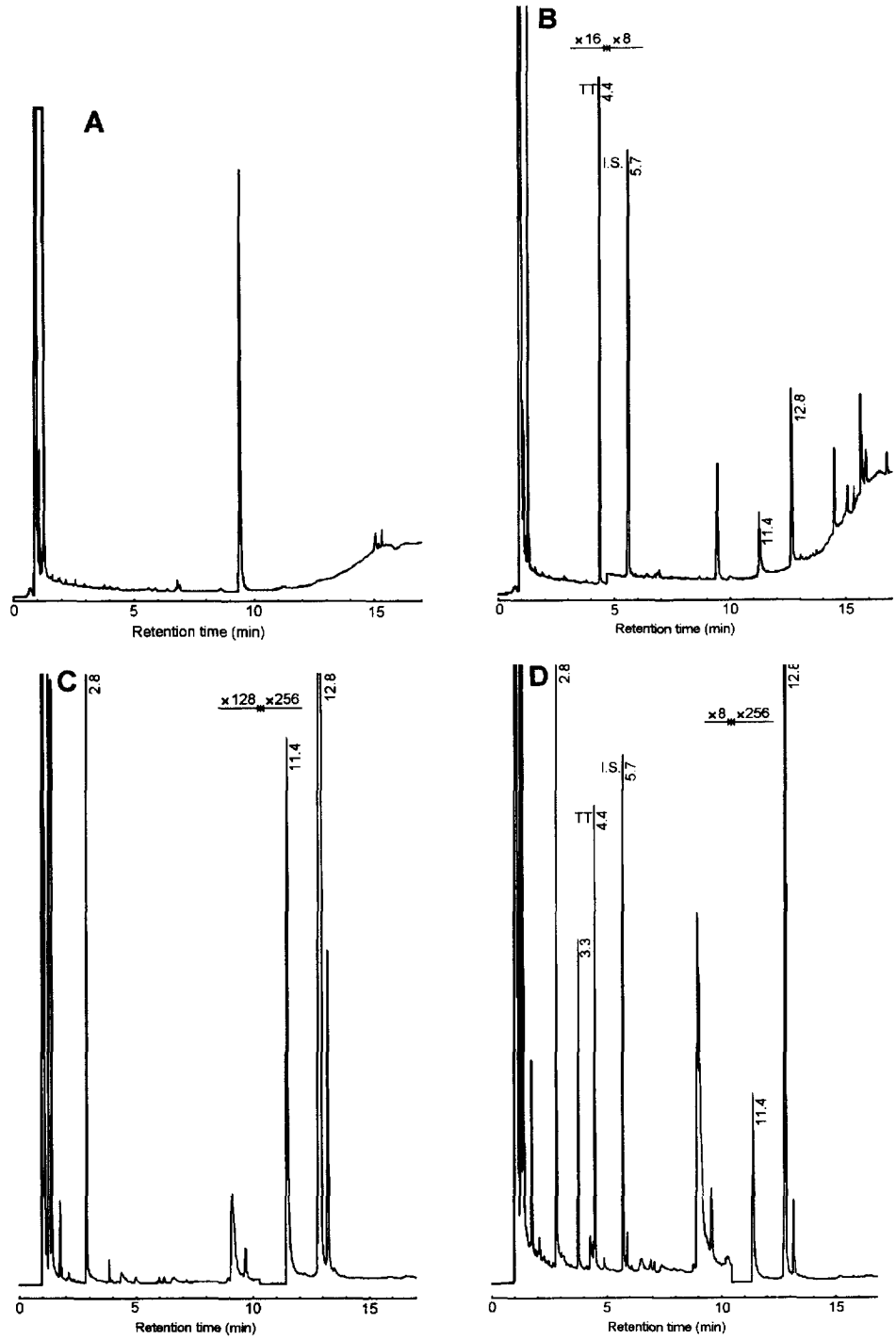

Fig. 2. (A) Patient plasma sample before treatment with thioTEPA. (B) Patient plasma sample after treatment with thioTEPA, at a concentration level of 5741 ng/ml. Peaks corresponding with retention times of 4.4 and 5.7 are thioTEPA and diphenlyamine, respectively. Peaks eluting at 11.4 and 12.8 min originate from cyclophosphamide. (C) Patient urine sample before treatment with thioTEPA. (D) Patient urine sample after treatment with thioTEPA, at a concentration level of 256 ng/ml. Peaks corresponding with retention times of 4.4 and 5.7 are thioTEPA and diphenlyamine, respectively. Peaks eluting at 11.4 and 12.8 min originate from cyclophosphamide.

