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Improved assay for determination of busulfan by liquid chromatography using postcolumn photolysis

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Abstract

A highly sensitive and time-reduced HPLC assay for the quantitative analysis of busulfan in plasma and aqueous samples is described. The assay is based on a precolumn derivatization of busulfan to 1,4-diiodobutane and UV-detection of iodide ions generated by a postcolumn photochemical dissociation of the derivative. The extraction and derivatization were carried out in a one-pot reaction without any solid phase extraction and is therefore suitable for high throughput analysis. Quantification was performed by using 1,5-pentanediol-bis-(methanesulfonate), a homologue of busulfan, as an internal standard. Linearity was demonstrated for concentrations from 50 to 10,000 ng/ml. The limit of detection was found at 10 ng/ml. Precision is indicated by an intra-day variety of 2.81% and by an inter-day variety of 6.61% for aqueous samples, 2.93 and 5.76% for plasma samples, respectively. The recovery of busulfan in plasma was more than 95%. No coelution with metabolites of busulfan or other drugs used in cancer therapy was found. The method was generated for measurements of busulfan in aqueous or plasma samples and applied in therapeutic drug monitoring of busulfan.
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Keyword: Busulfan

1. Introduction

1,4-bis(Methanesulfonyloxy)butane (busulfan), a bifunctional alkylating agent, is widely used as a cytostatic component in the preparative regimen for bone marrow transplantation. The individual bioavailability after oral busulfan administration differs highly and depends on many factors [1–6]. To reduce the variance in bioavailability of busulfan, different parenteral applications were tested in preclinical and clinical studies [7–11]. Furthermore, an influence of individual busulfan pharmacokinetics on engraftment and organ toxicity is discussed. [6,12–17]. It is most likely that, considering a possible correlation between busulfan plasma levels and the outcome of transplantation, the monitoring of busulfan pharmacokinetics have an important clinical impact [18,19]. Therefore, a highly sensitive and practicable method for analyzing plasma levels of busulfan is necessary.

Different busulfan assays were published. The described GC–MS methods [20–25] show a high reproducibility and sensitivity, but require a very expensive lab equipment.

Henner et al. [26] published a novel busulfan HPLC assay containing a precolumn diethyldithiocarbamate derivatization. With various results, other groups [27–31] attempted to reproduce and modify this method. Under the reaction and chromatographic conditions reported by Henner et al. [26]. Kazemifard and Morgan [28] described an incomplete derivatization reaction and a coelution of impurities. Based on the same derivatization of busulfan, Heggie et al. [29] described a very high recovery of the drug in plasma. Chow et al. [30] combined a diethyldithiocarbamate derivatization with a solvent- and solid-phase extraction but the overall recovery of busulfan decreased compared to the value published by Heggie et al. [29]. In addition, the assay showed increased between-day and within-day variations.

The busulfan determination carried out by an on-line LC–MS coupling method [32] did not require any

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derivatization. Compared with other assays the detection limit was unsatisfactory. Quernin et al. [33] developed a highly sensitive LC–MS method which is less expensive than previously published methods. However, the required devices for LC–MS analysis are not available in many laboratories.

Therefore, we updated a highly sensitive HPLC method using a postcolumn photolysis of the busulfan derivative 1,4-diiodobutane, published by Blanz et al. [34]. We introduced an internal standard and simplified the extraction and derivatization procedure. Without any solid phase extraction, busulfan and the internal standard were extracted and derived in a one-pot reaction. The changes resulted in a time reduced sample pretreatment, an increased reproducibility and an increased sensitivity of detection. This optimized HPLC method is suitable for a quantitative high throughput analysis of busulfan in plasma. The assay was applied for therapeutic drug monitoring of busulfan within a clinical study [35].

2. Experimental

2.1. Materials

Busulfan (98% purum), 1,4-diiodobutane and acetone were obtained from Sigma–Aldrich (Steinheim, Germany). HPLC grade ethyl acetate and sodium iodide (99.5%) were purchased from Merck (Darmstadt, Germany) and methanol was obtained from Riedel-de Haën (Seelze, Germany).

Water used for busulfan analysis was deionized and filtered with a Milli-Q plus PF water purification system (Millipore, Eschborn, Germany).

