Phytronix Breakaway Technologies

Sciex

Clinical

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Analysis of Busulfan in Plasma:

Quantification of Busulfan in plasma Using Luxon Ion Source®

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Introduction

Busulfan (Bu) is an alkylating agent commonly used in patients being prepared for hematopoietic stem cell transplantation (HSCT) for various types of hematologic malignancies such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in addition to non-malignancies (thalassemia)[1,2]. In the clinical doses, Bu is considered as a potent cytotoxic drug which causes severe and prolonged myelosuppression.

Our goal for this application note is to develop a diagnostic tool to rapidly quantify Busulfan in plasmas, which will allow a rapid diagnosis.

LDTD®-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled quantification method. To develop this application, we focused on performing an efficient sample preparation, which will increase the speed of the analysis process and confirm diagnoses more quickly.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing more precision, accuracy, and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic saturation characterize this type of ionization and is the result of the absence of solvent and mobile phase.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Stock solutions of Busulfan was prepared in acetonitrile at 1 mg/mL. Then, negative EDTA-K2 plasmas were spiked to generate a calibration curve and QCs.

Automated Sample Extraction

An automated system (**Figure 3**) is used to extract the samples using the following conditions:

100 µL of Internal standard in methanol:Water (10:90)
50 µL of Sample
- Vortex

400 µL of Acetonitrile
- Vortex and Centrifuge (3000 rpm/4 min.)

Spot 5 µL of upper layer on a LazWell™96 plate
- Dry 4 minutes at 40°C

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters

LDTD
Model: Luxon S-960, Phytronix
Carrier gas: 9.0 L/min (air)
Laser pattern:
- 3-second ramp to 45% power

MS/MS
MS model: QTrap® System 5500, Sciex
IonSpray Voltage: 5500
Temperature, GS1 and GS2 set to zero.
CUR: 30
Scan Time: 20 msec
Analysis Method: Positive MRM mode

Table 1 - MRM transitions for Luxon-MS/MS

	Transition	CE (V)
Busulfan-Quan	247.0 → 55.0	22
Busulfan-d8-Quan	255.0 → 62.0	22
Busulfan-Qual	247.0 → 151.0	11
Busulfan-d8-Qual	255.0 → 159.0	11

Results and Discussion

Validation Test

Calibration curves ranging from 25 to 2000 ng/mL were prepared in negative EDTA-K2 plasmas. A set of QCs were prepared in the same matrix. Replicate extractions were deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.995

Table 2 shows the inter-day correlation coefficients for Busulfan (Quan and Qual transition). Values greater than 0.995 are obtained. Figure 4 shows a typical calibration curve result for Busulfan-Quan.

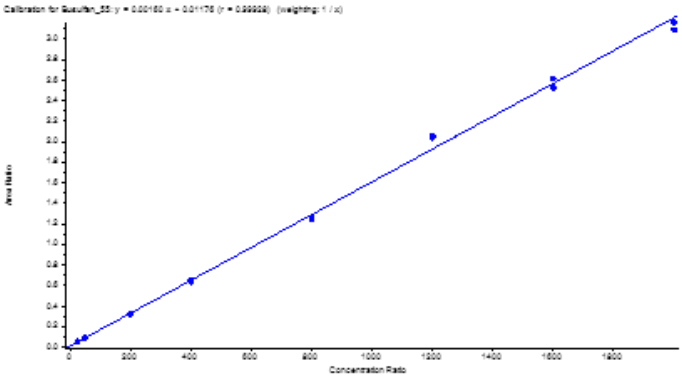

Figure 4 – Busulfan-Quan calibration curve

Table 2 – Inter-day calibration curve correlation coefficients

	Busulfan-Quan	Busulfan-Qual
Curve 1	0.99909	0.99936
Curve 2	0.99980	0.99979
Curve 3	0.99957	0.99982
Curve 4	0.99928	0.99979
Curve 5	0.99985	0.99982
Curve 6	0.99968	0.99976

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria were used:

- Each concentration must not exceed 15% CV.
- Each concentration must be within 100 ± 15% of the nominal concentration.

For the intra and inter-run precision and accuracy experiment, each QC was analyzed in sextuplicate, on six different runs **Table 3 and 4** show the intra and inter-run precision and accuracy results for Busulfan-Quan. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value. Similar results were obtained with the Busulfan-Qual transition.

Table 3 - Intra-Run Precision and Accuracy of Busulfan-Quan

Busulfan-Quan	LLOQ	QC-L	QC-M	QC-H	ULOQ
Conc (ng/mL)	25	75	1000	1500	2000
N	6	6	6	6	6
Mean (ng/mL)	28.1	76.2	987.4	1513.2	2060.8
%CV	4.7	1.4	2.4	0.8	1.9
%Nom	112.3	101.7	98.7	100.9	103.0

Table 4 - Inter-Run Precision and Accuracy of Busulfan-Quan

Busulfan-Quan	QC-L	QC-M	QC-H
Conc (ng/mL)	75	1000	1500
N	36	36	36
Mean (ng/mL)	76.8	980.6	1505.1
%CV	4.4	2.3	2.4
%Nom	102.4	98.1	100.3

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 1 day, sample extracts are spotted on a LazWell™ plate, dried and analyzed. The precision and accuracy results of QCs are reported in **Table 5**. All the results are within the acceptable criteria range for 1 day at 4°C.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 1 hour before analysis. The precision and accuracy results of QCs are reported in **Table 5**. All the results are within the acceptable criteria range for 1 hour at room temperature.

Table 5 - Wet and Dry Stability of Busulfan-Quan

Parameters	Dry stability (1 hour / RT)			Wet stability (1 day / 4°C)		
QC	QC-L	QC-M	QC-H	QC-L	QC-M	QC-H
Conc (ng/mL)	75	1000	1500	75	1000	1500
N	4	4	4	4	4	4
Mean (ng/mL)	77.0	1003.9	1510.7	77.7	985.1	1483.2
%CV	2.4	1.2	1.9	1.4	1.6	2.3
%Nom	102.6	100.4	100.7	103.6	98.5	98.9

Matrix effect study

Eight different plasmas were spiked at QC-M level, extracted, and analysed. The precision and accuracy results of the different matrix spiked are reported in **Table 6**. All the results are within the acceptable criteria range.

Table 6 – Matrix effect result

	M1	M2	M3	M4	M5	M6	M7	M8
Conc (ng/mL)	1000	1000	1000	1000	1000	1000	1000	1000
N	3	3	3	3	3	3	3	3
Mean (ng/mL)	978.2	1007.9	1020	986.5	988.8	1044	1036	1031
%CV	0.9	1.7	1.4	0.9	0.8	1.0	1.8	3.4
%Nom	97.8	100.8	102.0	98.7	98.9	104.4	103.6	103.1

Conclusion

The Luxon Ion Source® combined with the Sciex QTrap® 5500 mass spectrometer system enables the rapid analysis of Busulfan in plasmas This method of analysis can thus be applied at the clinical level in order to evaluate the concentration of Busulfan for a quick dosage adjustment.

References

1. Alatrash, G. *et al. Biol. Blood Marrow Transpl.* **17**, 1490–1496 (2011).
2. Chandy, M. *et al. Bone Marrow Transplant.* **36**, 839–845 (2005).