Table 1
Calibration concentrations back-calculated from peak areas for the determination of thioTEPA in plasma, measured by GC–FI-NPD

Concentration (ng/ml)	Calibration concentration (ng/ml)				C.V. (%)	RCR (%)
	Run 1	Run 2	Run 3	Mean		
2.0	2.1	1.8	2.2	2.0	10.2	—
3.0	2.9	3.4	2.9	3.1	9.4	3.3
5.0	4.8	4.9	4.8	4.9	1.2	−2.0
7.0	7.2	6.7	7.1	7.0	3.8	—
9.0	8.8	8.9	8.6	8.8	1.7	−2.2
10	10.0	10.2	10.4	10.2	2.0	2.0
10	12	10	11	11	9.1	10
20	18	18	21	19	9.1	−5.0
30	31	32	30	31	3.2	3.3
50	48	52	48	49	4.7	−2.0
70	71	69	71	70	1.6	—
90	90	86	85	87	3.0	−3.3
100	100	103	105	103	2.5	3.0
100	102	98	80	93	12.6	−7.0
200	201	195	194	195	2.9	−2.5
300	295	307	291	298	2.8	−0.7
500	496	500	532	509	3.9	1.8
700	720	714	752	729	2.8	4.1
900	869	898	879	882	1.7	−2.0
1000	1017	992	973	994	2.2	−0.6

C.V., coefficient of variation; RCR, relative concentration residual.

better, yet the percent relative concentration residuals (%RCR), calculated from Eq. (4) for calibration levels in the lower concentration range were more than 20% and are thus not acceptable [16].

$$\%RCR = 100 \times (RC - NC)/NC \quad (4)$$

where RC = interpolated concentration, and NC = nominal concentration.

Table 2
Calibration concentrations back-calculated from peak areas for the determination of thioTEPA in urine, measured by GC–FI-NPD

Concentration (ng/ml)	Calibration concentration (ng/ml)				C.V. (%)	RCR (%)
	Run 1	Run 2	Run 3	Mean		
10	11	—	10	11	3.7	10
20	19	20	19	19	3.0	−5.0
30	31	30	31	31	1.9	3.3
50	50	49	50	50	1.2	—
70	68	73	70	70	3.6	—
90	90	85	90	88	3.3	−2.2
100	101	103	100	101	1.5	1.0
100	85	105	95	95	10.5	−5.0
200	195	190	189	190	2.4	−5.0
300	311	306	322	313	2.6	4.3
500	517	509	505	510	1.2	2.0
700	705	686	695	695	1.4	−0.7
900	898	887	892	892	0.6	−0.9
1000	988	1017	1005	1003	1.5	0.3

C.V., coefficient of variation; RCR, relative concentration residual.

Table 3
Effect of calibration method on relative concentration residuals for the determination of thioTEPA in plasma, measured by GC–FI-NPD

Weight factor	Nominal concentration					
	2.0	5.0	50	100	500	1000
Decades[a]	—	−2.0	−2.0	3.0	1.8	−0.6
Unweighted	−495	−1010	−4.2	1.1	2.6	0.7
$1/x$	96	4.0	1.0	6.0	−3.0	−6.6
$1/x^2$	98	8.2	5.8	11	1.8	−2.1

[a]Calibration split up in three decades (2–10, 10–100 and 100–1000 ng/ml), unweighted; x represents the thioTEPA concentration.

To avoid biasing the calibration curve in favour of the high standards, weighting factors $1/x$ and $1/x^2$ were used, in which x represents the thioTEPA concentration [17,18]. The data in Table 3 indicate, however, that the least deviations of the nominal concentration were obtained by dividing the calibration curve in decades and this procedure needs therefore to be applied when the FI-NPD detector is used.