The following drugs of the respective suppliers were used to investigate the specificity of the method: heparin (5000 IU/ml) (Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany); diazepam (5 mg/ml) (AWD, Dresden, Germany); lorazepam (1 mg tablet) (Wyeth-Pharma GmbH, Münster, Germany); dolasetron (20 mg/ml) (Hoechst Marion Roussel, Bad Soden am Ts., Germany); tropisetron (1 mg/ml) (AWD, Dresden, Germany); cyclosporine (50 mg/ml) (Novartis Pharma GmbH, Nürnberg, Germany); prednisolone-21-hemisuccinate (5 mg/ml) (Jenapharm GmbH, Jena, Germany); allopurinol (300 mg tablet) (Hexal AG, Holzkirchen, Germany); methotrexate (2.5 mg/ml) (medac, Hamburg, Germany); cyclophosphamide (Sigma–Aldrich, Steinheim, Germany). The busulfan metabolites sulfolane (99%) and tetramethylene sulfoxide (96%) were obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Instrumentation

The HPLC system consisted of a Waters 626 pump (Waters, Eschborn, Germany) and a Waters 600s Controller connected to a Waters 717plus autosampler with an injection volume range of 0.1–2000 μl in 0.1 μl increments. The spectral detection was performed with a Spectra-Focus LC spectrometer (Spectra Physics, Egelsbach, Germany). Terminal output of the autosampler triggered the injection start signal to a Waters Interface module. Acquired data were processed by the Waters Maximum software. The solvent was degassed on-line by a vacuum membrane degasser (Thermo Separation Products, Egelsbach, Germany). Separation was performed on Lichrospher 100 RP-18 (5 μm) (LiChroCART HPLC Cartridge system 1254 mm × 4 mm; Merck, Darmstadt, Germany), protected with a LiChrospher 100 RP-18 (5 μm) guard column (LiChroCART, 4 mm × 4 mm, Merck). Samples were centrifuged with a ROTINA 48 R (Hettich Zentrifugen, Tuttlingen, F.R.G.).

2.3. Chromatographic conditions

The analysis was performed using an isocratic eluent system. The solvent system consisted of methanol and water (70:30; v/v). The flow rate was maintained at 1.2 ml/min. Chromatograms were monitored at 226 nm. The amounts of busulfan and the internal standard were evaluated using the respective peak areas. After finishing a sequence of plasma samples the HPLC column was washed with methanol for 1 h at a flow rate of 1.0 ml/min.

2.4. Synthesis of the internal standards

2.4.1. 1,3-Propanediol-bis-(methanesulfonate)

For the preparation of the propane homologue of busulfan, 0.25 mol methanesulfonylchloride and 0.5 mol pyridine were dissolved in dry chloroform (70 ml). 1,3-Propanediol (0.3 mol) dissolved in dry $CHCl_3$ (30 ml) was dropped slowly to the reaction mixture. Finally, the solution was stirred for 2 h. The reaction mixture was completely evaporated. The crude product was purified by recrystallisation in ethylacetate/hexane (60:40, v/v).

2.4.2. 1,5-Pentanediol-bis-(methanesulfonate)

The busulfan analog 1,5-pentanediol-bis-(methanesulfonate) was obtained from M. Hassan, Karolinska-Institutet (Huddinge, Sweden).

2.5. Sample treatment

Blood samples were obtained from patients receiving busulfan orally in 16 doses every 6 h on 4 consecutive days in the scope of a conditioning regimen before stem cell transplantation. The first dose of busulfan was fitted using ideal body weight (1 mg/kg). Further doses were adjusted to achieve busulfan plasma levels of 900 ± 100 ng/ml. Blood samples were collected just before and 1, 2, 4, and 6 h after application. The collected blood samples were placed into ice water (4 °C) immediately. After cooling, the samples were centrifuged for 10 min (400 × *g*) and the plasma layer

was transferred into cryo vials. Plasma samples were stored at −20 °C and thawed just prior to analysis.

2.6. Preparation of the standard solutions

The busulfan standards were set up as follows: 100 μl of a busulfan stock solution with a concentration of 500 ng/ml acetone and 5, 10, 20, 30, 40, 60, 80, 100, and 200 μl of another busulfan stock solution with a concentration of 50 μg/ml acetone were pipetted into a clean glass reaction vial. Furthermore, 40 μl of the internal standard solution (50 μg/ml acetone) were added. Then the acetone was evaporated to dryness. To obtain the final standard concentrations in a range of 50–10,000 ng/ml, busulfan and the internal standard were redissolved in 1 ml plasma or water by sonication for 5 min.

2.7. Preparation of the plasma samples

Forty microliters of the internal standard solution (50 μg/ml acetone) were placed into a clean glass reaction vial and the acetone evaporated to dryness. One-milliliter plasma was added and the standard dissolved by sonication for 5 min.

