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Short communication

High-performance liquid chromatographic method for quantification of busulfan in plasma after derivatization by tetrafluorothiophenol

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Abstract

A high-performance liquid chromatographic (HPLC) method was developed and validated for the determination of busulfan in plasma. Busulfan was extracted in toluene, derivatized by 2,3,5,6-tetrafluorothiophenol to obtain di-TFTP-butane, the derivatization product was then re-extracted in toluene and injected into the HPLC system with ultraviolet detection (wavelength: 275 nm). Recovery from extraction was 80%, the limit of quantification was 50 ng/ml and linearity ranged from 50 to 2000 ng/ml. In addition, forty-two samples obtained from pediatric patients treated with busulfan were analyzed by the HPLC and GC–MS assays based on the same derivatization procedure. The correlation between the di-TFTP-butane concentrations was highly significant ($p<0.0001$), demonstrating that the two methods were in good agreement.

Keywords: Derivatization, LC; Busulfan

1. Introduction

Busulfan (1,4-butanediol dimethanesulfonate) (Fig. 1A) is an alkylating agent, currently administered at high oral doses in preparative regimes for bone marrow transplantation for patients with haematological malignancies and nonmalignant disorders [1–3]. The pharmacokinetics and toxicity of busulfan are highly variable in treated patients [4] and the benefits of individual dose adjustment based on the determination of individual pharmacokinetic parameters are under investigation [5].

We initially developed a GC–MS method, based on the determination of derivatized busulfan (di-TFTP-butane) (Fig. 1B) by the tetrafluorothiophenol (TFTP) to determine busulfan concentrations in plasma [6]. However, although sensitive and easy to perform, the GC–MS method has disadvantages limiting its use for routine analysis, as the method is time consuming and expensive and a GC–MS apparatus is not available in every laboratory. Other

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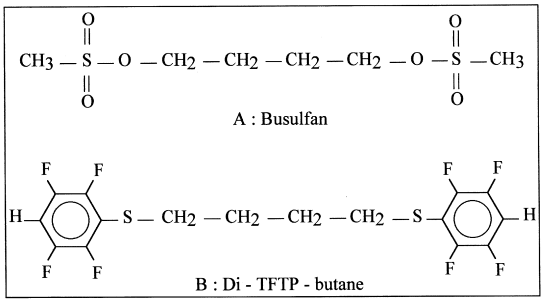

Fig. 1. Chemical structure of busulfan (A) and di-TFTP-butane (B)

methods have been described including HPLC–UV methods [7–13], but because busulfan has a poor absorption spectrum in ultraviolet, they are all based on derivatization. The derivatization used in our GC–MS assay appeared very stable and resistant to heating and this prompted us to validate an HPLC method with ultraviolet detection. This HPLC method is reported here and compared to the corresponding GC–MS method using the same derivatization.

2. Experimental

2.1. Reagents and materials

Busulfan and 2,3,5,6-tetrafluorothiophenol (TFTP) were purchased from Aldrich (Saint Quentin Fallavier, France). All the solvents used were analytical grade. Micro-spin filters with nylon 66 membrane in 0.45-μm pore sizes were obtained from Cluzeau Info-Labo (Sainte Foy La Grande, France).

2.2. HPLC system

The HPLC system consisted of a Spectra-Physics model 8800 pump (SP, Santa Clara, CA, USA), a Spectra-Physics SP8780 autosampler and a SP 8450 UV detector (275 nm). We used a C_{18} 5-μm particle size Ultrasphere ODS column (Beckman, Palo Alto, CN, USA). The mobile phase was a mixture of methanol/water (80:20, v/v) and the flow-rate was 1 ml/min.

2.3. Busulfan assay in plasma

A stock solution of busulfan (500 μg/ml) was prepared in acetone and stored in 1-ml aliquots at −20°C. Calibration standards (50 to 2000 ng/ml) and plasma controls (400 and 700 ng/ml) were prepared by appropriate dilutions of the stock solution in drug-free plasma. The controls were stable over one year at −20°C.

The derivatization and extraction procedures were adapted from our previous GC–MS method [6]. Briefly, 1 ml of plasma was extracted with 3 ml of toluene and the organic layer was evaporated under nitrogen. The residue was dissolved in a mixture of 1.5 *M* TFTP in methanol (20 μl), water (200 μl) and 1 *M* sodium hydroxide (20 μl) and heated at 70°C for 2 h. After derivatization, 3 ml of 1 *M* sodium hydroxide and 4 ml of toluene were added and the organic layer dried under nitrogen. The residue was dissolved in 500 μl of the mobile phase, filtered on a micro-spin filter and a 50-μl aliquot was injected into the HPLC system.

