วารสาร
เทคนิคการแพทย์และกายภาพบำบัด
JOURNAL OF MEDICAL TECHNOLOGY AND PHYSICAL THERAPY

นิพนธ์ต้นฉบับ
ORIGINAL ARTICLE

ความถูกต้องน่าเชื่อถือและความรวดเร็วในการวิเคราะห์ระดับยาบูซัลแฟนในพลาสมา ด้วยวิธีโซลิด เฟสเอ็กแทรคท์ชั่น แก๊สโครมาโตกราฟฟี่ แมสสเป็กโตมิตตรี้

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Received: *May 4, 2018*
Revised: *August 22, 2018*
Accepted: *October 2, 2018*

บทคัดย่อ

บูซัลแฟน เป็นยาเคมีบำบัดกลุ่มแอคิลเลทติ้ง เอเจนท์ ที่ใช้ร่วมกับไซโคลฟอสฟาไมด์ ซึ่งเป็นยากดภูมิคุ้มกัน บูซัลแฟน จะใช้เป็นยาควบคุมสภาวะของผู้ป่วยก่อนการปลูกถ่ายไขกระดูก ซึ่งต้องมีการติดตามการใช้ยาและการปรับขนาดยาของผู้ป่วยอย่าใกล้ชิด เพื่อเพิ่มประสิทธิภาพรักษา และต้องเฝ้าระวังระดับยาเป็นรายบุคคลไป ความน่าเชื่อถือและวิธีการวิเคราะห์อย่างรวดเร็วของการตรวจระดับยาบูซัลแฟนในพลาสมา จึงมีความสำคัญอย่างยิ่ง การศึกษานี้ใช้วิธีการตรวจวิเคราะห์ ด้วยเทคนิคจีซี เอ็มเอส ที่พัฒนาและตรวจสอบความถูกต้องของการตรวจวัดในพลาสมา ซึ่งใช้เทคนิคการสกัดยาจากตัวอย่างพลาสมา จากนั้นนำมาทำเป็นอนุพันธ์ด้วยโซเดียมไอโอไดด์ ก่อนที่จะวิเคราะห์ด้วยเครื่องจีซี เอ็มเอส การสกัดตัวอย่างด้วยเฟสของแข็งเป็นวิธีที่ใช้สำหรับการศึกษานี้ และวิธีการที่ใช้เป็นวิธีที่ได้พัฒนาและตรวจสอบความถูกต้องตามแนวทางขององค์การอาหารและยาแห่งสหรัฐอเมริกาแล้ว ขีดจำกัดของการวัดปริมาณต่ำสุดคือ 10 นาโนกรัมต่อมิลลิลิตร สำหรับการตรวจวิเคราะห์ระดับยา บูซัลแฟน นี้ได้รับการตรวจสอบอย่างถูกต้องครบถ้วน ทั้งด้านความแม่นยำ ความเป็นเส้นตรง (r^2>0.9994) ความคงตัวของยา และการสกัดคืนกลับของของยาซึ่งมีค่าตั้งแต่ 99.65-100.19% โดยการทำซ้ำ และวิธีการที่พัฒนาขึ้นนี้สามารถนำไปใช้สำหรับการวิเคราะห์ในห้องปฏิบัติการได้อย่างรวดเร็ว แม่นยำ และมีความน่าเชื่อถือสูง

คำสำคัญ: จีซี เอ็มเอส, บูซัลแฟน, ระดับยา, โซเดียมไอโอไดด์

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ORIGINAL ARTICLE

Reliability and rapid analysis method of plasma busulfan by solid phase extraction gas chromatography-mass spectrometry

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Abstract

Busulfan is an alkylating agent used in combination with cyclophosphamide as a conditioning agent prior to bone marrow transplantation. Therapeutic drug monitoring and dose adjustment are currently used to optimize and individualize therapy with busulfan. The reliability and rapid analysis method of plasma busulfan are important. GC-MS assay method was developed and validated for the determination of plasma busulfan. Busulfan was extracted from plasma samples and derivatized with NaI_2 prior to GC-MS determination. The Solid Phase Extraction (SPE) was used for extraction. The method was developed and fully validated according to USFDA guideline. The limit of quantification (LOQ) was 10 ng/mL for busulfan. The method was fully validated in terms of selectivity, accuracy, precision, linearity (r^2>0.9994) and stability. The recoveries of extraction ranged from 99.65-100.19% with repeatability. A rapid, sensitive, accurate and reproducible of GC-MS method for quantification of busulfan plasma levels were developed and validated, suggesting that this method could be used for routine analysis.

