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Short Communication

High-performance liquid chromatographic–mass spectrometric assay of busulfan in serum and cerebrospinal fluid

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ABSTRACT

A liquid chromatographic–mass spectrometric method has been developed to determine busulfan concentrations in the cerebrospinal fluid and serum of some children undergoing bone marrow autotransplantation. After two liquid–liquid extraction steps with dichloromethane on a biological matrix, the separation of busulfan was carried out by isocratic reversed-phase chromatography. The mass spectrometric system was operated in electron-impact mode. Principal ions at *m/z* 175, 111 and 79 were observed for busulfan, but only *m/z* 175 was chosen for the quantification of the analyte. The retention time of busulfan was 2.5 min. The detection limit of 100 ng/ml allowed the determination of cerebrospinal fluid and serum busulfan concentrations during the four days of high-dose (1 mg/kg) treatment prior to autotransplantation in five child patients.

INTRODUCTION

Busulfan (1,4-butanediol dimethanesulphonate) is used mainly in the treatment of chronic myelogenous leukaemia [1,2] and at high doses prior to autologous bone marrow transplantation [3–5]. Serum and cerebrospinal fluid (CSF) drug monitoring is particularly important in children treated with high-dose busulfan prior to autologous bone marrow transplantation, as they can develop secondary toxic effects [6].

Many techniques have been developed for the determination of busulfan in plasma samples: gas

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chromatography with electron-capture detection (GC–ECD) [7,8], gas chromatography–mass spectrometry (GC–MS) [9,10] and high-performance liquid chromatography (HPLC) with UV detection [11–13]. These techniques are very sensitive, but always involve pre-column or post-column derivatization of busulfan and time-consuming sample extraction procedures, which are unsuitable for the monitoring of the drug during high-dose treatment.

This paper describes a simple and reliable method to assay busulfan by HPLC–MS with selected-ion monitoring (SIM), using a particle beam interface. The assay, developed for serum and CSF samples, has been successfully applied in clinical routine.

EXPERIMENTAL

Chemical and reagents

Busulfan was obtained from Aldrich (Steinheim, Germany). Water used in the HPLC eluent was Milli-Q grade (Millipore, Rome, Italy). All solvents were analytical grade.

HPLC–MS analysis

Liquid chromatography was performed with a Waters 600 MS multi-solvent delivery system (Waters Chromatography Division, Rome, Italy) equipped with a U6K universal liquid chromatography injector. The chromatographic column was a reversed-phase μBondapak C_{18} steel column (10 μm particle size, 30 cm × 2.0 mm I.D.; Waters Chromatography Division). The elution was carried out with methanol–water (70:30, v/v) at a flow-rate of 0.5 ml/min. The HPLC system was connected to a LINC VG particle beam interface (VG, Biotech, Altrincham, UK).

The HPLC effluent was converted into an aerosol in a nebulizer, in which helium was introduced coaxially, and sprayed into a desolvation chamber maintained at a low pressure and at a temperature of 40°C. As the aerosol passed through the desolvation chamber, the volatile components, such as the mobile phase, were vaporized and the less volatile compounds, such as the analytes, condensed to form submicrometre particles. This mixture entered a skimmer area, which was pumped down and in which the sample molecules were separated from the solvent molecules. Finally, the beam of solvent-free particles was introduced into the ion source of a VG TRIO 2 mass spectrometer (VG, Biotech). The MS system was operated in electron-impact (EI) mode with an electron energy of 70 eV, an emission current of 350 μA and an ion source temperature of 250°C. Prior to the analysis, the instrument was tuned in the EI mode using the fragments m/z 69, 219, 220, 502, 503, 614 and 615 from perfluorotributylamine.

Standard solutions

A stock solution of busulfan was prepared in acetone at a concentration of 1 mg/ml. Serial dilution was performed to produce ethanolic or water spiking solutions ranging in concentration from 100 to 1 μg/ml. A volume of these solutions added to 1 or 0.5 ml of blank CSF or serum produced final concentrations ranging from 0.05 to 5 μg/ml. Spiked biological samples were processed according to the procedure described, and measured areas of busulfan were compared with those of standard solutions to determine analytical recovery.

Extraction procedure

Biological samples (serum or CSF, 0.5 or 1 ml) containing busulfan were pipetted into glass culture tubes. Dichloromethane (2 ml) was added, and the tubes were vortex-mixed for 1.5 min and centrifuged at 1000 *g* for 5 min. The supernatant was quantitatively transferred to a clean glass tube, and the biological sample was re-extracted with 2 ml of dichloromethane. The organic phases were collected, evaporated under a stream of nitrogen at room temperature, and redissolved in 100 μl of mobile phase. A 20-μl volume was injected into the HPLC column.

