ELSEVIER

Journal of Chromatography B, 716 (1998) 177–186

JOURNAL OF
CHROMATOGRAPHY B

Simultaneous determination of N,N′,N″-triethylenethiophosphoramide, cyclophosphamide and some of their metabolites in plasma using capillary gas chromatography

Alwin D.R. Huitema[a,*], Matthijs M. Tibben[a], Thomas Kerbusch[a], Jan W. Zwikker[b], Sjoerd Rodenhuis[c], Jos H. Beijnen[a]

[a]*Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute/Slotervaart Hospital, Louwesweg 6, 1066 EC Amsterdam, The Netherlands*

[b]*Department of Physical Organic Chemistry, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands*

[c]*Department of Medical Oncology, The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands*

Received 19 March 1998; received in revised form 4 June 1998; accepted 16 June 1998

Abstract

A sensitive assay for the simultaneous determination of N,N′,N″-triethylenethiophosphoramide (thioTEPA), its metabolite N,N′,N″-triethylenephosphoramide (TEPA), cyclophosphamide (CP) and its metabolite 2-dechloroethylcyclophosphamide (2-DCE-CP) in plasma has been developed and validated. The analytes were determined using gas chromatography with nitrogen/phosphorus selective detection after liquid–liquid extraction with chloroform using 100 μl of plasma. Diphenylamine (for TEPA, thioTEPA and 2-DCE-CP) and imipramine (for CP) were used as internal standards. The limits of quantitation for thioTEPA, TEPA, CP and 2-DCE-CP were 5, 5, 50 and 250 ng/ml, respectively. Linear calibration curves were observed over two decades of concentration. Accuracy, within-day and between-day precision were less than 13% for all analytes. Stability of the analytes proved to be satisfactory for at least 1 month, stored at −70°C. Analysis of samples obtained from patients receiving cyclophosphamide, thioTEPA and carboplatin in a high-dose regimen demonstrated the applicability of the assay. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: N,N′,N″-Triethylenephosphoramide; N,N′,N″-triethylenethiophosphoramide; Cyclophosphamide; 2-Dechloroethylcyclophosphamide

1. Introduction

Cyclophosphamide (CP, 2-[bis-(2-chloroethyl) amino]-tetrahydro-2H-1,3,2,-oxazaphosphorine-2-oxide, Endoxan®) and thioTEPA (N,N′,N″-triethylenethiophosphoramide, Ledertepa®) are both alkylating agents that have been used in cancer therapy for many years [1,2]. CP and thioTEPA have attracted renewed attention as they are part of various high-dose chemotherapy schedules with haematopoietic stem cell support [3]. In these high dose regimens the dose of common antineoplastic agents, such as the alkylating agents and the platinum containing compounds carboplatin and cisplatin, can be increased up to 10-fold, because bone marrow toxicity is no longer the dose-limiting toxicity. However,

*Corresponding author.

0378-4347/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(98)00300-4

other toxicities become more prominent [4]. CP and thioTEPA are frequently used simultaneously in these regimens because of their toxicity profile and potential synergistic antitumour activity [3].

CP is a prodrug which requires oxidation by the mixed-function oxidase cytochrome *P*450 enzymes to 4-hydroxycyclophosphamide which is in equilibrium with its tautomer aldophosphamide. Upon spontaneous elimination of acrolein, phosphoramide mustard is formed which is considered the ultimate alkylating species. Various inactivation routes for CP exist. One of these inactivation routes is the side chain oxidation of CP to 2-dechloroethylcyclophosphamide (2-DCE-CP) and chloracetaldehyde. Chloracetaldehyde is considered to be responsible for neurotoxicity [1].

ThioTEPA is rapidly metabolized by an oxidative desulfuration mediated by the cytochrome *P*450 to yield N,N′,N″-triethylenephosphoramide (TEPA). Like thioTEPA, TEPA exhibits alkylating activity and is therefore considered pharmacologically active [5]. Fig. 1 shows the structures of CP, 2-DCE-CP, thioTEPA and TEPA.

Little is known about the pharmacokinetics and its correlation with pharmacodynamic outcome of these agents in high-dose chemotherapy regimens. Pharmacokinetic monitoring can be a strategy to individualize dosing. Validated analytical methods are thus necessary in order to perform these pharmacokinetic studies.

Combined analysis of thioTEPA and TEPA has been described using gas chromatography (GC) with nitrogen/phosphorous selective flame ionisation detection (NPD), with various sample pretreatments such as liquid–liquid extraction (LLE) [6–8] or solid-phase extraction (SPE) [9]. For these methods relative large volumes of plasma were used. A laborious high-performance liquid chromatography (HPLC) method with fluorescence detection after SPE followed by derivatization has also been reported [10]. CP is usually also determined using GC with NPD or mass spectrometry (MS) detection with [11] or without [12–14] derivatization. However, HPLC [15] and polarographic methods [16] have also been described but lack the required sensitivity. For CP and four of its metabolites, a relative insensitive and laborious thin-layer chromatography system with densitometry for quantitation has been described [17]. The CP metabolite 2-DCE-CP (i.e. 3-dechloroethylifosfamide), can also be determined using GC [18]. For both thioTEPA and CP derivatization methods are often very labour-intensive making a method without derivatization preferable.