Keywords: GC-MS, Busulfan, Therapeutic drug monitoring, NaI_2

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INTRODUCTION

Busulfan (BU, 1, 4-butanediol dimethanesulfonate) is a bifunctional alkylating agent, used for the treatment of chronic granulocytic myeloid leukemia. It is frequently used in high-dose regimens in combination with cyclophosphamide for myeloablative treatment.[1-2] The high-dose busulfan given prior to the autologous bone marrow transplantation can exhibit significant non-hematological toxicity such as hepatic veno-occlusive disease (VOD).[3] The toxic effects are strongly related to high plasma concentrations of busulfan, while low levels are associated with increased incidence of graft rejection. Therefore, therapeutic drug monitoring (TDM) has been considered for individual optimization of busulfan therapy.[4]

Several methods have been developed for the determination of plasma concentrations of busulfan, including gas chromatography-mass spectrometry (GC-MS),[5] gas chromatography with electron capture detection (GC-ECD),[6-7] high-performance liquid chromatography with ultraviolet detection (HPLC-UV)[8] and Ultraper formance liquid chromatography (UPLC).[9] These methods have time-consuming due to many steps of derivatization and extraction,[10] hence they may not suitable for routine analysis. This study demonstrates a new method which takes shorter duration for derivatization and fewer steps for preparation. The new method will be useful for routine analysis.

MATERIALS AND METHODS

1. Chemicals

Busulfan and sodium iodide (NaI_2) were purchased from Sigma-Aldrich Ltd. (Steinheim, Germany). The HPLC grade acetonitrile, acetone and methanol were purchased from Labscan Ltd. (Bangkok, Thailand). Milli-Q water, Millipore Corporation (Massachusetts, USA) was used. Other chemicals were of analytical grade. Drug-free human plasma was derived from the Department of Transfusion Medicine, Siriraj Hospital, Bangkok, Thailand.

2. Instrumentations

The extraction and analysis of busulfan was carried out with CTC Combi AAL auto sampler injector and injected to a GC-MS (Agilent Technologies 7890A system, Santa Clara, CA, USA) and operated in the split/ splitless mode at an injection temperature of 260°C. The separation of target analysts was achieved on a DB-624 fused capillary column (30 m x 0.25 mm i.d. x 1.40 µm film thickness). Helium (carrier gas) was set to a constant flow rate of 2 ml/min with linear velocity of 40 cm/s. The GC column oven temperature program was set as follows. Initially set at 100°C for 1 min, ramped at 40°C/min to 180°C, then ramped to 260°C at 20°C/min, and finally to 260°C held for 3 min, for a total runtime of 10 min, The MS operation condition includes transfer line of 260°C, the ion source of 230°C, electron ionization (EI) of 70 eV. The optimization of methods was done in scan mode while quantitation was done in selected ion monitoring (SIM) mode. The developed method was fully validated according to the USFDA guideline[11] and EURACHEM guide.[12]

3. Standard stock solutions

Standard stock solutions of busulfan were prepared in acetone and were diluted with milli-Q water. The stock solutions of busulfan were diluted to the concentrations ranged from 10-5,000 ng/mL and used as working solutions. The Quality Control (QC) samples were prepared at 10, 100, 500, and 1000 ng/mL Solutions were stored at -20°C until used. The derivatization reagent was prepared by dissolving 1.0 mg NaI_2 in 5 ml of acetonitrile and 5 ml of methanol.

4. Sample extraction

The Solid Phase Extraction (SPE), Oasis HLB extraction cartridge 30 mg 1 mL was used for sample preparation. The SPE column were initially conditioned with 1 mL of methanol and equilibrated with 1 mL of Milli-Q water before use. A 200 µL of plasma samples, QC and standard solution were aspirated into the wetted preconditioned cartridges. Afterward, the sample components were washed with two steps of the wash solvent by 0.5 mL of D.I. water and 0.5 mL of 2%NH_4 in 5% methanol. Busulfan was subsequently eluted from the dried columns using 200 µL of derivatization reagent and injected into the GC-MS.

5. Bioanalytical method validation

Selectivity and sensitivity

The selectivity was examined using six sources of free drug plasma which were extracted and analyzed by the developed method. The result should not have any interfering peak of busulfan. The sensitivity at the limit of quantification (LOQ) was also examined by dilution of standard compounds in plasma which were extracted and then quantified for the lowest detectable concentration.

