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Short communication

Determination of treosulfan in plasma and urine by HPLC with refractometric detection; pharmacokinetic studies in children undergoing myeloablative treatment prior to haematopoietic stem cell transplantation

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Abstract

A direct and selective HPLC method with refractometric detection was worked out for determination of treosulfan in plasma and urine of children. Before injection onto reverse phase column plasma samples with treosulfan and barbital (I.S.) were clarified using filtration. The mobile phase was composed of phosphate buffer, pH 5 and acetonitrile. The linear range of the standard curve of treosulfan spanned concentrations of 10.0–2000.0 μg/ml and 50.0–10000.0 μg/ml in plasma and urine, respectively, and covered the levels found in biological fluids after infusion of the drug. The limit of detection amounted to 5 μg/ml for plasma and 25 μg/ml for urine. Intra- and inter-day precision and accuracy of the measurement fulfilled analytical criteria accepted in pharmacokinetic studies. Recovery of treosulfan as well as stability in biological fluids was also calculated. The validated method was successfully applied in pharmacokinetic studies of treosulfan administered to children prior to haematopoietic stem cell transplantation. Differences between pharmacokinetics of treosulfan in children and adults were also studied.
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Keywords: Alkylating compounds; Infusion; Microfiltration; Pharmacokinetic study; Pediatric patients

1. Introduction

Treosulfan (L-threitol-1,4-di-methanesulfonate) is a prodrug of a bifunctional alkylating agent with a structure similar to busulfan, belonging to the alkyl sulfonate group. It has a broad spectrum of anti-tumour activity and it is indicated for treatment of ovarian carcinoma and other solid tumours [1–5]. Moreover, the drug is toxic against all stem cell lines of the bone marrow and is administered prior to a donor bone marrow transplant or a peripheral stem cell transplant [6–9]. Literature of the subject presents only one validated direct method for determination of treosulfan in biological fluids, based on RP-HPLC with refractometric detection, cited in a few papers. However, details regarding validation of the method are insufficient [4,5,8]. Treosulfan can be also assayed indirectly by a gas chromatography method after ex vivo derivatization to L-(+)-diepoxybutane by alkaline treatment of samples [10]. Despite the clinical use of the oral and i.v. formulation, pharmacokinetic data for adult patients are limited [4,5,8,10] and there is no information about pharmacokinetics of the drug in children. In this paper the validated RP-HPLC method with refractometric detection for determination of treosulfan in human plasma and urine was described. The method was applied in pharmacokinetic studies of treosulfan in children after 2 h intravenous infusion of 10 g/m^2 or 12 g/m^2 dose of the drug as a part of the myeloablative regimen prior to haematopoietic stem cell transplantation.

2. Experimental

2.1. Materials

Treosulfan was obtained from Medac GmbH (Hamburg, Germany). Sodium barbital, potassium phosphate dibasic trihydrate ($K_2HPO_4 \cdot 3H_2O$) and ethylenediaminetetraacetic acid dipotassium salt dihydrate ($K_2EDTA \cdot 2H_2O$) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Eighty-five percent orthophosphoric acid of HPLC grade was obtained from Fluka (Buchs, Switzerland). Citric acid (P.O.Ch.,

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Gliwice, Poland) was also used. Acetonitrile (J.T. Baker, Deventer, The Netherlands) was of HPLC grade. Demineralised water (0.1 μS/cm) was always used (Seradest USF 1900, USF Seral, Germany). Microfiltration of plasma samples were carried out with microfilters Microcon (Millipore Corporation, Bedford, USA) with cut-off 10,000 Da.