Using the TI-NPD, a linear relationship was obtained over the whole concentration range 1–1000 ng/ml for both plasma and urine. The linear regression was weighted by $1/x^2$ yielding r^2 of 0.995 or better. Back-calculated concentrations gave deviations less than 10% of the nominal concentration (see Table 4 for plasma and Table 5 for urine). The goodness-of-fit test was performed to demonstrate linearity, resulting in P values >0.05. Thus, the linearity can be determined in one calibration curve with the use of the TI-NPD. A one-tailed F-test was used to compare the linearity between the FI-NPD and TI-NPD. The $\Sigma(\%RCR)^2$ of the overall cali-

Table 4
Calibration concentrations back-calculated from peak areas for the determination of thioTEPA in plasma, measured by GC–TI-NPD

Concentration (ng/ml)	Calibration concentration (ng/ml)				C.V. (%)	RCR (%)
	Run 1	Run 2	Run 3	Mean		
1	0.96	0.98	1.02	0.99	3.1	−1.3
5	6.1	5.2	4.7	5.3	13	6.0
10	9.7	11	9.6	9.9	9.9	−1.0
50	47	51	50	49	4.2	−2.0
100	97	94	96	96	1.6	−4.0
500	486	484	544	505	6.8	1.0
1000	984	978	1025	996	2.6	−0.4

C.V., coefficient of variation; RCR, relative concentration residual).

Table 5
Calibration concentrations back-calculated from peak areas for the determination of thioTEPA in plasma, measured by GC–TI-NPD

Concentration (ng/ml)	Calibration concentration (ng/ml)				C.V. (%)	RCR (%)
	Run 1	Run 2	Run 3	Mean		
1	1.00	1.00	1.01	1.00	0.58	0.33
5	4.6	4.9	4.7	4.7	3.09	−2.2
10	9.4	10.2	9.7	9.8	4.1	−2.2
50	53	53	51	52	2.4	4.0
100	107	107	107	107	—	7.0
500	502	505	500	502	0.5	0.5
1000	992	861	990	948	7.9	−5.2

C.V., coefficient of variation; RCR, relative concentration residual.

Table 6
Recoveries of thioTEPA from plasma and urine ($n=3$), measured by GC–FI-NPD

Concentration range (ng/ml)	Recovery (%)	
	Plasma	Urine
2–10	90 (±8.7)	—
10–100	94 (±6.1)	103 (±4.8)
100–1000	85 (±6.1)	99 (±3.5)

bration curve (5–1000 ng/ml for plasma and 10–1000 ng/ml for urine) for both detectors were calculated, and the F-test was performed resulting in a P value of 0.048 for plasma and 0.33 for urine. The linearity of the assay was thus dramatically improved using the TI-NPD in the range 5–1000 ng/ml for plasma. Linearity in urine was increased from 10–1000 to 1–1000 ng/ml.

3.5. Recovery

Recoveries of thioTEPA extraction from plasma or urine ranged from 85 to 100% (Table 6), corresponding with values described in other assays [7,8]. The recovery of diphenylamine at a concentration of 4 μg/ml from plasma and urine was 79 (±5.8) and 85% (±5.1), respectively. Recoveries of thioTEPA and diphenylamine from plasma were lower than from urine, which may be the result of irreversible binding to proteins. Extractions were initially performed in the absence of NaOH at neutral pH, according to the method of Hagen et al. [7]. Experiments showed, however, that the extractions under these conditions gave lower and irregular recoveries for thioTEPA from plasma (76%) resulting in poor linearity. Extraction at alkaline pH however gave cleaner blank chromatograms and higher analyte recoveries. Lower recoveries for both thioTEPA and diphenylamine (55–75%) were obtained when the organic layer was evaporated under nitrogen to complete or near dryness. The loss of thioTEPA can be ascribed here to evaporation [8], as prolonged exposure to a stream of nitrogen resulted in progressively lower thioTEPA concentrations. Also, absorption of thioTEPA at the vials during evaporation to complete dryness, after which thioTEPA was only partially reconstituted, might result in lower recoveries. Adding acetic acid to the ethyl acetate extract with the purpose of protonating thioTEPA and diphenylamine, which might prevent the protonated components being evaporated, gave a loss of 85% of thioTEPA. This is probably due to the chemical instability of thioTEPA in acidic medium [19,20]. To gain a good sensitivity without complete evaporation of the organic layer, extractions with 100–300 μl ethyl acetate were also tested. Reducing the amount of organic solvent resulted in recoveries of 70–80% and was therefore excluded. Addition of other organic solvents with higher boiling points to prevent evaporation to dryness, such as 1-pentanol, 1-butanol or n-heptane, improved the recovery of thioTEPA but gave extensive drifts in the baseline of the chromatograms. The most satisfactory results were obtained by addition of 40 μl 1-propanol with evaporation to a final volume of approximately 20 μl. The evaporation step at ambient temperature under nitrogen took about 20 min.