2.8. Extraction and derivatization procedure

A total of 1.5 ml of 8 M aqueous sodium iodide solution and 200 μl of HPLC grade methanol were added to each reaction vial containing the plasma. The samples were vortexed. After adding of 500 μl ethyl acetate the reaction vials were closed firmly. The bi-phasic solution in each vial was stirred with a micro-magnet for 60 min in a 70 °C hot water-bath. After cooling to room temperature, the organic phase (upper layer) was moved to an eppendorf cup and centrifuged for 5 min at 10,000 × g. An aliquot of 20 μl was injected into the HPLC column.

2.9. Assay validation

Calibration curves for plasma and water samples were set up in the following concentrations: 50, 250, 500, 1000, 1500, 2000, 3000, 4000, 5000, and 10,000 ng/ml. Linearity was checked by analysis of 10 sets of calibration curve samples. The intra-day variation was determined by analysis of five calibration curves. To investigate the inter-day variation, 10 sets of busulfan standard curve samples prepared on 10 different days were compared. The measurements were implemented in each case for plasma and aqueous samples. Accuracy and precision were evaluated using plasma control samples containing 500 and 4000 ng/ml busulfan, respectively, which were prepared at the start of the study and analyzed in the course of the investigation using currently prepared standard curves ($n = 10$).

The specificity of the assay was established by investigation of busulfan-free plasma from patients receiving several drugs in therapeutic doses and blank plasma spiked with the following busulfan metabolites or drugs used commonly in cancer therapy: sulfolane; tetrahydrothiophene-1-oxide (tetramethylene sulfoxide); heparin; diazepam; lorazepam; dolasetron; tropisetron; cyclosporine; prednisolone-21-hemisuccinate; allopurinol and cyclophosphamide.

The derivatization rate of two different busulfan concentrations was examined (500 and 5000 ng/ml). After the conversion of busulfan dissolved in water to 1,4-diiodobutane, the peak area was compared with the peak area of equimolar 1,4-diiodobutane added to the reaction solution instead of busulfan and worked up under the same reaction conditions ($n = 10$). To verify the extraction and derivatization of busulfan in plasma, the experiment was repeated for plasma samples. In an analogous procedure, the recovery of the internal standard was determined.

The limit of detection was found by examining the following busulfan concentrations in plasma: 1, 5, 10, 20, 50, 100 ng/ml.

For investigation of the stability of the busulfan derivative a calibration curve was set up with plasma. After the chemical reaction, the organic phase was removed and stored at room temperature. On 5 consecutive days, 20 μl of every sample were injected into the HPLC column and the peak areas were compared.

3. Results and discussion

3.1. Quantitative analysis

Quantification of busulfan were performed by plotting the peak area ratios of busulfan standard solutions consisting the internal standard (peak $\text{area}_{\text{busulfan}}$/peak $\text{area}_{\text{internal standard}}$) against known concentrations of spiked plasma or aqueous samples. Linearity was demonstrated for concentrations from 50 to 10,000 ng/ml. The coefficient of correlation was 0.999. Under the described conditions the peaks were detected with a retention time of 11.1 min for busulfan and 14.9 min for the internal standard. A summary of the statistical data for the measured calibration curves is given in Table 1. Fig. 1 shows chromatograms of blank plasma, plasma and aqueous samples spiked with busulfan resulting in a concentration of 50 and 500 ng/ml, respectively, and plasma from a patient receiving 1 mg/kg busulfan p.o.

Table 1
Statistical data for the calibration curves of busulfan

	Plasma samples	Aqueous samples
n	15	15
Concentration range (ng/ml)	50–10 000	50–10 000
Limit of detection (ng/ml)	10	10
Correlation coefficient	0.9998	0.9999
Intra-day variation ($n = 5$) (%)	2.93	2.81
Inter-day variation ($n = 10$) (%)	5.76	6.61

