Development and validation of a novel method for concomitant busulfan and treosulfan determination in plasma and Mitra™ microsampling device

Arkadiusz Kocur[1], Agnieszka Czajkowska[2], Paulina Michalczuk[1], Norbert Patalas[1], Aleksandra Minkowska[3], Iwona Malinowska[3], Paweł Łaguna[3], Tomasz Pawiński[1]

[1] Department of Drug Chemistry, Pharmaceutical and Biomedical Analysis, Medical University of Warsaw - Warsaw (Poland),
[2] Therapeutic Drug Monitoring, Clinical Pharmacokinetics and Toxicology Laboratory, The Children's Memorial Health Institute - Warsaw (Poland),
[3] Department of Pediatric Haematology and Oncology UCC WUM - Warsaw (Poland)

Contact: arkadiusz.kocur@wum.edu.pl

MEDICAL UNIVERSITY OF WARSAW
FACULTY OF PHARMACY

BACKGROUND

- Busulfan and treosulfan are alkylating agents widely used in conditioning regimens prior to hematopoietic stem cell transplantation (HSCT).
  - Busulfan (Bu) requires therapeutic drug monitoring (TDM) because of its narrow therapeutic window and substantial interindividual pharmacokinetic variability.
  - Treosulfan (Treo) is increasingly used as an alternative conditioning agent, particularly in pediatric patients, although its pharmacokinetics remain less extensively characterized.
- Volumetric absorptive microsampling (VAMS) using the Mitra™ device enables standardized collection of only 10 µL of capillary blood and may facilitate minimally invasive pharmacokinetic monitoring in children.
- The aim of the study was to develop and validate a rapid LC–MS/MS method for the simultaneous determination of busulfan and treosulfan in plasma and Mitra™ VAMS samples and to assess its applicability to pharmacokinetic studies and therapeutic drug monitoring in pediatric HSCT patients.

METHODS

- An LC–MS/MS method for the simultaneous determination of busulfan and treosulfan in Mitra™ VAMS samples was developed.
- Analytes were extracted from VAMS tips as presented on Fig. 2. Plasma samples were prepared with protein precipitation with acetonitrile.
- Chromatographic separation was performed on a reversed-phase (CN) LC column using gradient elution with water and acetonitrile containing formic acid, with MS detection using MRM pairs. Bu-d_8 was used as internal standard for both analytes.
- Method was preliminary applied for TDM of two clinical cases (Figure 3).



Figure 1. Sample pretreatment protocol to LC-MS/MS analysis of Bu and Treo.

RESULTS

Validation parameter	Busulfan – Plasma	Busulfan – Mitra™ VAMS	Treosulfan – Plasma	Treosulfan – Mitra™ VAMS
Calibration range	10–2,000 ng/mL	10–2,000 ng/mL	0.5–100 µg/mL	0.5–100 µg/mL
r^2	0.9987	0.9979	0.9991	0.9983
LLOQ	10 ng/mL	10 ng/mL	0.5 µg/mL	0.5 µg/mL
LLOQ accuracy	103.4%	105.8%	97.6%	102.9%
LLOQ precision	7.2% CV	9.1% CV	6.4% CV	8.7% CV
Within-run precision	2.8–6.4% CV	3.7–8.6% CV	2.5–5.9% CV	3.4–8.1% CV
Between-run precision	3.6–7.1% CV	4.5–9.3% CV	3.1–6.7% CV	4.2–9.0% CV
Accuracy	96.2–104.8%	94.5–106.7%	97.1–104.2%	95.0–106.1%
Extraction recovery	91.6 ± 4.8%	87.9 ± 5.6%	93.2 ± 4.1%	85.7 ± 6.2%
Matrix effect	97.8 ± 5.2%	94.1 ± 6.4%	101.6 ± 4.7%	92.8 ± 7.1%
IS-normalized matrix factor CV	4.6%	6.8%	4.2%	7.5%
Carryover	3.1% of LLOQ	4.4% of LLOQ	2.6% of LLOQ	3.8% of LLOQ
Dilution integrity – accuracy	101.8%	103.5%	98.6%	102.1%
Dilution integrity – precision	4.2% CV	5.8% CV	3.9% CV	5.4% CV
Bench-top stability	97.4–103.1%	95.8–104.6%	96.5–102.8%	94.9–105.2%
Autosampler stability	98.2–102.6%	96.4–104.1%	97.8–103.0%	95.7–104.8%
Freeze–thaw stability	96.8–103.7%	95.1–105.4%	97.0–103.5%	94.6–105.8%
Long-term stability	97.1–102.9%	95.4–104.7%	96.3–103.6%	94.8–105.5%
Overall validation	PASS	PASS	PASS	PASS

Figure 2. Table with preliminary results of method validation (performed according to ICH M10 guidelines).