2.2. Chromatographic conditions

The mobile phase was prepared by mixing 150 ml of acetonitrile with 850 ml of phosphate buffer consisted of 1 g $K_2HPO_4 \cdot 3H_2O$ and 0.1 g $K_2EDTA \cdot 2H_2O$ per 1 l H_2O adjusted to pH 5 with 85% H_3PO_4. The aqueous solution was filtered through a 0.45 μm cellulose acetate membrane filter (Sartorius AG, Goettingen, Germany). Determination of treosulfan and barbital (I.S.) in biological fluids was performed in a Chromatograph model HP 1100 (Hewlett-Packard, Waldbronn, Germany). The HPLC system consisted of a quaternary pump model G1311A set at a flow rate of 1 ml/min, degasser model G1322A and refractometric detector model G1362A. The samples of 100 μl were injected into the system using manual injector model G1328A. The separation was performed on a 250 mm × 4.6 mm Nucleosil 100 (C18) column packed with 5 μm particles, with a guard column (Nucleosil C18), both from Alltech Associates Inc. (Deerfield, USA). HP1100 apparatus was equipped with ChemStation used for instrument control, data acquisition and data analysis.

2.3. Plasma sample preparation

Stock solution of treosulfan was prepared with 40 mg/ml in deionised water. Barbital sodium was dissolved in water to obtain a concentration of acidic form of barbital (I.S.) of 50 mg/ml. Then, standard solutions: 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 mg/ml of treosulfan and 10.0 mg/ml of I.S. were prepared also in water. The volumes of 25 μl of the treosulfan and I.S. solutions were transferred to a vial containing 250 μl human plasma, adjusted to pH of 5.5 by 1 M citric acid to avoid artificial ex vivo degradation. The resulting plasma contained: 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, 1000.0 and 2000.0 μg/ml of treosulfan and 1000.0 μg/ml of I.S. After centrifugation at 10,000 × *g* for 15 min and microfiltration (cut-off 10,000 Da) a volume of 100 μl was applied to the HPLC system. Plasma samples of children were processed in the same manner, except that each 250 μl of plasma was spiked with 25 μl of water and 25 μl of I.S.

2.4. Urine sample preparation

Stock solution of treosulfan and barbital sodium was prepared with respectively 40 mg/ml and 50 mg/ml (of acidic barbital) in deionised water. Then, standard solutions: 0.1, 0.2, 0.4, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/ml of treosulfan and 20.0 mg/ml of I.S. were prepared also in water. The volumes of 25 μl of the treosulfan and I.S. solutions were transferred to a vial containing 100 μl human urine previously acidified with 1 M citric acid. The resulting urine contained: 25.0, 50.0, 100.0, 250.0, 500.0, 1000.0, 2500.0, 5000.0 and 10000.0 μg/ml of treosulfan and 5000.0 μg/ml of I.S. Then, urine samples were diluted with 350 μl water and a volume of 100 μl was injected onto the column. Urine samples of patients were processed in the same manner, except that each 100 μl of urine was spiked with 25 μl of water and 25 μl of I.S.

2.5. Validation parameters

2.5.1. Specificity

Drug-free human plasma and urine were tested for the examination on the presence of disturbing endogenous compounds on chromatograms. The samples were prepared according to the above procedure. Then, chromatograms of plasma and urine samples containing treosulfan and I.S. were compared with chromatograms of the blank plasma and urine.

2.5.2. Linearity of the calibration curve

Linearity of the calibration curve was estimated for the peak area of treosulfan to I.S. ratio as a function of the treosulfan concentration ranging from 10.0 to 2000.0 μg/ml and 50.0–10000.0 μg/ml in plasma and urine, respectively. These ranges cover the concentration values typically found in human plasma and urine after administration of treosulfan. The equations of calibration curves were used to calculate unknown treosulfan concentration in human plasma and urine. A correlation coefficient r was calculated to confirm linearity of the calibration curves.

2.5.3. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD for treosulfan was determined as a signal to noise baseline ratio of 3:1, both for plasma and urine. The LOQ is defined as the lowest concentration of treosulfan of the calibration curves in plasma and urine at which the coefficient of variation CV $\leq$ 15% of the nominal concentration.