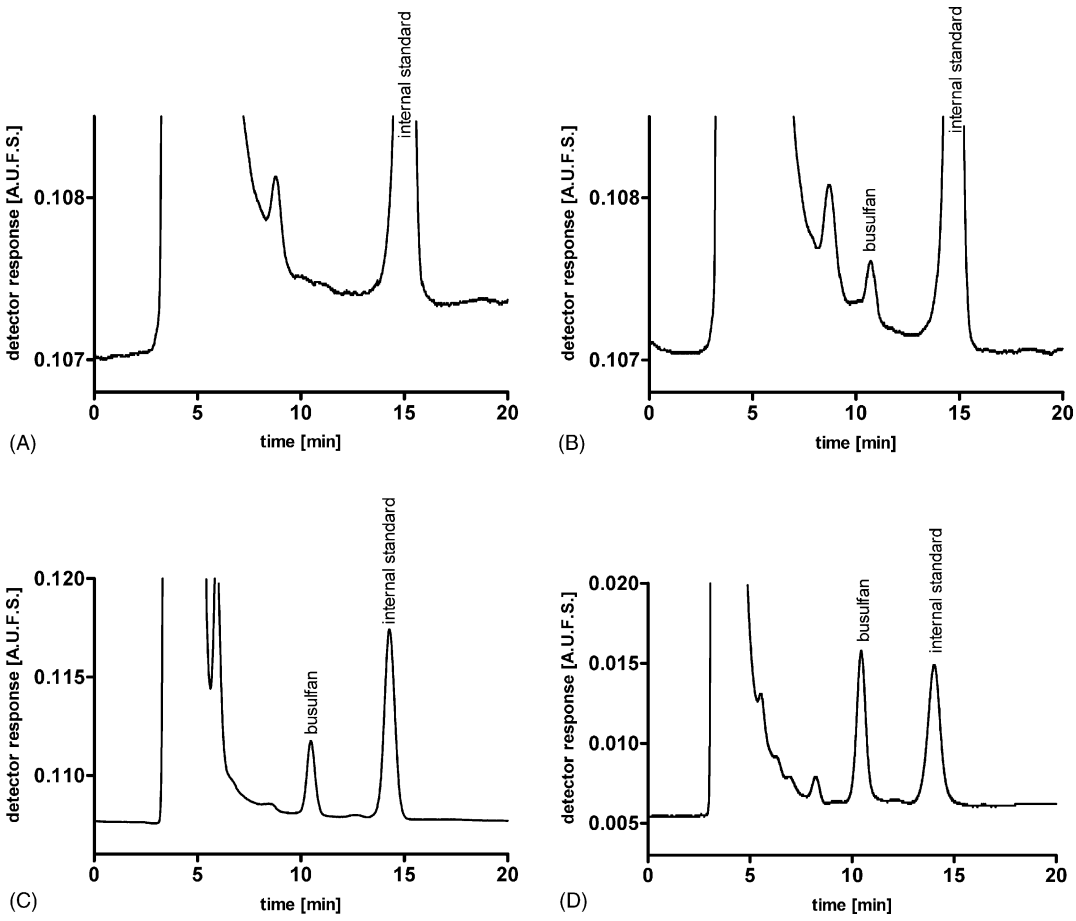

Fig. 1. (A) Chromatogram of busulfan free plasma sample; (B) pooled plasma spiked with 50 ng/ml busulfan (same scaling like chromatogram A); (C) aqueous sample containing 500 ng/ml busulfan; (D) plasma sample obtained from a patient 1 h after oral application of busulfan. The measured busulfan concentration was 1054 ng/ml.

3.2. Accuracy and precision

Precision and accuracy were determined for busulfan plasma concentrations of 500 and 4000 ng/ml. Precision, described as coefficient of variation ($n = 10$), was less than 5% for both concentrations. The accuracy was expressed as relative deviation of the mean calculated value from the spiked concentrations ($n = 10$) and was within an acceptable range. A summary of the results is shown in Table 2.

3.3. Reproducibility

To evaluate the reproducibility of the assay, the intra-day and the inter-day variation were determined. Standard solutions of different concentrations ranging between 50 and 10,000 ng/ml were set up fivefold and analyzed on one day. An intra-day variation of $2.81 \pm 0.04\%$ for water samples and $2.93 \pm 0.02\%$ for plasma samples, respectively, was detected (Table 3). The inter-day variation ($n = 10$) in quantification of busulfan was $6.61 \pm 0.06\%$ in aqueous samples, and $5.76 \pm 0.06\%$ in plasma, respectively (Table 4). Furthermore, standard solutions of 1,4-diiodobutane which were stored at room temperature were analyzed on 5 consecutive days. The results showed a high stability of the busulfan derivative (variation of 1.59%).

3.4. Sensitivity

The limit of detection was determined at a busulfan concentration of 10 ng/ml. At this concentration, the peaks of

Table 2
Precision and accuracy of the assay (C.V.: coefficient of variation), $n = 10$

Nominal busulfan plasma conc. (ng/ml)	Mean calculated conc. (ng/ml)	Precision (C.V., %)	Accuracy ((mean calculated conc./nominal conc.) × 100) (%)
500	515 ± 16.8	3.26	103.1
4000	3992 ± 31.5	0.79	99.8

Table 3
Intra-day precision of busulfan calibration curves in plasma and aqueous samples (S.D.: standard deviation, C.V.: coefficient of variation)