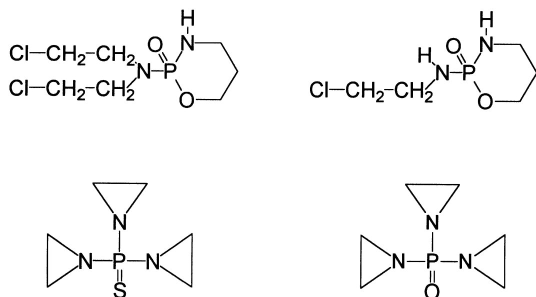

Fig. 1. Chemical structures of CP (upper left), 2-DCE-CP (upper right), thioTEPA (lower left) and TEPA (lower right).

A method for the simultaneous determination of thioTEPA, CP and some of their metabolites would be very convenient for pharmacokinetic studies within high-dose chemotherapy, but has never been reported. Hence, the aim of our study was to develop and to validate a sensitive method using micro volumes (100 μl) of biological matrix preferably without derivatization.

2. Experimental

2.1. Chemicals

ThioTEPA (Ledertepa) was obtained from AHP Pharma (Hoofddorp, Netherlands), CP, 2-DCE-CP, 4-ketocyclophosphamide, 4-hydroperoxycyclophosphamide, phosphoramide mustard and carboxyphosphamide were a kind gift of Dr. Pohl (Asta Medica, Frankfurt am Main, Germany). TEPA was synthesized at the Faculty of Chemistry, University of Utrecht, according to the method described by Craig and Jackson [19]. Identity and purity of TEPA were assessed by GC, GC–MS, ^{1}H-, ^{13}C- and ^{31}P-NMR. Purity turned out to be >98%. All gasses used were obtained from Hoekloos (Schiedam, The Netherlands). Ethanol, methanol and chloroform were purchased from Biosolve (Valkenswaard, The Netherlands). Diphenylamine (Merck, Amsterdam, The Netherlands) and imipramine HCl (OPG, Amster-

dam, The Netherlands) were used as internal standards. Blank plasma was obtained from the Central Laboratory of Blood Transfusion (Amsterdam, The Netherlands). Distilled water was used throughout and all other chemicals used were of analytical grade and used without further purification.

2.2. Instrumentation

A gas chromatograph (HP 5890 series II, Hewlett-Packard, Amstelveen, The Netherlands) equipped with a flame ionization nitrogen/phosphorous selective detection system (NPD), a split/splitless injector and an autosampler (model HP 6890, Hewlett-Packard) was used. A 25 m×0.32 mm CP Sil-13 CB (Chrompack, Middelburg, The Netherlands) column with a film thickness of 0.25 μm was used. The injector and detector temperatures were 250 and 275°C, respectively. Splitless injection was used. Helium was used as carrier gas at a flow-rate of 3 ml/min. The flow-rates of the gasses for the detector were: hydrogen 3 ml/min, air 95 ml/min and make-up (helium) 25 ml/min. The oven temperature program started at 100°C and was ramped directly at 10°C/min to 180°C, then to 220°C at 40°C/min and kept at 220°C for 6 min. Hereafter the temperature was ramped to 275°C at 40°C/min. Finally, the oven was kept at 275°C for 2 min, resulting in a total run time of 19 min. Data were recorded with a Spectra Physics model 4270 integrator (Spectra Physics, Santa Clara, CA, USA) and reprocessed using the PC1000 system (Thermo Separation Products, Breda, The Netherlands).

MS measurements were performed on a Finnigan MAT GCQ GC–MS combination equipped with a A200S autosampler (Finnigan MAT, Veenendaal, The Netherlands). The same capillary column was used, the oven temperature started at 100°C and was ramped at 10°C/min to 180°C, at 40°C/min to 220°C, and then kept at 220°C for 8 min. The transfer line was maintained at 275°C. The measurements were performed in the positive ion electron impact (EI) mode with an electron energy of 70 eV and a scan range of 70–400 m/z.

2.3. Sample pretreatment

Samples of 100 μl plasma (diluted with blank human plasma if necessary) were transferred to a 1.5-ml eppendorf tube. A 100-μl volume of water, 20 μl of a 20 μg/ml diphenylamine solution in methanol (internal standard for thioTEPA, TEPA and 2-DCE-CP), 20 μl of a 25 μg/ml imipramine HCl solution in water (internal standard for CP) and 10 μl of a 1 *M* NaOH solution and 750 μl of chloroform were added. This mixture was vortexed for 30 s and then centrifuged for 5 min at 10 500 *g*. The plasma layer was removed and the chloroform layer was transferred into a 1.5-ml eppendorf tube. The organic layer was evaporated under a gentle stream of nitrogen at ambient temperature to a volume of 50–100 μl. A 100-μl volume of ethanol was then added and, again, it was evaporated to reach the same volume. Again, 100 μl ethanol were added and this mixture was evaporated to a final volume of 50–100 μl. Of this mixture, 1 μl was injected into the GC system.