3.6. Accuracy and precision

Data of the assay performance are presented in Table 7. The accuracy, and within- and between-run precision were less than 10%. Both accuracy and precision are thus far within the acceptable criteria of 10–25% deviation [17].

Table 7
Accuracy and precision of thioTEPA extraction from plasma and urine, measured by GC–FI-NPD

Nominal concentration (ng/ml)	Accuracy (%)		Within-run precision (%)		Between-run precision (%)	
	Plasma	Urine	Plasma	Urine	Plasma	Urine
5	2.0	—	5.5	—	5.7	—
50	4.0	2.2	5.3	4.4	9.0	4.3
500	5.6	8.0	6.5	1.3	4.3	4.2

3.7. Specificity

Analysis of plasma and urine samples of six different individuals showed that there were no endogenous components interfering with the determination of thioTEPA and diphenylamine.

3.8. Sensitivity

Using the FI-NPD, the limit of detection (LOD) for thioTEPA in plasma was 0.5 ng/ml at a signal-to-noise ratio of 3, and the lower limit of quantification (LLQ) at which the concentration can be determined with an accuracy of less than 20% was 2.0 ng/ml. The LOD of thioTEPA in plasma increased with a factor 5 to a concentration of 0.1 ng/ml, using the TI-NPD. The TI-NPD detector is equipped with a electrically heated ceramic-coated thermionic source, which operates in a diluted hydrogen/air environment. A hot chemical reactive gas layer is formed around the heated source in which N- or P-containing compounds collide. This results in a lower noise level and subsequently in an increased signal-to-noise ratio, resulting in a higher sensitivity of the TI-NPD in comparison with the FI-NPD. The present assay with the FI-NPD was thus 2–10 fold more sensitive than other methods using the FI-NPD and 10–50 fold more sensitive by using the TI-NPD detector. In other studies, thioTEPA was extracted with ethyl acetate [7,9] or with SPE [8], yielding LODs of 1–5 ng/ml using a sample volume of 400 μl or more.

3.9. Stability

ThioTEPA was stable for at least 3 months in plasma kept at −20°C. The plasma concentration was about 98±1.5%. In urine a concentration-dependent decrease was observed. A decrease of 37, 21 and 8% at 5, 50 and 500 ng/ml, respectively, was observed. Kept at −80°C, no significant decrease of thioTEPA in urine was found.

Residues of 1-propanol from plasma extracts spiked with thioTEPA were stable in the autosampler over a period of 24 h. Concentrations were about 99% of the initial concentration.

3.10. Patient samples

In Fig. 3, the thioTEPA plasma concentration of two patients is shown. The plasma concentration