Concentration (ng/ml)	Peak area busulfan/peak area standard							
	Plasma samples				Aqueous samples			
	Mean	S.D. (ng/ml)	C.V. (%)	*n*	mean	S.D. (ng/ml)	C.V. (%)	*n*
50	0.035	0.0029	8.23	5	0.036	0.0049	13.71	5
250	0.181	0.0082	4.54	5	0.163	0.0070	4.29	5
500	0.354	0.0067	1.88	5	0.332	0.0039	1.18	5
1000	0.726	0.0179	2.47	5	0.690	0.0112	1.62	5
1500	1.099	0.0206	1.87	5	1.043	0.0066	0.63	5
2000	1.379	0.0230	1.67	5	1.415	0.0153	1.08	5
3000	2.236	0.0651	2.91	5	2.107	0.0176	0.83	5
4000	2.944	0.0387	1.31	5	2.848	0.0491	1.72	5
5000	3.569	0.0933	2.62	5	3.554	0.0415	1.17	5
10000	7.237	0.1304	1.80	5	7.188	0.1320	1.84	5

busulfan and the internal standard were detectable with a signal-to-noise ratio of 5. Regarding the sensitivity, no difference between water and plasma samples was observed.

3.5. Recovery

To investigate the extraction and derivatization behavior of busulfan in plasma, the analyses were carried out at concentrations of 500 and 5000 ng/ml. For both concentrations the average peak areas of several busulfan preparations and the derivative 1,4-diiodobutane, injected directly in equimolar concentrations, were determined ($n = 20$). The recovery of busulfan was calculated by comparing the respective peak areas. Consequently, the recovery of busulfan in plasma was 95% at a concentration of 500 ng/ml and 96.2% at a concentration of 5000 ng/ml.

In an analogous manner, the recovery of busulfan dissolved in water was investigated. Assuming that the extraction rate of busulfan from water is almost 100%, the recovery depends on derivatization only. Accordingly, the derivatization of busulfan in water is 96.4% at a concentration of 500 ng/ml, and 96.8% at 5000 ng/ml, respectively.

Under acceptance of a constant derivatization behavior in plasma and water (under constant reaction conditions) the extraction of busulfan from plasma was calculated by comparing the recoveries of busulfan dissolved in water and in plasma. The resulted average busulfan extraction from plasma was found to be 98.9±0.4%. The mean derivatization of busulfan was 96.6 ± 0.2%. The overall recovery of the determination of busulfan in plasma can be indicated with 95.6 ± 0.6%.

3.6. Specificity

In clinical investigations a high specificity of the busulfan analyses is required. Therefore, the elution behavior of the following drugs was investigated: heparin, diazepam, lorazepam, dolasetron, cyclosporine, prednisolone-21-hemisuccinate, allopurinol, methotrexate, cyclophosphamide, sulfolane and tetramethylene sulfoxide. The analyzed drugs and busulfan metabolites were eluted with the solvent front or not detected, respectively. Interferences with busulfan and the internal standard were not observed.

In addition, busulfan-free plasma from patients who received several of these drugs was checked. There was no coelution with busulfan in all investigated samples.

Table 4
Inter-day precision of busulfan calibration curves in plasma and aqueous samples (S.D.: standard deviation, C.V.: coefficient of variation)

Concentration (ng/ml)	Peak area busulfan/peak area standard							
	Plasma samples				Aqueous samples			
	Mean	S.D. (ng/ml)	C.V. (%)	*n*	Mean	S.D. (ng/ml)	C.V. (%)	*n*
50	0.028	0.0068	24.23	9	0.028	0.0069	24.82	8
250	0.148	0.0094	6.33	10	0.162	0.0136	8.36	9
500	0.323	0.0161	4.98	10	0.342	0.0166	4.87	10
1000	0.685	0.0196	2.86	10	0.697	0.0288	4.13	10
1500	1.034	0.0391	3.78	10	1.056	0.0369	3.49	10
2000	1.384	0.0286	2.07	10	1.432	0.0526	3.67	10
3000	2.184	0.0752	3.45	10	2.193	0.0910	4.15	10
4000	2.907	0.0651	2.24	10	2.827	0.1194	4.22	10
5000	3.657	0.1243	3.40	10	3.502	0.1526	4.36	10
10000	7.368	0.2734	3.71	9	7.332	0.2971	4.05	9

3.7. Internal Standard

1,3-Propanediol-bis-(methanesulfonate) and 1,5-pentanediol-bis-(methanesulfonate), two substances of the homologous series of busulfan, were tested for use as an internal standard. Both compounds are subject to the same derivatization reaction as busulfan. Iodide anions detected at $\lambda_{max} = 226$ nm were split by a photochemical reaction of the diiodo-derivatives. In contrast to assays published by other groups [27,28], the detected equivalents of the pre-column synthesized derivatives of the internal standard and of busulfan are identical, which allowed to analyze them at the same wavelength with a maximum absorption.