Accuracy and precision

Accuracy and precision were examined by six replicate analyses of plasma spiked with four different concentrations (LOQ, 50, 500 and 1000 ng/mL) for three separate days. The percentage of relative error (%RE), indicating accuracy, was calculated as the measured concentration divided by the spiked concentration. The percentage of coefficient of variation (%CV), indicating precision, was obtained from the ratios of standard deviation (SD) to the mean of the measured concentration. Both %RE and %CV should be within ± 20% at LOQ and 15% at other concentrations.

Linearity and calibration curve

A calibration curve was represented by a linear regression model, y=mx+b and weighting by 1/x, where y is the ratio of peak area of analyze to the peak area of IS, x is the concentration at different levels including 10, 50, 100, 500, 1,000 and 5,000 ng/mL. All calibration ranges yielded linear relationships with coefficients of determination (r^2) and their value must exceed 0.995.

Recovery of extraction

The recovery method was performed by comparing concentration of the extracted samples at three different concentrations (50, 500 and 1000 ng/mL) with a concentration of non-extracted standard solutions at the same concentrations. The percentage of absolute recovery (%RV) was the ratio of the measured extracted concentration to the non-extracted concentration.

Stability

The stability of analysis was performed by three replicate analyses of plasma spiked with three different concentrations (50, 500 and 1000 ng/mL) under various conditions with freshly prepared samples. The first condition was freeze and thaw in which plasma samples were frozen at -20°C and thawed at room temperature for three cycles before analysis. For short-term stability tests, the plasma samples were stored at room temperature for 6 hours before analysis. Post-preparative stability tests were done by placing the vials of plasma samples in the auto-sampler at 25°C for 10 hours before analysis. Lastly, for long-term stability, plasma samples were frozen at -20°C for 1 month before analysis. The acceptable percentage of variation in each condition must be within ±15%.

RESULTS

The results obtained for validation parameters described in the methods section were satisfactory for the studied analysts. No interferences from endogenous matrix compounds in blank blood from other drugs in spiked samples were observed. LODs ranged from 1 to 10 ng/mL for the substances investigated, except for busulfan that had a LOD of 1 ng/mL (Figure 1). The method was linear in the range from the LOQ (10ng/ml) to 5000 ng/mL for stimulants, giving a regression coefficient (R^2) always higher than 0.9994 **(Figure 2)**.

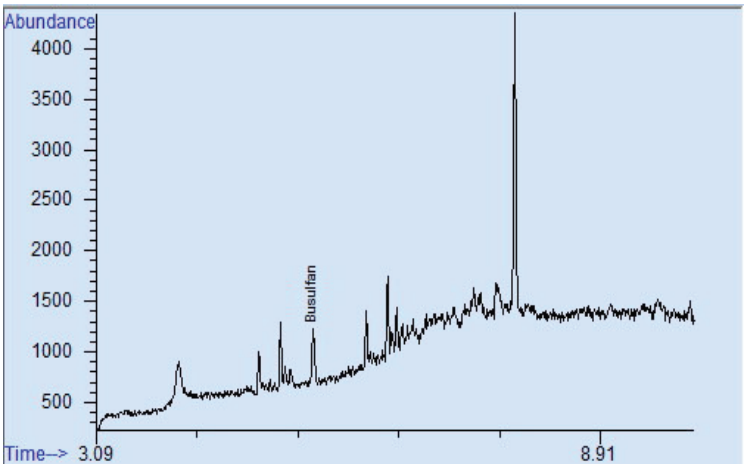

Figure 1 Chromatogram of extracting and derivatized of busulfan plasma spiked with concentration 1 ng/ml