2.5.4. Precision and accuracy

Intra-day precision of the elaborated method was calculated for 50.0 and 1000.0 μg/ml and for 100.0 and 5000.0 μg/ml of treosulfan concentrations in plasma and urine, respectively, for five samples of each concentration. Inter-day precision was estimated for all concentrations within the calibration curve range. The precision was expressed as %CV. Accuracy was estimated for the same range of treosulfan concentrations as for evaluation of the method precision. It was expressed by the percent difference between the mean concentration determined and the nominal concentration: %error = [(mean concentration − nominal concentration)/nominal concentration] × 100.

2.5.5. Recovery

The recovery of 50.0 and 1000.0 μg/ml treosulfan concentrations in plasma was evaluated. First series consisted of five 250 μl blank acidified plasma spiked with 25 μl of 0.5 or 10.0 mg/ml treosulfan solution and 25 μl of 10.0 mg/ml I.S. solution. After centrifugation and microfiltration a sample of 100 μl

was injected onto the column. Then, five blank acidified plasma samples of second series were supplemented with I.S. only. Treosulfan was added after centrifugation and microfiltration and a sample with a volume of 100 μl was injected onto the HPLC system. The recoveries were calculated as the peak area ratio of treosulfan to I.S., from the first and the second series. The recoveries were calculated using the formula:

$$\%\text{recovery} = \frac{P^{\mathrm{I}}_{\text{treosulfan/I.S.}}}{P^{\mathrm{II}}_{\text{treosulfan/I.S.}}} \times 100$$

where $P^{\mathrm{I}}{}_{\text{treosulfan/I.S.}}$ and $P^{\mathrm{II}}{}_{\text{treosulfan/I.S.}}$ are the peak areas of treosulfan to peak area of I.S. from the first and the second series.

2.6. Freeze and thaw stability test

The stability of treosulfan samples with concentration of 50.0 and 1000.0 μg/ml in plasma and of 100.0 and 5000.0 μg/ml in urine was analyzed before and after two freeze and thaw cycles. The samples were prepared according to the procedure described above.

2.7. In vivo application

A number of 5 children (1 girl and 4 boys) aged between 2 and 15 years, with acute myeloid leukemia (AML), Hodgkin's disease, acute biphenotypic leukemia (AML/ALL) and severe acquired aplastic anaemia (SAA) were included in the study. The investigation has been approved by the Human Investigation Committee at the University of Medical Sciences in Poznań. Treosulfan was administered at three doses of 12 g/m^2 (4 patients) and 10 g/m^2 (1 patient) each in myeloablative regimen prior to haematopoietic stem cell transplantation. Before infusion treosulfan was dissolved in sterile water for injection to obtain a concentration of 50 mg/ml. After dilution to a defined dose it was intravenously administered for 2 h. Aliquots of blood after single dose were drawn via an indwelling venous access at following time: before infusion and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after the start of infusion. Blood samples were adjusted to a final pH of 5.5 by the addition of 50 μl 1 M citric acid per 1 ml of blood immediately after collection to avoid artificial ex vivo degradation of treosulfan and then centrifugated to obtain plasma. The resulting plasma samples were kept frozen at −20 °C until analysis. Moreover, urine samples of patients were collected into separate containers over a time course of 24 h after the start of infusion and treated with 50 μl of 1 M citric acid per 1 ml of urine to guarantee a pH < 6. The first sample of urine was collected before infusion of treosulfan to patient. The volume of each urine sample was determined and after centrifugation kept frozen at −20 °C until analysis.

2.8. Pharmacokinetic analysis

The plasma treosulfan concentrations were used to calculate pharmacokinetic parameters based on two-compartment disposition modeling. The Topfit 2.0 software package was used for the calculation. Area under the curve ($AUC_{0\to\infty}$) was estimated by trapezoidal rule with extrapolation to infinity using the ratio C_n/k_{el} where C_n was the last measurable concentration. The elimination rate constant (k_{el}) was estimated from the terminal linear segment of the log plasma concentration/time data. The elimination half-life ($t_{0.5}$) was calculated from $\ln 2/k_{el}$; t_{max} was estimated from a treosulfan concentration/time curve and C_{max} was read at t_{max}. Clearance of the drug (Cl_{tot}) was calculated by dividing the total dose (D) of treosulfan by $AUC_{0\to\infty}$, taking into consideration a complete bioavailability ($F = 1$). Volume of distribution (V_{ss}) was estimated from the following equation: $V_{ss} = Cl_{tot} \times MRT_{disp}$.