Drug administration

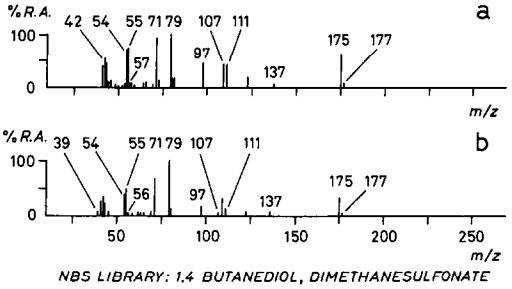
Five child patients with acute myeloblastic leukaemia were studied. High-dose busulfan (1 mg/kg) every 6 h per os was administered for four days, prior to the treatment with cyclophospha-

mide and autologous bone marrow transplantation. Blood samples (5 ml) were collected before the first dose and 6 h after the last dose of busulfan. They were centrifuged at 1000 *g* for 5 min, and the serum was collected. CSF (1 ml) was collected from all patients by lumbar puncture before and 6 h after the last day of treatment. Biological fluids collected before the first dose were used as blanks.

RESULTS AND DISCUSSION

HPLC–MS analysis

The EI mass spectrum of busulfan was recorded during an HPLC run, by repeatedly scanning the quadrupole mass filter every 1 s from *m/z* 50 to 250 with an interscan time of 0.1 s. It was identified by comparing it against the computer-stored list of NBS library spectra (Fig. 1). Quantitation was performed by focusing the instrument in the SIM mode. Fragment ions *m/z* 79, 111, 175 and 177 were monitored for busulfan. However, only *m/z* 175 was used for the quantitation because it showed the best correlation with the concentration of the analyte. Typical SIM chromatograms obtained from spiked serum and CSF of a patient are shown in Fig. 2. No detectable interferences were observed. The retention time of busulfan was 2.0 min. With this method,



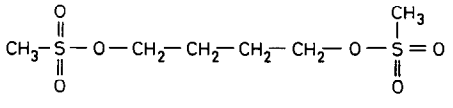


Fig. 1. Mass spectrum of busulfan (a) compared with that stored in NBS computer library spectra (b).

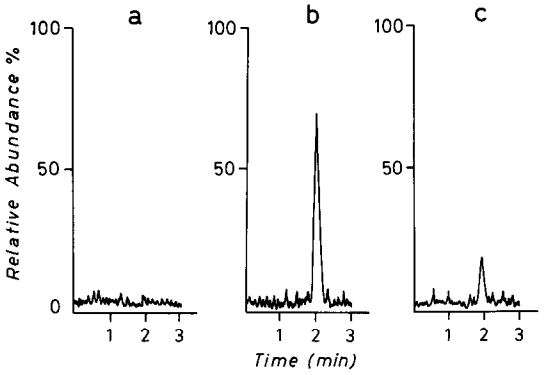


Fig. 2. Recordings of *m/z* 175 obtained by HPLC–MS analysis. (a) Serum blank; (b) extract of a 0.5-ml serum sample spiked with 500 ng/ml busulfan; (c) extract of a 1-ml CSF sample containing 107 ng/ml busulfan.

concentrations as low as 100 ng/ml could be precisely quantitated at a signal-to-noise ratio of 3 with a sensitivity limit of 50 ng/ml (signal-to-noise ratio of 2).

Calibration curve and recovery

The calibration curve of the peak at *m/z* 175 (counts $\times 10^{-6}$) (*y*) *versus* the concentration of the analyte (ng/ml) (*x*) was prepared and checked daily from spiked serum and spiked CSF carried through the entire procedure; it was linear over the range 0.1–5 μg/ml (regression equation: $y = 4.3x - 0.15$, $r = 0.98$). Analytical recoveries from serum and CSF containing 100 ng/ml, 500 ng/ml and 1 μg/ml added busulfan were assessed in ten replicate samples (Table I).

TABLE I

RECOVERY OF BUSULFAN FROM SPIKED BIOLOGICAL SAMPLES

Concentration added (ng/ml)	Recovery (mean ± S.D., $n = 10$) (%)	
	Serum	CSF
100	97.07 ± 1.50	97.54 ± 1.23
500	97.06 ± 1.06	97.49 ± 1.16
1000	96.77 ± 1.24	96.99 ± 1.74

TABLE II

WITHIN-DAY AND BETWEEN-DAY PRECISION

Concentration added (ng/ml)	Serum		CSF	
	Mean concentration found (ng/ml)	R.S.D. (%)	Mean concentration found (ng/ml)	R.S.D. (%)
Within-day (n = 4)				
500	490.0	0.58	492.6	0.60
1000	979.5	0.27	988.1	0.29
Between-day (n = 6)				
500	483.5	1.67	484.3	1.27
1000	962.7	1.67	968.6	1.80

Within-day and between-day precision

The within-day precision was checked by determining four serum and four CSF samples spiked with two different concentrations of busulfan. For the between-day precision, two concentrations of the analyte were determined in duplicate every day for six days (Table II).

CONCLUSION

The method described here is simple and easy to perform. The main advantages are the rapid sample extraction and the possibility of determining busulfan without any derivatization. Even if the limit of quantitation is high, the determination of busulfan concentrations in serum and CSF was possible during all the days of treatment. An example of the method's application was the quantitation of serum and CSF concentrations of busulfan in five child patients, 6 h after the last day of high-dose treatment (1 mg/kg). A mean serum level of 451 ± 125 ng/ml and a mean CSF level of 264 ± 94 ng/ml were observed, in agreement with data reported elsewhere [10,14].

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