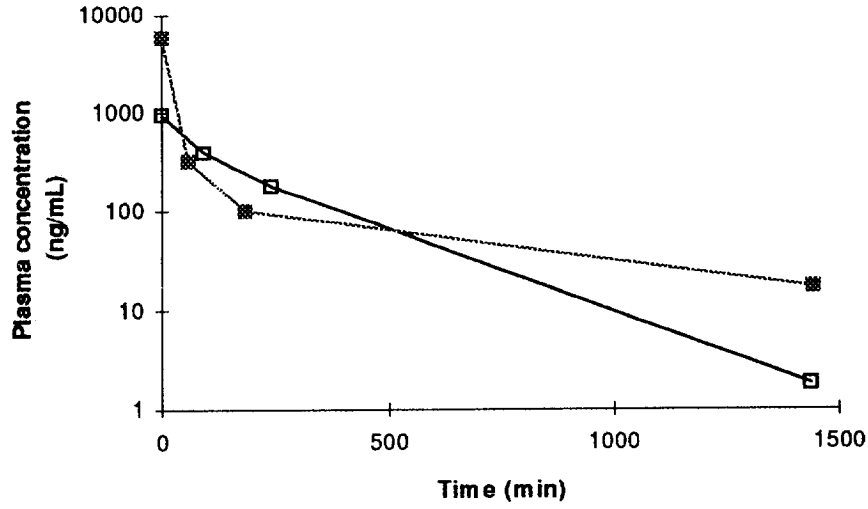

Fig. 3. Mean plasma concentrations of thioTEPA determined in two patients treated with 80 (□) or 120 mg/m^2 (■) (t=0; end of 30-min intravenous infusion).

decreases bi-exponentionally, comparable with literature data [2,21].

3.11. GC–MS

The GC–MS studies of patient plasma and urine samples show that the peak eluting at 4.4 min had a molecular mass of 189 (MH^+, 190), which resembles the molecular mass of thioTEPA (Fig. 4A). Fragments with m/z 147 and m/z 115 originate from $[M-C_2H_4N]^+$ and $[M-C_2H_4N-S]^+$, respectively. Fig. 4B shows the mass spectrum of diphenylamine (MH^+, 170), which elutes at 5.6 min. These results prove that the above-described assay determines thioTEPA in the biological specimens studied.

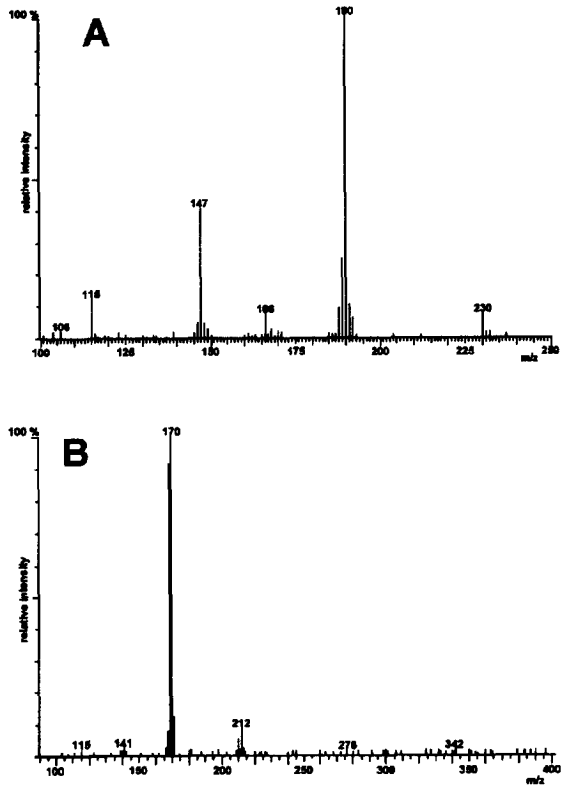

Fig. 4. (A) Mass spectrum of thioTEPA in a patient plasma sample. Fragments m/z 190, m/z 147 and m/z 115 originate from $[MH]^+$, $[M-C_2H_4N]^+$ and $[M-C_2H_4N-S]^+$, respectively. (B) Mass spectrum of diphenylamine extracted from a spiked plasma sample. Fragment m/z 170 originates from $[MH]^+$.

4. Conclusion

The described method is suitable for the determination of thioTEPA in plasma and urine of patients treated with the drug. With a limit of quantification of 0.1 ng/ml (using TI-NPD), starting with micro volumes of biological material, the applied method is more sensitive than previously described methods, and can be used for kinetic studies. The TI-NPD gave, compared with the FI-NPD, a better linearity and an increase in sensitivity with a factor 5.

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