3. Results and discussion

3.1. HPLC conditions for resolution of treosulfan

Preliminary studies on determination of treosulfan in biological fluids according to the previously reported technique [4] failed in our HPLC system. For over 24 h the refractometric detector was not balanced. Therefore, a composition of mobile phase was modified as described in Section 2.2. Addition of a small volume of acetonitrile caused that a base line was stable after 16 h. Under these HPLC conditions many compounds (busulfan, 3-nitrobenzenesulfonic acid, barbital, codeine, prazosin, isoniazid, gliclazide, alendronate sodium, nicotinic acid, ephedrine, papaverine) were tested. Barbital and codeine appeared to be the most optimal compounds to serve as I.S., finally barbital was chosen. Busulfan, which is similar in structure to treosulfan, appeared to have a migration time very similar to that of endogenous compounds.

3.2. Validation parameters

3.2.1. Specificity

Chromatograms with separated peaks of treosulfan and I.S. in plasma and urine are presented in Figs. 1 and 2. Under the described chromatographic conditions the retention time of treosulfan and I.S. was 5.9 and 11.6 min, respectively. However, the chromatograms presented also other additional peaks originated from endogenous compounds of plasma and urine. These peaks, with retention time of about 3 min, did not interfere with treosulfan and I.S. peaks. The young patients were treated also with antibiotics, antifungal agents, vitamins, ranitidine, amlodipine, prednisone and others. The medicines did not interfere with treosulfan and I.S. Derivatives of NSAIDs, cardiovascular, anticonvulsant and other drugs were also spiked to HPLC column to investigate specificity of the designed technique (Table 1). The drugs did not disturb determination of treosulfan in samples of plasma or urine. The results confirmed specificity and usefulness of the elaborated technique for determination of treosulfan in clinical samples.

3.2.2. Linearity, LOD and LOQ

Standard curves for treosulfan proved to be linear in the concentration range of 10.0–2000.0 μg/ml in plasma and of

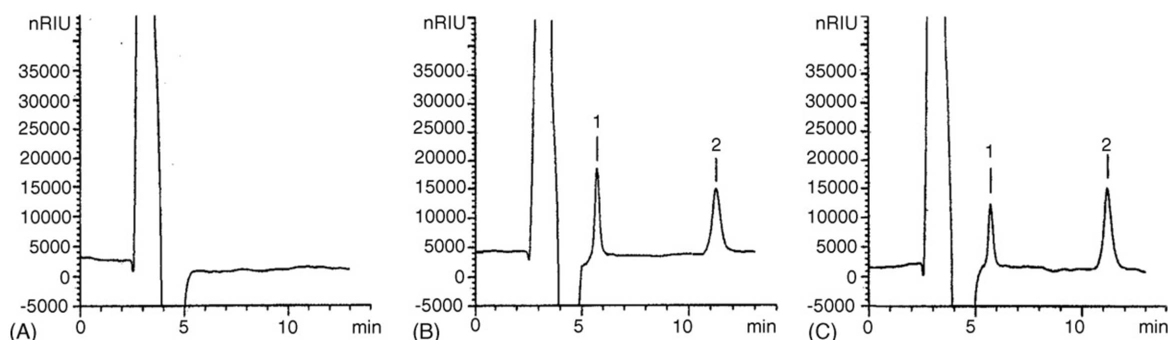

Fig. 1. Chromatograms obtained after microfiltration of human plasma samples: (A) blank plasma; (B) 250 μl of plasma spiked with 25 μl of 10 mg/ml concentration of treosulfan (1) and I.S. (2) (final concentration of each analyte in plasma was 1 mg/ml); (C) plasma sample of a child obtained at 1 h after the start of infusion (determined concentration of treosulfan was 560 μg/ml).