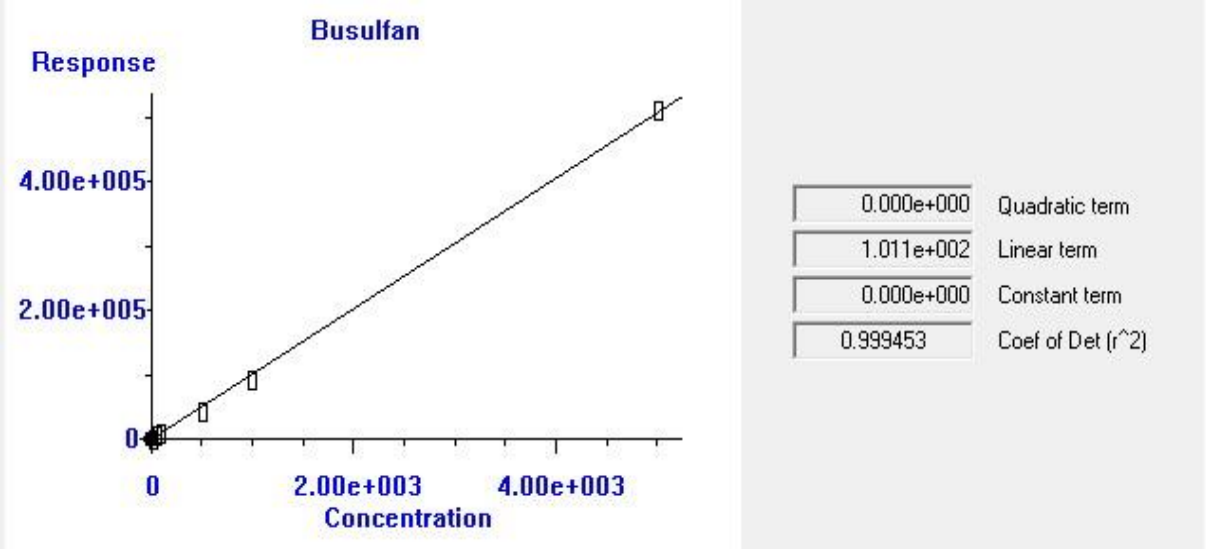

Figure 2 Linearity study for the calibration curve of busulfan by GC-MS in the calibration range 10–5000 ng/ml

Table 1. The recovery of busulfan in plasma samples preparation by SPE extraction.

Concentration (ng/ml)	Recovery of extraction (%RV)	Precision(%CV)
50 (n=6)	99.65	0.39
100 (n=6)	99.94	1.45
1000 (n=6)	100.19	13.18

n = Number of replicates

Table 2. The precision and accuracy (within-day and between-day).

Expected concentration (ng/ml)	Within-day (n=6)			Between days (n=18)		
	Mean±S.D. (ng/ml)	Precision (%CV)	Accuracy (%RE)	Mean±S.D. (ng/ml)	Precision (%CV)	Accuracy (%RE)
10 (LOQs)	10.03±0.07	0.01	100.30	10.02±0.07	0.01	100.20
50	49.94±0.75	0.56	99.88	49.91±0.54	0.29	99.82
100	100.52±1.22	1.48	100.52	100.25±1.16	1.36	100.25
1000	1000.65±3.47	12.06	100.01	946.28±3.79	14.40	94.62

n = Number of replicates

Table 3 Variation of busulfan level in spiked plasma samples under four different conditions (n=3)

Stability test	Variation (%)		
	LQC (50 ng/ml)	MQC (100 ng/ml)	HQC (1000 ng/ml)
Freeze and thaw (3 cycles)	-1.18	4.22	0.37
Short term (6 hr.)	6.01	2.07	-0.13
Long term (30 days)	3.16	3.00	-0.08
Post- preparative (24 hr.)	0.32	-2.13	0.13

n = number of replicates

Accuracy and precision, both intra- and inter day, were acceptable, giving values of percentage of coefficient of variation and percentage of relative error lower than 15%. The analysts were stable in the auto sampler for 24 hours at room temperature, without showing a significant decrease in the concentrations. The recoveries for the three concentrations studied (50, 500 and 1000 ng/mL) were in the range from 99.65 to 100.19% for the analysts **(Table 1)**. No carryover was observed for any of the analysts. Validation results are presented in **Table 2** (LOQs precision, accuracy and process efficiency) and **Table 3** (stability).

DISCUSSION

The extraction method in this study was rapid and reliable for plasma busulfan analysis. This method was highly sensitive, precise and accurate. For the extraction, the process of eluting with derivative reagent was reactive between busulfan and iodine in the process then used few minutes for the process. The previously published method used liquid-liquid for extraction, derivatization and incubation that took a long period.

CONCLUSION

This paper presented a rapid extraction method of busulfan analysis by GC–MS. It was based on derivatization with NaI_2, which was rapid, quantitative and easy. This method was suitable for routine drug monitoring analysis. It will be beneficial for the patients who need urgent determination of busulfan level.

ACKNOWLEDGEMENTS

The authors wish to thank Siriraj Poison Control Center, Faculty of Medicine Siriraj Hospital for laboratory setup and chromatographic analysis process.

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