2.4. Calibration

Drug-free human plasma (100 μl) was spiked with 100 μl of a solution of thioTEPA, TEPA (both in concentrations of 5, 10, 25, 50, 100, 250 and 500 ng/ml), CP and 2-DCE-CP (both in concentrations of 50, 100, 250, 500, 1000, 2500 and 5000 ng/ml) in water in appropriate concentrations. To this mixture 20 μl of a 20 μg/ml diphenylamine solution in methanol, 20 μl of a 25 μg/ml imipramine HCl solution in water, 10 μl of a 1 *M* NaOH solution and 750 μl of chloroform were added. This mixture was further processed as described in Section 2.3.

2.5. Validation

A three-run validation on the following parameters was performed: accuracy, within-day and between-day precision, linearity, selectivity and specificity, extraction efficacy, stability, limit of detection (LOD) and lower limit of quantitation (LLQ).

2.6. Accuracy, precision and linearity

Quality control samples were prepared at concentrations of 5, 25, 250 en 500 ng/ml for thioTEPA and TEPA and 50, 250, 2500, 5000 ng/ml for CP and 2-DCE-CP. Four replicates of each quality control sample were analyzed simultaneously with calibration curves in three consecutive runs. The

accuracy was defined as the percentage of the mean deviation from the nominal concentration.

The within-day and between-day precision were calculated with a one-way analysis of variance (ANOVA) using the analytical run as the group variable. From the ANOVA analysis the day mean square (DayMS), error mean square (ErrMS) and grand mean (GM) were obtained. Within-day and between-day precision were defined using Eqs. (1) and (2), respectively, where n is the number of replicates.

$$\text{Within-day precision}(\%) = 100 \times (\text{ErrMS})^{0.5}/\text{GM} \quad (1)$$

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

The linearity of three calibration curves for each analyte was tested with the F-test for lack of fit [20], using a weight factor of $1/(\text{conc.})^2$ in order to avoid biasing in favour of the high standards. The deviation from the theoretical concentration is calculated for each calibration concentration.

2.7. Specificity and selectivity

Possible interference from endogenous compounds was investigated by the analysis of six different blank plasma samples. All known metabolites of CP were investigated for interference with the analytical method: 4-ketocyclophosphamide, carboxyphosphamide, 4-hydroxycyclophosphamide, phosphoramide mustard and didechloroethylcyclophosphamide. Carboplatin, granisetron, acetaminophen, temazepam, dexamethasone, oxazepam and sodium-2-mercaptoethane sulphonate (MESNA) were tested comedication frequently given in high-dose chemotherapy. All compounds were tested in a final concentration of 20 μg/ml in plasma.

2.8. Extraction efficacy

The extraction efficacy was determined at 25, 100 and 500 ng/ml for thioTEPA and TEPA and at 250, 1000 and 5000 ng/ml for 2-DCE-CP and CP. Four replicates were analyzed at each concentration level. Because the exact final volume of the samples after the sample pretreatment is unknown, the recovery of the extraction was determined using the external standard, methaqualone. During the sample pretreatment exactly 600 μl of the chloroform layer were transferred to the second eppendorf tube and after the sample pretreatment 10 μl of a 10 μg/ml solution of methaqualone in ethanol were added to processed and nonprocessed samples. The ratios of the areas of the analytes and methaqualone were used and the extraction efficiencies were calculated by dividing these ratios for the processed and the nonprocessed samples, corrected for the volume of the chloroform layer transferred.

2.9. Limit of detection and limit of quantitation

The LOD for thioTEPA, TEPA, 2-DCE-CP and CP was determined using a signal-to-noise ratio of 3. The LLQ was defined as the lowest concentration with a deviation from the nominal concentration (measure of accuracy) and a relative standard deviation (measure of precision) of less than 20%. The upper limit of quantitation (ULQ) was arbitrarily defined as 500 ng/ml for thioTEPA and TEPA, and 5000 ng/ml for 2-DCE-CP and CP.

2.10. Analysis of patient samples

Plasma samples were analyzed, obtained from a patient treated with a high-dose chemotherapy regimen consisting of 1000 mg/m^2 CP by a 1-h infusion ($t=0$) followed by 265 mg/m^2 carboplatin by a 1-h infusion and 40 mg/m^2 thioTEPA in a 0.5-h infusion. ThioTEPA was given each 12 h for 4 days, CP and carboplatin were administered each day for 4 consecutive days. Blood samples were obtained during the first day before start of the infusions and at $t=30$, 60, 90, 120, 150, 165, 180, 210, 285, 330 and 390 min and the next day before the infusions. Blood samples were centrifuged immediately for 3 min at 2500 g at 4°C and plasma was stored at −70°C until analysis. All samples were analyzed within 2 weeks of collection.