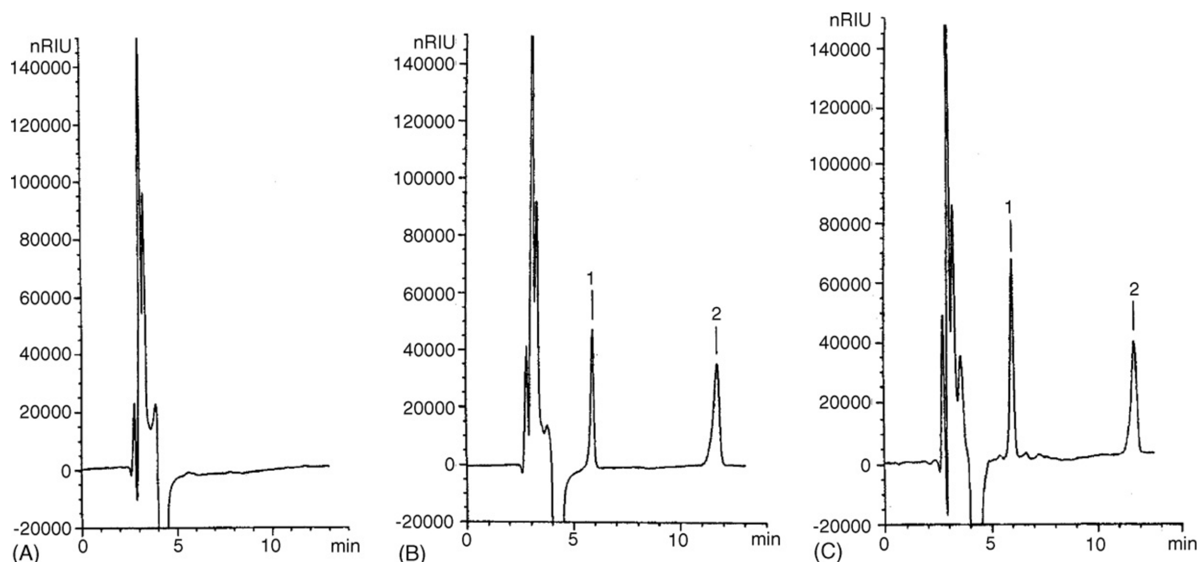

Fig. 2. Chromatograms of human urine samples: (A) blank urine; (B) 100 μl of urine spiked with 25 μl of 20 mg/ml concentration of treosulfan (1) and I.S. (2) (resulting in 5 mg/ml concentration of each analyte); (C) sample of a child obtained at 3 h after the start of infusion (6.8 mg/ml of treosulfan).

50.0–10000.0 μg/ml in urine. Equations of the standard curves are presented in Tables 2 and 3. The established equations of treosulfan were used to calculate plasma and urine concentrations in children following infusion of the drug. For the worked out technique LOD (S/N = 3:1) was 5.0 μg/ml in plasma and 25.0 μg/ml in urine. LOQ, defined as the lowest concentration in the standard curve at which CV $\leq$ 15% amounted to 10.0 μg/ml and 50.0 μg/ml in plasma and urine, respectively. Since the concentration of treosulfan in plasma and urine after infusion was high, the method was sensitive enough and suitable for pharmacokinetic studies of the drug in biological fluids.

3.2.3. *Precision, accuracy and recovery*

The designed technique was characterized by high precision of estimation of treosulfan concentrations within a day and between days both in plasma and urine, as proved by low values of the CV (<13%). Also the accuracy of calculation fitted the range accepted for an analytical method designed for pharmacokinetic studies, in which a percent error of concentration estimation cannot exceed 15% (Tables 2 and 3). Total recovery of treosulfan from plasma following centrifugation and microfiltration was in the range of 40.0–48.1% and was sufficient for pharmacokinetics studies of treosulfan in plasma.