2.4. Comparison of the GC–MS and HPLC methods

Forty-two plasma samples were obtained from pediatric patients treated with busulfan at the oral dose of 1 mg/kg every 6 h for 16 doses and plasma concentrations of di-TFTP-butane were determined with the two methods. During the GC–MS assay procedure, plasma samples containing busulfan-d8 as internal standard were extracted in ethylacetate and derivatized with TFTP. After derivatization, di-TFTP-butane and di-TFTP-butane-d8 were re-extracted by ethyl acetate.

The following drugs, potentially co-administered with busulfan were tested for interferences with the assay procedure: omeprazole, fluconazole, sulfamethoxazole, heparin, trimethoprim, acyclovir, clonazepam, ondansteron.

Statistical calculations were performed using a

commercially available program (STATVIEW, Abacus Concepts, Berkeley, USA).

3. Results

3.1. HPLC analysis

Under the chromatographic conditions that we used, the retention time of di-TFTP-butane was 27.0±0.8 min. No interferences were detected at the retention time of di-TFTP-butane (Fig. 2A). A representative chromatogram obtained 30 min after busulfan intake is shown in Fig. 2B. The identification of di-TFTP-butane was confirmed by GC–MS according to our method previously published. The mass spectrum of di-TFTP-butane is presented Fig. 3.

Recovery from extraction measured at 250 ng/ml busulfan was 80% ($n=10$).

The sensitivity of the assay, corresponding to a signal-to-noise ratio of 3, was 25 ng/ml. The limit of quantification of the assay was 50 ng/ml.

For the calibration curves, the areas of di-TFTP-butane were plotted against the corresponding busulfan concentrations. The calibration curves were linear over the concentration range of 50 and 2000 ng/ml. The correlation equation was $Y=138.3X$ and the coefficient of variation of the slope of the calibration curves was 1.9% ($n=4$).

The intra and inter-assay coefficients of variation, determined from measurements of di-TFTP-butane from calibration standards and two quality controls (400 and 700 ng/ml) were less than 10% and recoveries were between 92 and 102% (Table 1).

3.1.1. Comparison with the GC–MS assay using the same TFTP derivatization

Forty-two plasma samples were obtained from paediatric patients receiving busulfan for therapeutic reasons and busulfan concentrations were determined by GC–MS and HLPC. The plasma concentrations were 660±399 ng/ml and 642±352 ng/ml (mean±standard deviation) respectively with the GC–MS (range 80–1821 ng/ml) and the HPLC (range 124–1644 ng/ml) assays. There was a significant correlation between the concentrations measured by the two methods ($r^2=0.958$, $p<0.0001$) (Fig. 4).

4. Discussion

Busulfan concentrations in plasma were initially determined in our laboratory by GC–MS. All the GC–MS methods reported include a derivatization of busulfan either with sodium iodide or with tetrafluorothiophenol (TFTP). We have previously shown that the derivatization with TFTP is simple, performed in a single phase, stable and resistant to heating. In addition we have shown the good correlation between the concentrations of di-TFTP-butane and diiodobutane measured by GC–MS [6]. However, the routine use of GC–MS assays is time consuming and expensive and a GC–MS apparatus is not commonly available unlike HPLC systems.

The absorption of busulfan in the ultra violet wavelength region is poor and in the literature, the HPLC methods for the quantification of busulfan require a derivatization step. Derivatization was performed either with sodium iodide and post-column photolysis [7], or with thiocresol using direct quantification by UV detection [8] or with sodium diethyldithiocarbamate requiring on-line derivatization [9] or with direct UV detection [10–13].

We adapted our previous GC–MS assay in order to quantify busulfan by HPLC. Following the same derivatization procedure, we modified the extraction and used toluene instead of ethylacetate to clean the samples and to obtain chromatograms free of interferences. Recoveries from extraction measured at 250 ng/ml busulfan were similar: 78% in ethylacetate (GC–MS) versus 80% in toluene (HPLC). However, the GC–MS assay has a better sensitivity (limit of quantification 20 ng/ml) than the HPLC assay (limit of quantification 50 ng/ml). This should not be a disadvantage for the HPLC assay, as in our experience with the pharmacokinetics of busulfan in children treated with high doses, a sensitivity of 50 ng/ml was sufficient.