2.11. Stability

The stability of thioTEPA, TEPA, CP and 2-DCE-CP stored at −70°C for a period of 1 month, including three freeze–thawing cycles, was investigated by analyzing 20 patient samples obtained as described in Section 2.10 immediately after collection and 1 month after the first analysis.

The stability of the processed samples during storage in the autosampler was tested over a period of 30 h by reanalyzing all calibration samples after 30 h. The recovery after this period was calculated by dividing the slope of the calibration curve after 30 h in the autosampler by the slope of the initial calibration curve.

2.12. Statistics

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 6.1 (SPSS, Chicago, IL, USA). Correlations were considered statistically significant if calculated *P* values were 0.05 or less.

3. Results and discussion

3.1. Chromatography and detection

Fig. 2 shows two chromatograms of patient samples obtained before and 5 h after the last infusions (methaqualone was added as an external standard). Due to the thermal instability of underivatized CP in the GC system, two peaks originating from CP appear in the chromatogram [11,14]. In our chromatogram two peaks were also identified representing the product of CP formed by intramolecular cyclization (Fig. 3) and the parent. The formation of the intramolecular cyclization product of CP was increased using a high injector temperature (250°C) in combination with a low starting temperature of the oven temperature program (100°C). Using this combination the peak area of the parent was less than 15% of the peak area of the intramolecular cyclization product over the whole concentration range and a good linear relationship between concentration and peak area of the intramolecular cyclization product was found. The ratio between parent CP (peak 6) and

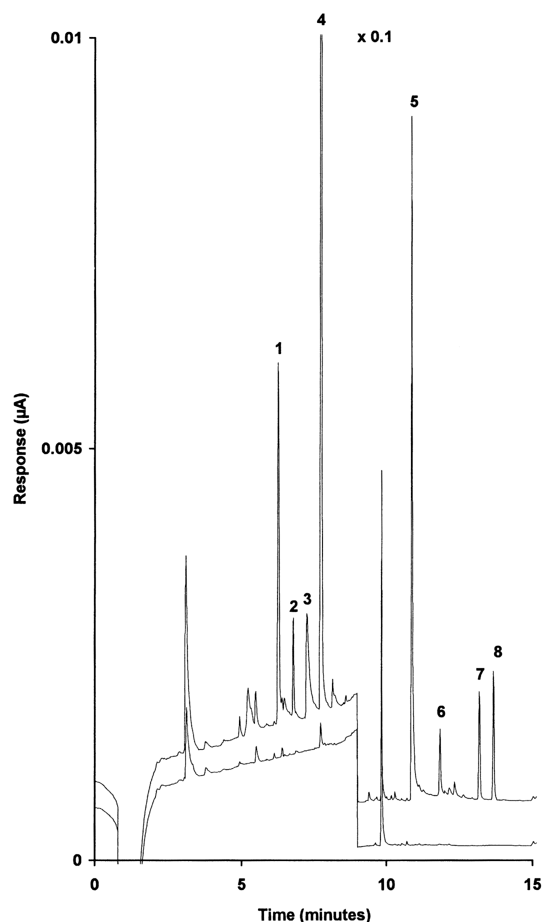

Fig. 2. Chromatograms of patient samples obtained before (lower line) and after CP and thioTEPA infusions (upper line). Peaks and corresponding concentrations: 1 = TEPA (307 ng/ml), 2 = thioTEPA (30 ng/ml), 3 = 2-DCE-CP (concentration <LLQ), 4 = diphenylamine, 5 = intramolecular cyclization product of CP (44 μg/ml), 6 = CP, 7 = methaqualone and 8 = imipramine.

the intramolecular cyclization product (peak 5) remained constant. In Fig. 4a and b the mass spectra of CP and its intramolecular cyclization product are depicted. The retention times for TEPA, thioTEPA, 2-DCE-CP, diphenylamine, the intramolecular cyclization product of CP, CP, methaqualone and imi-

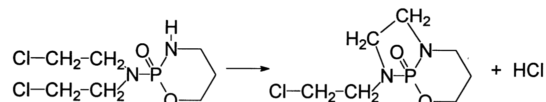

Fig. 3. Intramolecular cyclization of CP.