Table 1
Tested medicines to confirm selectivity of the HPLC method

Medicines spiked into HPLC column	Medicines co-administered with treosulfan to patients
NSAIDs: ibuprofen, naproxen, diclofenac, ketoprofen, piroxicam	Antibiotics: amphotericin, cetepime dihydrochloride, co-trimoxazole, merpenem, teicoplanin, ticarcillin and clavulanic acid
Cardiovascular: prazosin, terazosin, losartan, furosemide	Antifungals: fluconazole, itraconazole
Anticonvulsant: carbamazepine, lamotrygine	Vitamins: K, D_3, folic acid
Other: codeine, phenacetine, busulfan, *p*-aminosalicylic acid, moclobemid, gliclazide	Other: ranitidine, ondansetron, clonazepam, cetirizine dihydrochloride, aminophylline, amlodipine, prednisone, estradiol

Table 2
Validation parameters of standard curve for analysis of treosulfan in human plasma[a]

Nominal concentration (μg/ml)	Mean assayed value (μg/ml)	Precision (%CV)	Accuracy (%error)
Intra-day repeatability ($n = 5$)			
50.0	45.19	6.41	9.61
1000.0	1030.35	2.58	3.04
Inter-day reproducibility ($n = 5$)			
10.0	9.90	7.40	1.02
25.0	24.08	8.78	3.69
50.0	48.15	7.61	3.69
100.0	106.47	7.19	6.47
250.0	230.60	7.29	7.76
500.0	472.71	1.57	5.46
1000.0	1032.37	3.67	3.24
2000.0	2001.07	1.36	0.31

[a] Equation of calibration curve for treosulfan: $y = 7.499 \times 10^{-4} \times c - 2.703 \times 10^{-3}$ ($r = 0.9997$).

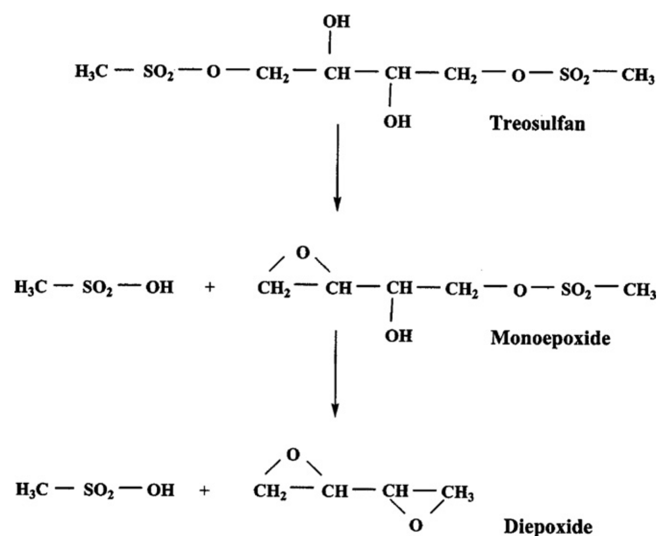

Fig. 3. Conversion pathway of treosulfan to active metabolites [11,12].

3.2.4. Stability of treosulfan; thaw and freeze test

The monoepoxide and diepoxide metabolites of treosulfan (Fig. 3) are formed by a nonenzymatic pH- and temperature-dependent intramolecular nucleophilic substitution, but at pH below 6.0 no transformation of treosulfan occurs [4,5]. The result caused that blood samples were acidified immediately after collecting of blood to avoid artificial excess. Improper preparation of blood samples would cause false pharmacokinetic results and in consequence would bias therapy of the ill children.

Treosulfan proved to be stable in acidified plasma and urine after two thaw and freeze cycles as demonstrated by CV < 9% and %error of estimation of treosulfan concentration <7% (Table 4).