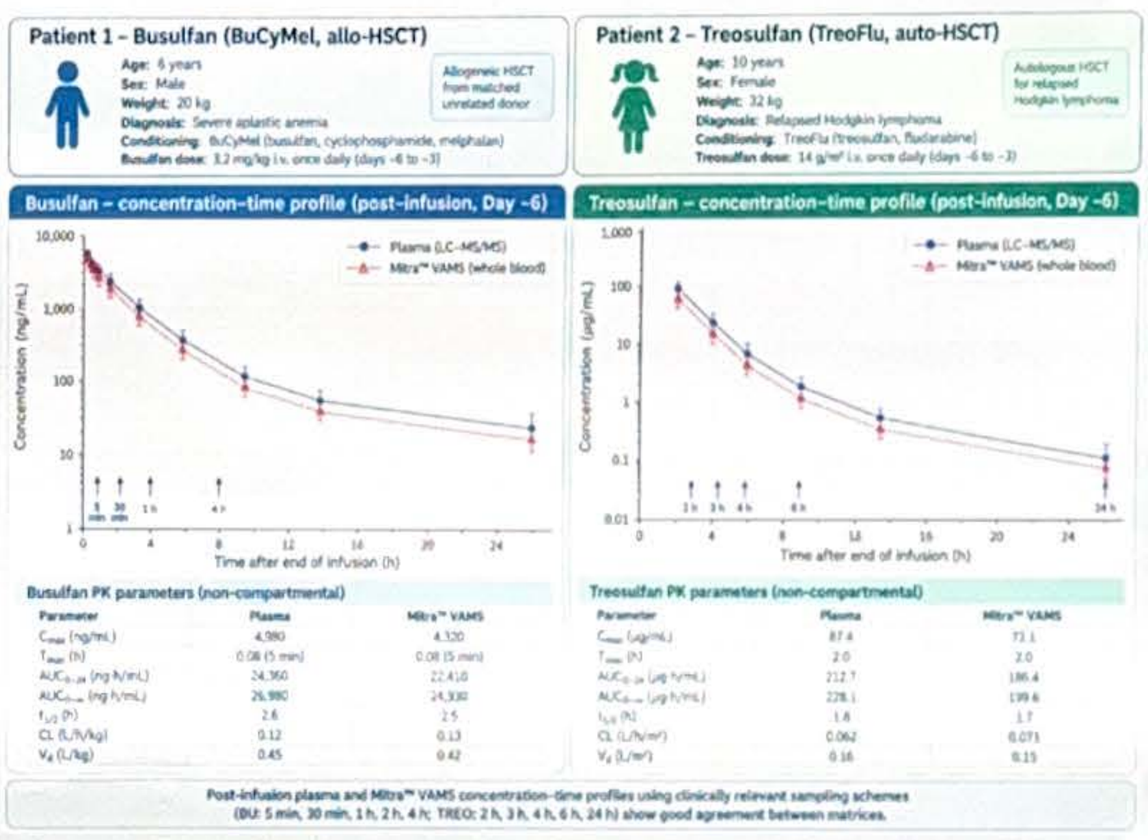


Figure 3. Representative post-infusion pharmacokinetic profiles of busulfan and treosulfan in paired plasma and Mitra™ VAMS samples from two pediatric HSCT patients. The left panel shows busulfan concentrations in a 6-year-old boy (20 kg) with severe aplastic anemia undergoing BuCyMel conditioning before allo-HSCT, with samples collected at 5 min, 30 min, 1 h, 2 h, and 4 h after end of infusion (EOI). The right panel shows treosulfan concentrations in a 10-year-old girl (32 kg) with neuroblastoma undergoing TreoFlu conditioning before auto-HSCT, with samples collected at 2, 3, 4, 6, and 24 h after EOI. Plasma and Mitra™ VAMS concentration–time profiles showed good agreement for both analytes. Based on AUC_{0-24}, the VAMS/plasma ratio was 0.92 for busulfan and 0.88 for treosulfan, indicating close matrix comparability for busulfan and slightly lower whole-blood exposure for treosulfan.

KEY FINDINGS

- Simultaneous quantification of **busulfan and treosulfan** was achieved using a single LC–MS/MS assay.
- Only **10 µL of blood** is required for Mitra™ VAMS analysis.
- The assay demonstrated satisfactory linearity, precision and accuracy. Matrix effects and carry-over were minimal.
- Both analytes were stable under the evaluated storage and processing conditions (Mitra™ protected with citric acid to limit the conversion to S,S-EBDM epoxide ex vivo)
- **The method is suitable for pharmacokinetic studies requiring repeated low-volume sampling.**

WHAT CAN BE CHANGED IN PRACTICE?

10 µL blood is sufficient for simultaneous busulfan and treosulfan analysis.

The developed method:

- reduces the volume of blood required for pharmacokinetic monitoring,
- facilitates repeated sampling in pediatric patients,
- enables simultaneous analysis of two major HSCT conditioning agents,
- supports more intensive pharmacokinetic profiling,
- provides an analytical basis for future individualized treosulfan exposure assessment.

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