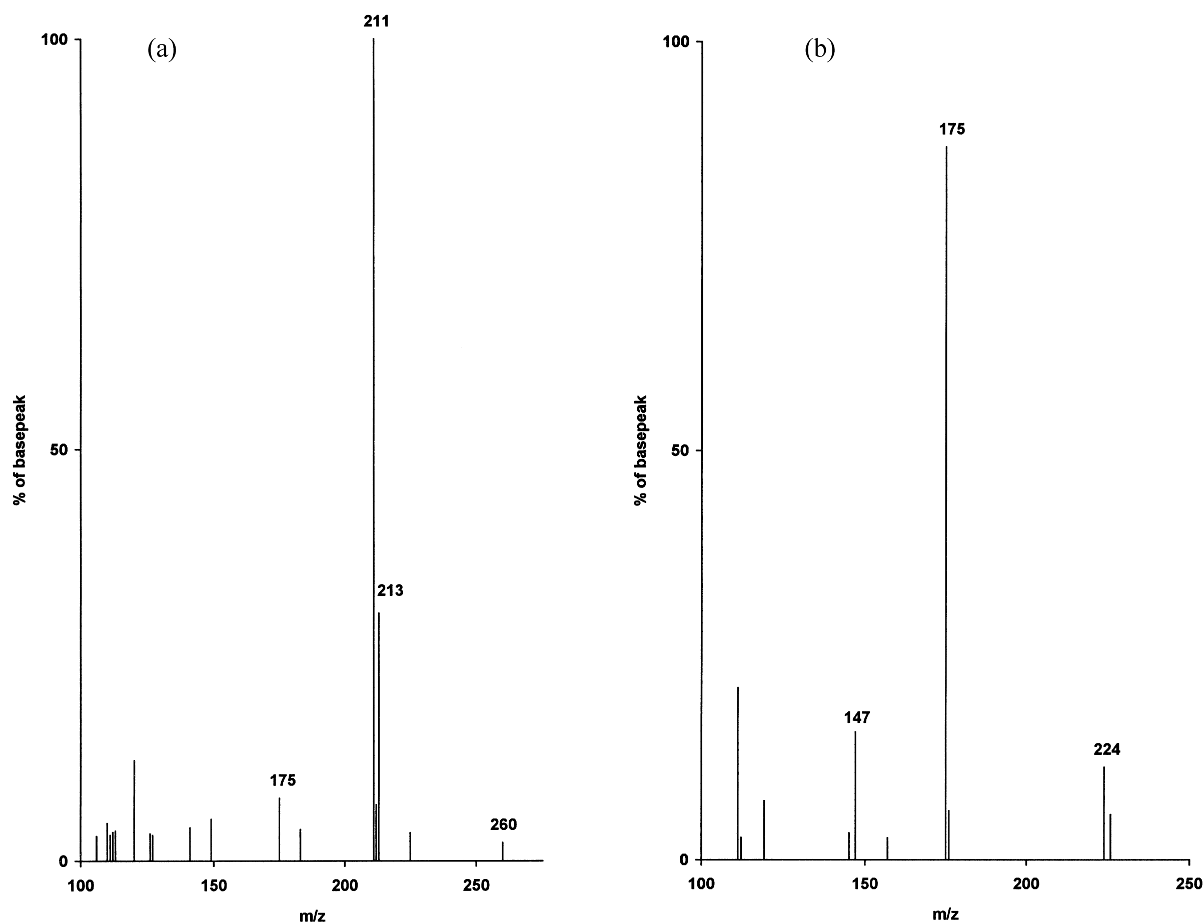

Fig. 4. (a) Mass spectrum of CP. The fragments with $m/z=211$ and $m/z=213$ originate from $[M-CH_2\text{-}Cl]^+$, $m/z=260$ from $[M]^+$. (b) Mass spectrum of the intramolecular cyclization product of CP. The fragment with $m/z=175$ originates from $[M-CH_2\text{-}Cl]^+$, $m/z=224$ originates from $[M]^+$.

pramine were 6.5, 7.0, 7.5, 8.0, 11.0, 11.8, 13.2 and 13.6 min, respectively. The chromatographic conditions provide a good separation between the analytes. However, a very small peak ($<1\%$ compared to diphenylamine) originating from blank plasma has the same retention time as diphenylamine. This small peak did not influence the analytical performance. With GC–MS all analytes and the internal standards were identified to be the original products, the peak with retention time 9.8 min was identified to be caffeine. Diphenylamine can be used as internal standard for all analytes. However, since CP and diphenylamine were eluted at very different temperatures, the use of a second internal standard increased the robustness of the method. Facchinetti et al. used imipramine as internal standard for CP after LLE with ethylacetate [12]. Imipramine also proved to be a good internal standard for CP in our assay.

MS was tested as detection method but NPD gave superior detection sensitivity. An advantage of MS detection is its high selectivity but the selectivity of the assay using NPD detection in combination with the chromatographic system proved to be satisfactory.

3.2. Extraction

LLE with chloroform was chosen as the single extraction method. For TEPA and thioTEPA SPE [10] and LLE with ethylacetate [6] or chloroform [7] have been described. CP and 2-DCE-CP can be extracted with ethylacetate [12,18], although for CP

Table 1
Extraction efficacy of thioTEPA, TEPA, 2-DCE-CP and CP from plasma

Concentration 2-DCE-CP and CP (ng/ml)	Concentration thioTEPA and TEPA (ng/ml)	Extraction efficacy (%)±S.D.				
		TEPA	thioTEPA	2-DCE-CP	CP	n
250	25	70 (±2.1)	83 (±1.5)	21 (±2.8)	87 (±5.1)	4
1000	100	65 (±1.8)	88 (±2.8)	22 (±2.3)	76 (±3.4)	4
5000	500	71 (±4.1)	94 (±5.0)	29 (±2.8)	81 (±2.9)	4

S.D.=standard deviation.

various other extraction methods have been described including extraction with chloroform [14] and SPE [11,15,17]. Chloroform based extractions provided the best recoveries for thioTEPA, TEPA and CP and therefore this solvent was chosen as extraction medium.