Both substances, 1,3-propanediol-bis-(methanesulfonate) and 1,5-pentanediol-bis-(methanesulfonate), satisfy the demands on an internal standard, but they differed in their retention time. 1,3-Propanediol-bis-(methanesulfonate) was detected at 8.2 min and 1,5-pentanediol-bis-(methanesulfonate) at 14.9 min. Because of coelution of 1,3-propanediol-bis-(methanesulfonate) with coextracted plasma components, 1,5-pentanediol-bis-(methanesulfonate) was chosen for using as internal standard.

By quantitative analysis of the internal standard, linearity was proven for concentrations from 50 to 10,000 ng/ml with a correlation coefficient of 0.999. The overall recovery of the internal standard in plasma was 94.2%. No interferences were detectable with the examined drugs and metabolites.

3.8. Therapeutic drug monitoring

The assay was used to verify busulfan plasma levels in patients receiving busulfan as part of a preparative regimen before human stem cell transplantation. We evaluated a therapeutic drug monitoring in thirteen patients with myelodysplastic syndrome or relapsed acute myeloid leukemia with high risk of regimen related toxicity undergoing stem cell transplantation. Busulfan dosages were adjusted to achieve busulfan Css (concentration at steady state) of 900 ± 100 ng/ml. All patients received a dose reduced conditioning regimen consisting of targeted busulfan and fludarabin intravenously (4×30 mg/m^2). Busulfan was administered orally in 16 doses every 6 h. The first dose of busulfan was fitted using ideal body weight (1 mg/kg). Busulfan plasma levels were determined after dose 1, 5, 9 and if necessary after dose 13. Fig. 1 shows a representative chromatogram of a plasma sample, obtained from a 34 year old male 1 h after oral application. A busulfan plasma concentration of 1054 ng/ml was measured. The respective pharmacokinetic curve (Fig. 2) was fitted with the TOPFIT-software [36] using an one-compartment model.

Corresponding the measured Css levels busulfan doses 4, 8, 12, and 16 were adjusted. The mean starting dose of busulfan was 0.89 ± 0.097 mg/kg actual body weight (1 mg/kg ideal body weight). Dose modifications lead to the expected changes in drug exposure. The correlation between dose adjustments and resulting changes of plasma concentrations is shown in Fig. 3. In 10 patients a dose reduction undergoing the administration of busulfan was necessary to achieve the target levels. The totally administered dose of busulfan ranged from 9.9 to 18 mg/kg with a median dosage of 13.8 mg/kg. The average Css after doses 5 and 9 ranged from 808 to 1155 ng/ml with a median value of 934 ng/ml ($n = 13$). An average exposure over 4 days of treatment including plasma levels of dose 1, 5, 9, and 13 could be obtained in seven patients and ranged from 806 to 1128 ng/ml (median 973 ng/ml). All patients receiving the described conditioning regimen had a stable engraftment of peripheral blood stem cells from all related and unrelated donors.

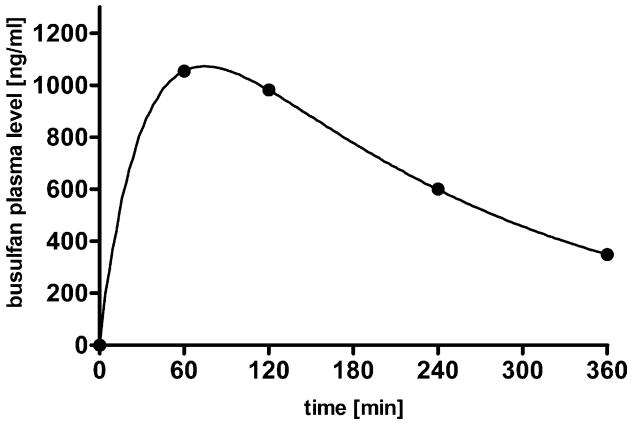

Fig. 2. Concentration versus time curve of busulfan in plasma after first dose of 1 mg/kg.

During the whole time period for this study no problems with the HPLC equipment or an instability of the described assay proofed by the plasma control samples occurred, demonstrating the ability of the method for routine just-in-time measurements. In conclusion, the therapeutic drug monitoring of busulfan using the described HPLC method is a feasible procedure in patients receiving human stem cell transplantation to minimize the risk of regimen related toxicity.