The HPLC method described above is simple. It does not involve post-column derivatization and di-TFTP-butane is measured directly by UV detection at 275 nm. In addition, and in contrast with the

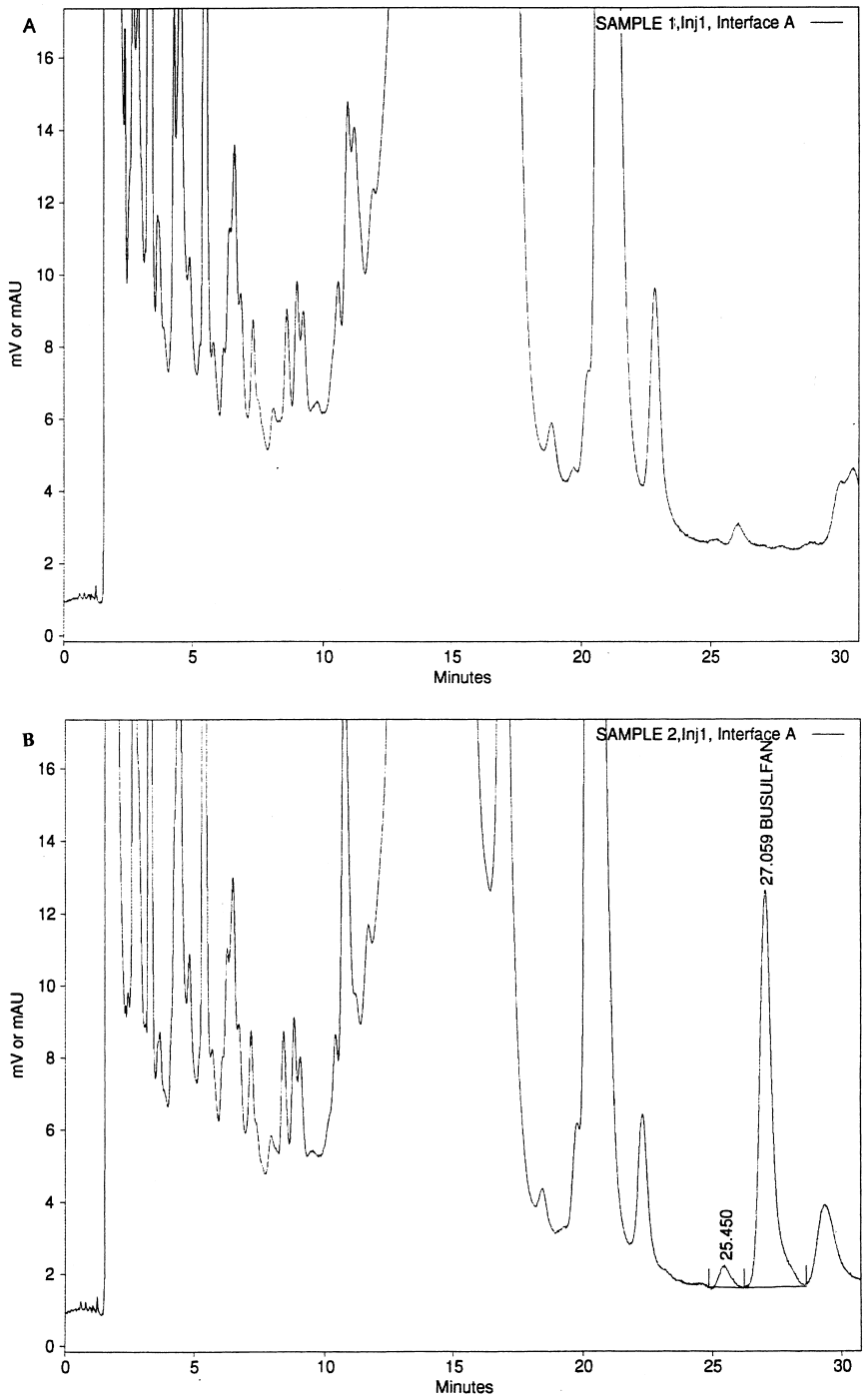

Fig. 2. Representative chromatograms obtained from a patient: (A) plasma before busulfan intake (B) plasma obtained 30 min after an oral administration of 1 mg/kg and containing 672 ng/ml of busulfan.