Very low recoveries with a high degree of variability for TEPA, thioTEPA and diphenylamine (5–20%) were obtained when the chloroform layer was evaporated under nitrogen at ambient temperature to complete dryness. The loss of thioTEPA and TEPA may be explained by evaporation [6,9]. Also adsorption to the tube resulting in only partial reconstitution of the analytes may contribute to the observed low recoveries. Because chloroform is incompatible with the NPD detector we used, the chloroform layer was partially evaporated and ethanol was added. After two cycles virtually all chloroform is evaporated and the remaining solution can be injected into the GC.

In Table 1 the extraction efficacy of TEPA, thioTEPA, CP and 2-DCE-CP are summarized. The extraction efficacy for TEPA, thioTEPA and CP were all >65%. The extraction efficacy of 2-DCE-CP was 20%. We were, however, able to quantitate 2-DCE-CP with acceptable accuracy and precision. Using ethylacetate as extraction medium a recovery of 2-DCE-CP from plasma of 44% has been described [18]. Due to the polar nature of 2-DCE-CP relatively low recoveries were obtained using LLE.

3.3. Specificity and selectivity

Analysis of plasma samples obtained from six different individuals showed no interference of endogenous compounds with the determination of the analytes and the internal standards used.

The metabolites of CP and the selected comedicated drugs did not interfere with the analysis. Carboxyphosphamide and MESNA were not extracted at the high pH used. 4-Hydroxycyclophosphamide is very unstable leading to the formation of phosphoramide mustard, which was also not extracted. All other metabolites and selected comedicated drugs gave no peaks in the chromatogram.

3.4. Accuracy, precision and linearity

Data of the assay performance are presented in Tables 2 and 3 . For TEPA, thioTEPA and CP both within-day and between-day precisions were less than 10%. For 2-DCE-CP within-day and between-day precisions were less than 13%. Accuracy was

Table 2
Accuracy and precision of thioTEPA and TEPA analysis

Nominal conc. (ng/ml)	Accuracy (%)				Within-day precision (%)		Between-day precision (%)	
	TEPA	95% C.I.	thioTEPA	95% C.I.	TEPA	thioTEPA	TEPA	thio-TEPA
5	103.0	98.8–107.3	103.7	99.3–108.1	5.3	5.0	4.4	5.2
25	101.1	97.6–104.7	98.6	95.5–101.7	4.6	5.2	3.4	[a]
250	104.4	100.4–108.4	97.9	94.8–101.0	5.1	5.2	3.9	[a]
500	105.0	102.9–107.2	98.6	96.0–101.2	1.7	4.4	3.2	[a]

95% C.I.=95% confidence interval.
[a] No statistically significant deviation between days of analysis was observed additional to the within-day precision.

Table 3
Accuracy and precision of 2-DCE-CP and CP analysis

Nominal conc. (ng/ml)	Accuracy (%)				Within-day precision (%)		Between-day precision (%)	
	2-DCE-CP	95% C.I.	CP	95% C.I.	2-DCE-CP	CP	2-DCE-CP	CP
50	–	–	106.1	99.8–112.3	–	8.1	–	5.1
250	101.0	95.5–106.5	102.4	97.7–107.0	7.9	6.5	3.4	3.6
2500	96.3	93.3–99.2	101.5	96.2–106.9	12.7	3.2	9.7	9.0
5000	104.1	99.4–108.8	101.0	95.0–107.0	5.6	6.9	5.0	7.3

95% C.I., 95% confidence interval.

less than 10% for all analytes in the concentration range tested. Both accuracy and precision were thus within the acceptable criteria.

For all analytes the *F*-test for lack of fit for linearity was performed for three calibration curves and all *P* values were higher than 0.05 indicating a linear relationship between response and concentration in the range tested. In Table 4 the deviation from the theoretical concentration and the relative standard deviation is given for each analyte at all calibration concentrations. In Table 5 slopes, intercepts and correlation coefficients for representative calibration curves are given.

3.5. Stability

For thioTEPA, TEPA, 2-DCE-CP and CP recoveries after storage for 1 month at −70°C including three freeze–thawing cycles were 99.4, 91.0, 96.6 and 95.3%, respectively which is in accordance with the results of van Maanen et al. [6] and Kaijser et al. [18].

Table 5
Slope, intercept and correlation coefficient for representative calibration curves for all analytes

	TEPA	thioTEPA	CP	2-DCE-CP
Slope	.00202	0.0104	1.21	0.0630
Intercept	.00068	0.00053	0.0022	−0.0072
R^2	0.998	0.998	0.998	0.995

All analytes were stable in processed samples during storage for 30 h in the autosampler at ambient temperature. Recoveries were 101.8, 103.6, 111.4 and 103.3% for thioTEPA, TEPA, 2-DCE-CP and CP, respectively.