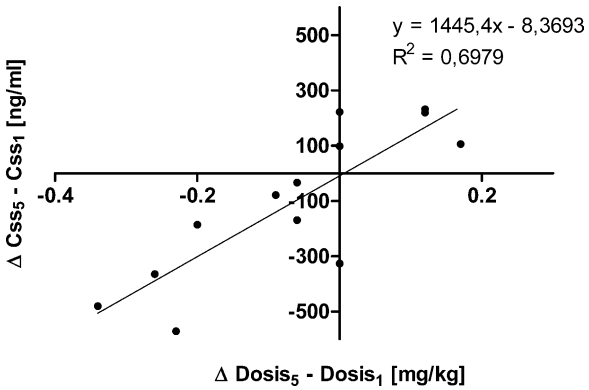

Fig. 3. Correlation of dose adjustment and change in following Css level.

4. Conclusion

An HPLC method for quantitative analysis of busulfan was developed, characterized by high sensitivity and precision and by a time reduced sample pretreatment. The advantages of the assay published by Blanz et al. [34], e.g. the photochemical online derivatization and the excellent selectivity, were combined with a simple and robust sample pretreatment. Furthermore, an internal standard was introduced increasing the safety of the measurement results. Completing the extraction and derivatization in one step without a solid phase extraction, the efficiency of the assay was improved and the financial costs per measurement were diminished. After finishing the one-pot reaction an aliquot of the upper layer was directly injected on the column without an exchange of the solvent, resulting in a simplified analysis, too. The small number of preparation steps, necessary for working up a busulfan plasma sample, decreased the error rate. This was a prerequisite for a high precision of the assay. These advantages combined with a time reduced sample pretreatment enabled a therapeutic drug monitoring of busulfan using HPLC.

The therapeutic drug monitoring of busulfan is a practicable procedure to minimize the risk of regimen related toxicity. Prospective and randomized clinical studies are warranted to investigate the impact of busulfan dose adjustment on the outcome after allogeneic blood stem cell transplantation.

The method described here is suitable for the quantitative analysis of busulfan in plasma or aqueous samples in clinical settings by means of HPLC.