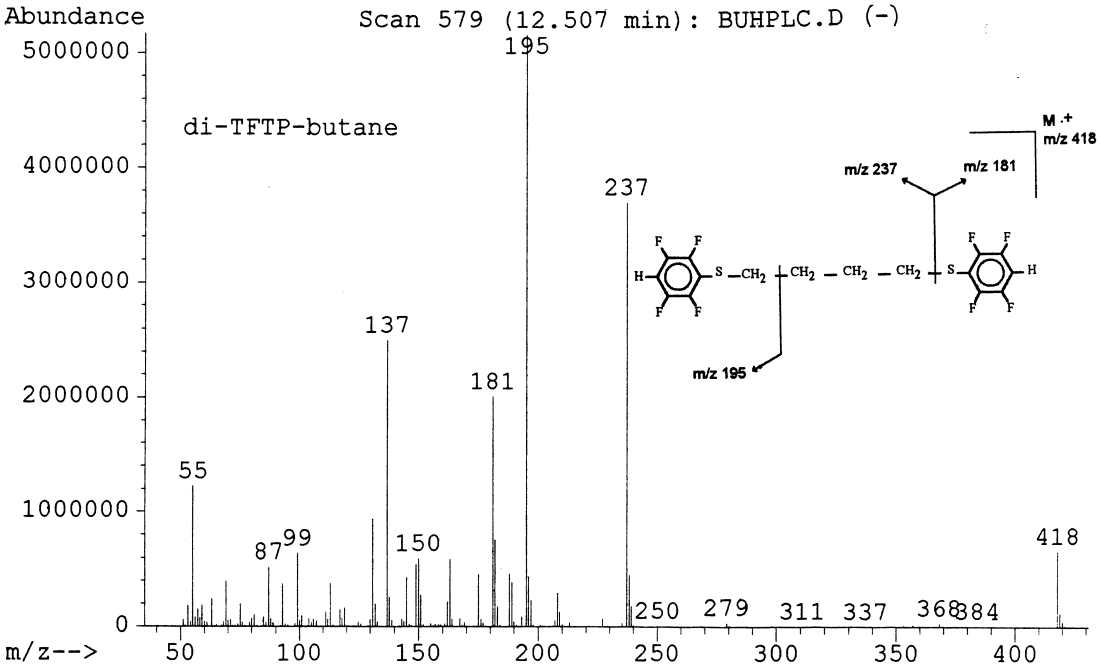

Fig. 3. Mass spectra of di-TFTP-butane after EI (electron impact).

GC–MS assay, it requires an apparatus available in most laboratories. The correlation between the GC–MS and HPLC methods using the same derivatization was very good.

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Table 1
Accuracy and precision of the method of quantification of busulfan in plasma by HPLC–UV detection following derivatization with tetrafluorothiophenol

Busulfan concentration (ng/ml)	*n*		Mean concentration found (ng/ml)		C.V. (%)		Recovery (%)	
	Intra-assay	Inter-assay	Intra-assay	Inter-assay	Intra-assay	Inter-assay	Intra-assay	Inter-assay
Calibration standards								
50	4	4	50.0	52.5	3.2	9.1	100.0	105.5
100	4	4	91.8	100.7	8.9	8.8	91.8	100.7
250	4	4	247.8	247.0	5.0	4.5	99.1	99.8
500	4	4	494.8	508.7	6.5	2.0	99.0	101.7
1000	4	4	1006.8	992.5	1.1	1.1	100.7	99.7
2000	4	4	2013.8	2007.0	1.1	0.5	100.7	100.4
QC samples								
400	4	4	401.2	405.0	0.8	0.6	100.3	101.3
700	4	4	702.0	707.5	0.78	0.7	100.3	101.7

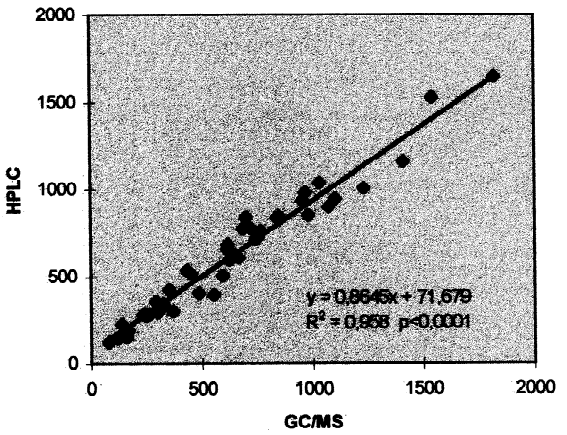

Fig. 4. Comparison of the concentration of di-TFTP-butane in forty-two plasma samples with GC–MS and HPLC.

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