3.6. Limit of detection and lower limit of quantitation

The LODs for TEPA, thioTEPA, CP and 2-DCE-CP were 3, 2, 5, 50 ng/ml, respectively. At the lowest calibration concentration (5 ng/ml for thioTEPA and TEPA, 50 ng/ml for CP and 250 ng/ml for 2-DCE-CP) both accuracy and precision

Table 4
Calibration curves: deviation from the theoretical concentration and relative standard deviation for the analytes at all tested calibration concentrations

Concentration CP and 2-DCE-CP (ng/ml)	Concentration TEPA and thioTEPA (ng/ml)	TEPA		thioTEPA		CP		2-DCE-CP	
		Dev. (%)	R.S.D. (%)	Dev. (%)	R.S.D. (%)	Dev. (%)	R.S.D. (%)	Dev. (%)	R.S.D. (%)
50	5	+0.4	9.6	−0.2	1.8	+0.5	4.2		
100	10	+1.2	8.8	+2.0	3.1	+0.0	5.4		
250	25	−6.2	4.9	−2.7	7.4	−4.0	6.5	+4.0	7.7
500	50	−0.1	7.1	+4.9	7.4	+2.0	3.1	−4.0	6.3
1000	100	+1.9	7.5	+4.3	7.4	−3.4	8.0	−3.0	6.2
2500	250	−0.2	7.4	−2.3	5.9	+3.7	9.7	+0.0	7.2
5000	500	+2.1	9.0	−5.1	6.5	+1.8	7.5	+4.8	3.1

Dev. = deviation from the theoretical concentration; R.S.D. = relative standard deviation.

were <20%, therefore these concentrations were accepted as LLQs.

3.7. Patient samples

In Fig. 5a and b the plasma concentration versus time curves of thioTEPA, TEPA and CP for a patient treated with high-dose chemotherapy are shown. The plasma concentration of thioTEPA decreases bi-exponentionally as described earlier [6,8]. A substantial amount of TEPA was still present before the next infusion of thioTEPA resulting in accumulation of TEPA during the 4 days of treatment. The observed first-order elimination of CP is in accordance with literature data [4]. 2-DCE-CP could be detected in patient samples but concentrations were usually less than the LLQ. As depicted in Fig. 5b some patients have relatively high concentrations of 2-DCE-CP which may be correlated to a high risk of developing neurotoxicity. Because of the sensitivity of the assay, the described method can also be used for the