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References

[1] M. Hassan, P. Ljungman, P. Bolme, O. Ringden, Z. Syruckova, A. Bekassy, J. Stary, I. Wallin, N. Kallberg, Blood 84 (1994) 2144.
[2] M. Hassan, H. Ehrsson, P. Ljungman, Leuk. Lymphoma. 22 (1996) 395.
[3] K. Voges, H.J. Kolb, J. Mittermüller, U. Jehn, W. Wilmanns, V. Heinemann, Ann. Hematol. 70 (Suppl. II) (1995) A128.
[4] P.J. Shaw, C.E. Scharping, R.J. Brian, J.W. Earl, Blood 84 (1994) 2357.
[5] U. Schuler, S. Schroer, A. Kuhnle, J. Blanz, K. Mewes, I. Kumbier, B. Proksch, K.P. Zeller, G. Ehninger, Bone Marrow Transplant. 14 (1994) 759.
[6] I. Buggia, F. Locatelli, M.B. Regazzi, M. Zecca, Ann. Pharmacother. 28 (1994) 1055.
[7] G. Ehninger, U. Schuler, U. Renner, M. Ehrsam, K.P. Zeller, J. Blanz, R. Storb, H.J. Deeg, Blood 85 (1995) 3247.
[8] M. Hassan, Z. Hassan, C. Nilsson, M.A. Rehim, S. Kumlien, B. Elfsson, N. Kallberg, Cancer Chemother. Pharmacol. 42 (1998) 471.
[9] H.J. Deeg, U.S. Schuler, H. Shulman, M. Ehrsam, U. Renner, C. Yu, R. Storb, G. Ebninger, Biol. Blood Marrow Transplant. 5 (1999) 316.
[10] U.S. Schuler, M. Ehrsam, A. Schneider, H. Schmidt, J. Deeg, G. Ehninger, Bone Marrow Transplant. 22 (1998) 241.
[11] H.P. Bhagwatwar, S. Phadungpojna, D.S.L. Chow, B.S. Andersson, Cancer Chemother. Pharmacol. 37 (1996) 401.
[12] G. Vassal, S. Koscielny, D. Challine, D. Valteau-Couanet, I. Boland, A. Deroussent, J. Lemerle, A. Gouyette, O. Hartmann, Cancer Chemother. Pharmacol. 37 (1996) 247.
[13] J.T. Slattery, J.E. Sanders, C.D. Buckner, R.L. Schaffer, K.W. Lambert, F.P. Langer, C. Anasetti, W.I. Bensinger, L.D. Fisher, F.R. Appelbaum, et al., Bone Marrow Transplant. 16 (1995) 31.
[14] E. Masson, W.C. Zamboni, Clin Pharmacokinet. 32 (1997) 324.
[15] W.I. Bensinger, K.S. Schiffman, L. Holmberg, F.R. Appelbaum, R. Maziarz, P. Montgomery, E. Ellis, S. Rivkin, P. Weiden, K. Lilleby, S. Rowley, S. Petersdorf, J.P. Klarnet, W. Nichols, A. Hertler, R. McCroskey, C.H. Weaver, C.D. Buckner, Bone Marrow Transplant. 19 (1997) 1183.
[16] S.M. deMagalhaes, E.J. Bloom, A. Donnenberg, J. Lister, S. Pincus, W.B. Rybka, E.D. Ball, Bone Marrow Transplant. 17 (1996) 329.
[17] L.B. Grochow, Semin. Oncol. 20 (1993) 18.
[18] J.T. Slattery, R.A. Clift, C.D. Buckner, J. Radich, B. Storer, W.I. Bensinger, E. Soll, C. Anasetti, R. Bowden, E. Bryant, T. Chauncey, H.J. Deeg, K.C. Doney, M. Flowers, T. Gooley, J.A. Hansen, P.J. Martin, G.B. McDonald, R. Nash, E.W. Petersdorf, J.E. Sanders, G. Schoch, P. Stewart, R. Storb, K.M. Sullivan, E.D. Thomas, R.P. Witherspoon, F.R. Appelbaum, Blood 89 (1997) 3055.
[19] H.J. Deeg, H.M. Shulman, J.E. Anderson, E.M. Bryant, T.A. Gooley, J.T. Slattery, C. Anasetti, A. Fefer, R. Storb, F.R. Appelbaum, Blood 95 (2000) 1188.
[20] M. Hassan, H. Ehrsson, J. Chromatogr. 434 (1988) 330.
[21] G. Vassal, A. Gouyette, O. Hartmann, J.L. Pico, J. Lemerle, Cancer Chemother. Pharmacol. 24 (1989) 386.
[22] T.L. Chen, L.B. Grochow, L.A. Hurowitz, R.B. Brundrett, J. Chromatogr. 425 (1988) 303.
[23] L. Embree, R.B. Burns, J.R. Heggie, G.L. Phillips, D.E. Reece, J.J. Spinelli, D.O. Hartley, N.J. Hudon, J.H. Goldie, Cancer Chemother.Pharmacol. 32 (1993) 137.
[24] W.K. Lai, C.P. Pang, L.K. Law, R. Wong, C.K. Li, P.M. Yuen, Clin. Chem. 44 (1998) 2506.
[25] M. Abdel-Rehim, Z. Hassan, L. Blomberg, M. Hassan, Ther. Drug Monit. 25 (2003) 400.
[26] W.D. Henner, E.A. Furlong, M.D. Flaherty, T.C. Shea, W.P. Peters, J. Chromatogr. 416 (1987) 426.
[27] J.J. MacKichan, T.P. Bechtel, J. Chromatogr. 532 (1990) 424.
[28] A.G. Kazemifard, D.J. Morgan, J. Chromatogr. 528 (1990) 274.
[29] J.R. Heggie, M. Wu, R.B. Burns, C.S. Ng, H.C. Fung, G. Knight, M.J. Barnett, J.J. Spinelli, L. Embree, J. Chromatogr. B. Biomed. Sci. Appl. 692 (1997) 437.
[30] D.S. Chow, H.P. Bhagwatwar, S. Phadungpojna, B.S. Andersson, J. Chromatogr. B. Biomed. Sci. Appl. 704 (1997) 277.
[31] N. Bleyzac, P. Barou, G. Aulagner, J. Chromatogr. B. Biomed. Appl. 742 (2000) 427.
[32] S. Pichini, I. Altieri, A. Bacosi, S. Di-Carlo, P. Zuccaro, P. Iannetti, R. Pacifici, J. Chromatogr. 581 (1992) 143.
[33] M.H. Quernin, M. Duval, C. Litalien, E. Vilmer, E.J. Aigrain, J. Chromatogr. B. Biomed. Appl. 763 (2001) 61.
[34] J. Blanz, C. Rosenfeld, B. Proksch, G. Ehninger, K.P. Zeller, J. Chromatogr. 532 (1990) 429.
[35] M. Bornhauser, B. Storer, J.T. Slattery, F.R. Appelbaum, H.J. Deeg, J. Hansen, P.J. Martin, G.B. McDonald, W.G. Nichols, J. Radich, A. Woolfrey, A. Jenke, E. Schleyer, C. Thiede, G. Ehninger, C. Anasetti, Blood 102 (2003) 820.
[36] G. Heinzel, R. Woloszczak, P. Thomann, Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC, Gustav Fischer, Stuttgart, Jena, New York, 1993.