3.3. Pharmacokinetic parameters

The elaborated method was used in pharmacokinetic studies on treosulfan after 2 h infusion of the drug. The pharmacokinetic profile in plasma was followed for up to 24 h after start of the infusion (Fig. 4). The time-related changes in plasma concentrations of treosulfan were described by a two-compartmental model. The mean terminal half-life of treosulfan in plasma was 1.9 h, in agreement with previously published reports related to adult patients treated with treosulfan administered orally or as 0.25 h, 0.5 h, 1.0 h, 2 h infusion [4–6,8]. Moreover, values of other pharmacokinetic parameters such as AUC, total clearance and volume of distribution were comparable to the literature data related to effects of 2 h infusion of treosulfan at different doses to adult patients (Table 5). After doses of 8–14 g/m^2 the respective results were similar to data obtained in presented study. However, differences appeared when treosulfan was administered in higher doses (20–56 g/m^2) and involved greater AUC and lower values of Cl and V_{ss}. Urinary excretion of the drug in children was amounted to about 30% of the total dose and varied over a wide range (21–45%), depending on renal function in a child. Analysis of treosulfan in biological fluids as the prodrug of active mono- and diepoxybutane derivatives

Table 3
Validation parameters of standard curve for analysis of treosulfan in urine[a]

Nominal concentration (μg/ml)	Mean assayed value (μg/ml)	Precision (%CV)	Accuracy (%error)
Intra-day repeatability ($n = 5$)			
100.0	89.70	6.81	10.30
5000.0	5024.85	1.77	0.50
Inter-day reproducibility ($n = 5$)			
50.0	44.65	12.79	10.71
100.0	95.16	10.64	4.84
250.0	230.31	6.95	7.88
500.0	511.54	6.22	2.31
1000.0	1046.69	4.69	4.67
2500.0	2566.15	7.00	2.65
5000.0	4893.79	3.11	2.12
10000.0	10029.63	1.70	0.30

[a] Equation of calibration curve for treosulfan: $y = 1.465 \times 10^{-4} \times c + 6.259 \times 10^{-3}$ ($r = 0.9998$).

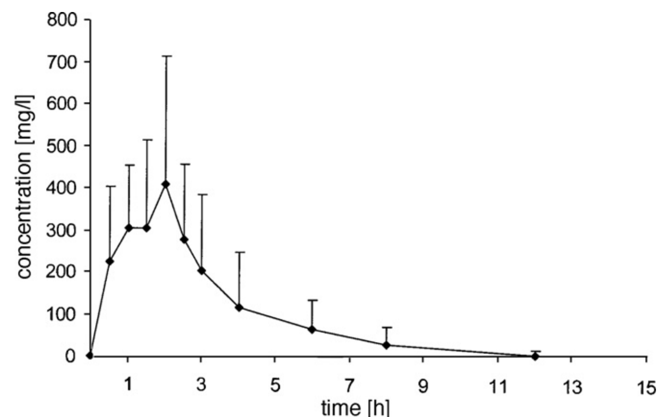

Fig. 4. Mean plasma treosulfan concentration as a function of time after 2 h infusion of the drug at dose of 5–23 g to five children.

Table 4
Stability of treosulfan in plasma and urine after thaw and freeze cycles

Matrix	Nominal concentration (μg/ml)	Mean assayed value (μg/ml)			Mean ± SD (μg/ml)	Precision (%CV)	Accuracy (%error)
		0 h	24 h	48 h			
Plasma	50.0	45.33	48.00	46.66	46.66 ± 1.34	2.87	6.68
	1000.0	988.00	978.00	1017.73	994.58 ± 20.66	2.08	0.54
Urine	100.0	97.20	86.96	104.03	96.08 ± 8.61	8.96	3.92
	5000.0	4912.90	4796.86	4322.46	4677.41 ± 312.83	6.69	6.45

Table 5
Comparison between pharmacokinetic parameters (mean ± SD or ranges) of treosulfan after single 2 h infusion to children and adults