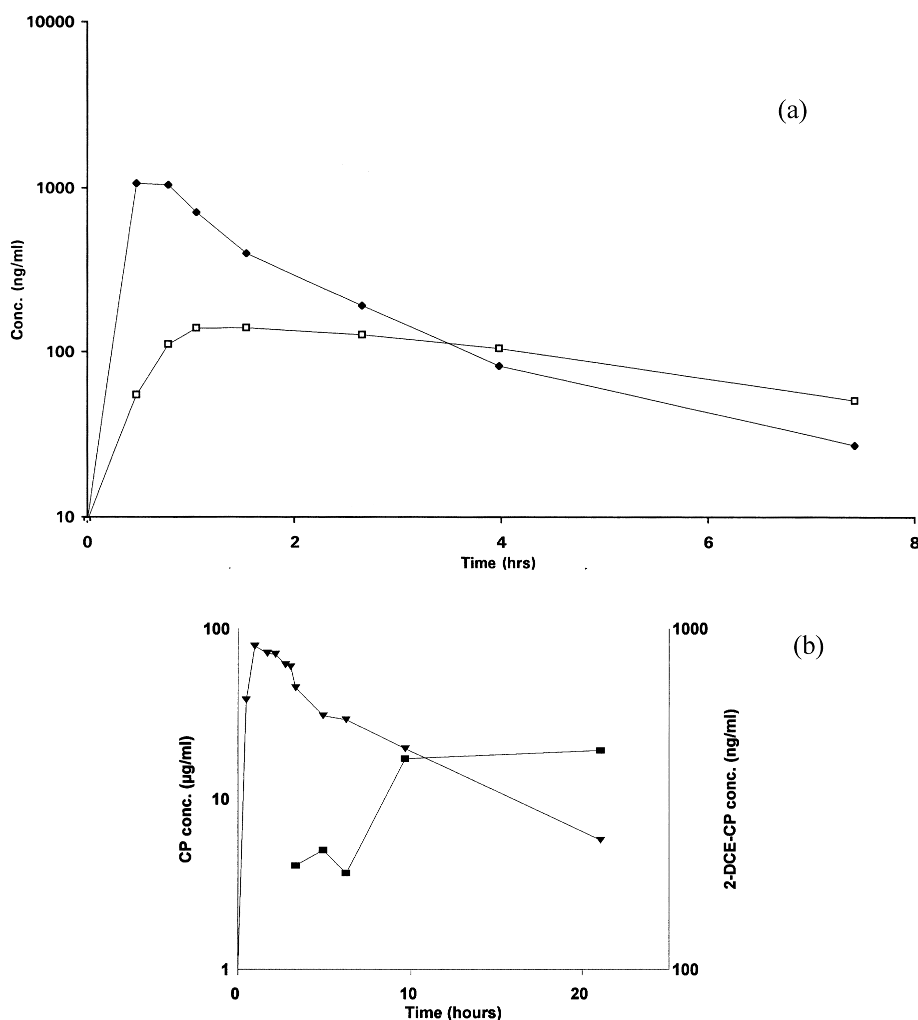

Fig. 5. (a) Plasma concentrations of thioTEPA (♦) and TEPA (□) determined in a patient treated with thioTEPA (40 mg/m^2) and CP (1000 mg/m^2) in combination with carboplatin (265 mg/m^2). (b) Plasma concentrations of CP (▼) and 2-DCE-CP (■) determined in a patient treated with thioTEPA (40 mg/m^2) and CP (1000 mg/m^2) in combination with carboplatin (265 mg/m^2).

determination in plasma of patients treated with conventional doses of CP or thioTEPA.

4. Conclusion

The described GC method using LLE with chloroform and NPD detection is suitable for the simultaneous determination of thioTEPA, TEPA, CP and 2-DCE-CP in plasma of patients treated with CP and/or thioTEPA, both in high-dose or conventional dose regimens. The presented method has been implemented in pharmacokinetic studies of thioTEPA and CP in our hospital.

Acknowledgements

This research was supported with a grant from the Dutch Cancer Society (project NKI 97-1439). The authors would like to thank R. van Gijn for his technical assistance and suggestions.

References

[1] M.J. Moore, Clin. Pharmacokinet. 20 (1991) 194.
[2] M.P. Sykes, D.A. Karnofsky, F.S. Philips, J.H. Burchenal, Cancer 6 (1953) 142.
[3] E. van der Wall, J.H. Beijnen, S. Rodenhuis, Cancer Treat. Rev. 21 (1995) 105.
[4] S. Rodenhuis, A. Westermann, M.J. Holtkamp, W.J. Nooijen, J.W. Baars, E. van der Wall, I.C.M. Slaper-Cortenbach, J.H. Schornagel, J. Clin. Oncol. 14 (1996) 1473.
[5] B.E. Cohen, M.J. Egorin, E.A. Kohlhepp, J. Aisner, P.L. Gutierrez, Cancer Treat. Rep. 70 (1986) 859.
[6] R.J. van Maanen, R.D. van Ooijen, J.H. Beijnen, J. Chromatogr. B 698 (1997) 111.
[7] M.J. Egorin, B.E. Cohen, E.A. Kohlhepp, P.L. Gutierrez, J. Chromatogr. 343 (1985) 196.
[8] P.J. O'Dwyer, F. LaCreta, P.F. Engstrom, R. Peter, L. Tartaglia, D. Cole, S. Litwin, J. DeVito, D. Poplack, R.J. DeLap, R.L. Comis, Cancer Res. 51 (1991) 3171.
[9] B.J. McDermott, J.A. Double, M.C. Bibby, D.E.V. Wilman, P.M. Loadman, R.L. Turner, J. Chromatogr. 338 (1985) 335.
[10] A. Sano, S. Matsutani, S. Takitani, J. Chromatogr. 458 (1988) 295.
[11] G. Momerency, K. Van Cauwenberghe, P.H.Th.J. Slee, A.T. Van Oosterom, E.A. De Bruijn, Biol. Mass Spectrom. 23 (1994) 149.
[12] T. Facchinetti, M. D'Incalci, G. Martelli, L. Cantoni, G. Belvedere, M. Salmona, J. Chromatogr. 145 (1978) 315.
[13] E.A. De Bruijn, U.R. Tjaden, A.T. Van Oosterom, P. Leeflang, P.A. Leqlerq, J. Chromatogr. 279 (1983) 603.
[14] N. van den Bosch, O. Driesen, A. Emonds, A.T. van Oosterom, P.J.A. Timmermans, D. de Vos, P.H.Th.J. Slee, Methods Find. Exp. Clin. Pharmacol. 3 (1981) 377.
[15] L.C. Burton, C.A. James, J. Chromatogr. 431 (1988) 450.
[16] M.M. Ellaithy, M. Fayez El-Tarra, N.B. Tadros, M.M. Amer, J. Assoc. Off. Anal. Chem. 67 (1984) 679.
[17] A.F.A. Hadidi, J.R. Idle, J. Chromatogr. 427 (1988) 121.
[18] G.P. Kaijser, J.H. Beijnen, A. Bult, G. Wiese, J. de Kraker, H.J. Keizer, W.J.M. Underberg, J. Chromatogr. 583 (1992) 175.
[19] A.W. Craig, H. Jackson, Br. J. Pharmacol. 10 (1955) 321.
[20] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Micotte, L. Kaufman, Chemometrics: a Textbook, Elsevier, New York, 1988, p. 84.