Pharmacokinetic parameters	Children	Adults (literature data)				
	10–12 g/m^2	8 g/m^2 [4]	10 g/m^2 [4]	12 g/m^2 [6]	14 g/m^2 [6]	20–56 g/m^2 [8]
$t_{0.5}$ (h)	1.88 ± 0.36	1.75 ± 0.06	1.99 ± 0.61	2.1 ± 0.5	2.0 ± 0.6	2.02 ± 0.46
C_{max} (μg/ml)	402 ± 258	181 ± 36	306 ± 94	260 ± 35	322 ± 47	562–1660
$AUC_{0\to\infty}$ (μg/ml h)	1260 ± 949	541 ± 107	940 ± 293	898 ± 104	1104 ± 173	2120–8040
Cl_{tot} (ml/min)	265 ± 137	255 ± 59	190 ± 63	225 ± 23	216 ± 32	145.4 ± 30.3
V_{ss} (l)	29 ± 14	30 ± 8	26 ± 12	34 ± 5	31 ± 7	19.5 ± 3.8

may provide an indicator of its alkylating potency in individual patients.

4. Conclusion

The designed HPLC method with refractometric detection fulfils the validation requirements for quantitative analysis of drugs in biological samples. It is appropriately fast and enables the analysis of treosulfan within 15 min. Moreover, the method is specific, repeatable, reproducible, adequately accurate and precise therefore, it was successfully applied in pharmacokinetic studies on treosulfan after 2 h infusion of the drug to younger patients undergoing myeloablative treatment prior to haematopoietic stem cell transplantation. Pharmacokinetics of treosulfan in biological fluids of children after infusion of the drug demonstrates a similar pattern as in adults.

References

[1] M. Gropp, W. Meier, H. Hepp, Gynecol. Oncol. 71 (1998) 94.
[2] U. Keilholz, R. Schuster, A. Schmittel, N. Bechrakis, J. Siehl, M.H. Foerster, E. Thiel, Eur. J. Cancer 40 (2004) 2047.
[3] P. Kopf-Maier, G. Sass, Cancer Chemother. Pharmacol. 37 (1996) 211.
[4] R.A. Hilger, A. Harstrick, W. Eberhardt, C. Oberhoff, M. Skorzec, J. Baumgart, S. Seeber, M.E. Scheulen, Cancer Chemother. Pharmacol. 42 (1998) 99.
[5] R.A. Hilger, G. Jacek, C. Oberhoff, S. Kredtke, J. Baumgart, S. Seeber, M.E. Scheulen, Cancer Chemother. Pharmacol. 45 (2000) 483.
[6] D.W. Beelen, R. Trenschel, J. Casper, M. Freund, R.A. Hilger, M.E. Scheulen, N. Basara, A.A. Fauser, B. Hertenstein, H.A. Mylius, J. Baumgart, U. Pichlmeier, J.R. Hahn, E. Holler, Bone Marrow Transplant. 35 (2005) 233.
[7] J. Casper, W. Knauf, T. Kiefer, D. Wolff, B. Steiner, U. Hammer, R. Wegener, H.D. Kleine, S. Wilhelm, A. Knopp, G. Hartung, G. Dölken, M. Freund, Blood 103 (2004) 725.
[8] M.E. Scheulen, R.A. Hilger, C. Oberhoff, J. Casper, M. Freund, K.M. Josten, M. Bornhäuser, G. Ehninger, W.E. Berdel, J. Baumgart, A. Harstrick, P. Bojko, H.H. Wolf, A.E. Schindler, S. Seeber, Clin. Cancer Res. 6 (2000) 4209.
[9] M. Markiewicz, M. Wojciechowska, I. Wylezol, K. Wozniczka, S. Giebel, J. Wojnar, M. Krawczyk-Kulis, E. Mendek-Czajkowska, J. Holowiecki, Bone Marrow Transplant. 37 (2006) 231.
[10] J. Welsh, J.F.B. Stuart, M. Soukop, D. Cunningham, R. Blackie, G. Sangster, K.C. Calman, Br. J. Cancer 46 (1982) 467.
[11] J.A. Hartley, C.C. O'Hare, J. Baumgart, Br. J. Cancer 79 (1999) 264.
[12] A. Boobis, M. Rawlins, S. Thomas, M. Wilkins, in: C. Dollery (Ed.), The Drugs, Churchill Livingstone, Edinburgh, 1999, p. 161.