Simultaneous Determination of Treosulfan and Fludarabine in Plasma by LC-MS/MS

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Background

Myeloablative agents (MAs) are given to patients prior to hematopoietic stem cell transplantation (HSCT) to reduce tumor burden and prevent graft rejection, but they can also cause serious side effects. Treosulfan (Treo) is a prodrug that undergoes sequential, nonenzymatic conversion to two epoxide species with alkylating activity in physiological conditions. A structural analog of the alkylating agent busulfan, Treo exhibits comparable myeloablative activity while causing fewer adverse effects. Treo is currently being considered for FDA approval in combination with fludarabine (Flu), one of the most commonly used MAs. Because there is significant interindividual variation in plasma concentrations of both Treo and Flu, therapeutic drug monitoring (TDM) is warranted to ensure that dosages are given that maximize efficacy and minimize toxicity.

Objective

To develop a rapid, accurate turbulent flow liquid chromatography-tandem mass spectrometry (TFLC-MS/MS) assay capable of simultaneously quantifying Treo and Flu in plasma to facilitate TDM of both compounds.

Materials and Methods

All patient specimens, calibrators, and controls must be acidified with 1 M citric acid (50 µL/mL) to prevent conversion of Treo into the epoxide species.

Standards and Controls

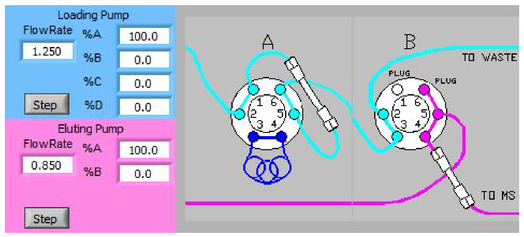
	Treo (µg/mL)	Flu (ng/mL)
Calibrator 1	31.3	78.2
Calibrator 2	125	312.5
Calibrator 3	250	625
Calibrator 4	500	1,250
Calibrator 5	1,000	2,500
Calibrator 6	2,000	5,000
Low Control	200	250
Mid Control	750	1,500
High Control	1,500	3,500

Sample Preparation

- Extract Treo and Flu from 100 µL acidified patient plasma, calibrator, or control with 200 µL methanol containing internal standards Treo-d_4 and Flu-$^{13}C^{15}N_3$
- Vortex and centrifuge
- Inject 10 µL of the extract into the TFLC-MS/MS system.

TFLC-MS/MS Method

- TFLC system: Thermo Fisher Scientific Aria TLX-2 controlled by Aria 1.6.3
- MS/MS: Thermo Fisher Scientific TSQ Quantum Ultra with electrospray ionization (ESI) source operating in positive ion mode and compounds detected using multiple reaction monitoring (MRM)
- TurboFlow column: Cyclone-P
- Analytical column: Accucore PFP
- Mobile Phase A: 0.1% formic acid in water
- Mobile Phase B: 0.1% formic acid in methanol



Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	1.25	Step	100.0	-	-	-	====	out	0.85	Step	100.0	-
2	0.50	45	0.25	Step	100.0	-	-	-	T	in	0.85	Step	100.0	-
3	1.25	60	1.25	Step	-	-	100.0	-	====	in	0.85	Step	70.0	30.0
4	2.25	45	1.25	Step	60.0	40.0	-	-	====	out	0.85	Ramp	5.0	95.0
5	3.00	45	1.25	Step	60.0	40.0	-	-	====	in	0.85	Step	5.0	95.0
6	3.75	90	1.25	Step	100.0	-	-	-	====	out	0.85	Step	100.0	-

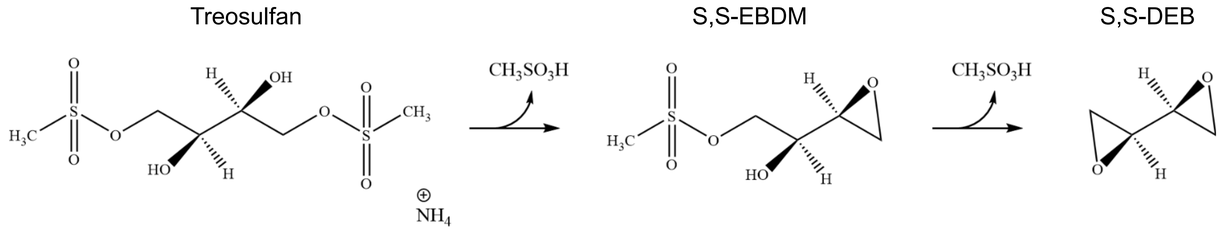
Mass Transitions (m/z)

- Treo: 296.0 → 87.1 & 183.1
- Treo-d_4: 300.0 → 91.2 & 187.1
- Flu: 286.1: → 134.1 & 154.1
- Flu-$^{13}C^{15}N_3$: 290.1 → 138.0 & 158.0

Results

Precision

Within-day precision was assessed by performing 10 extractions of each level of control and testing them in a single batch. Between-day precision was evaluated by testing controls extracted in triplicate each day for 20 days.



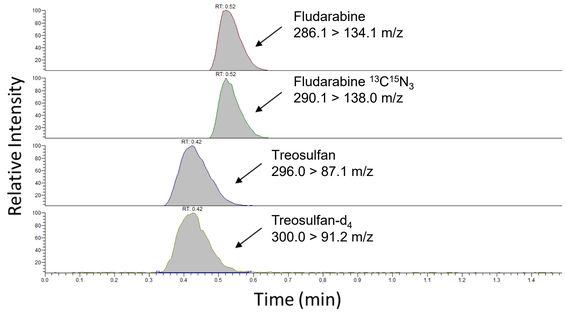
Accuracy

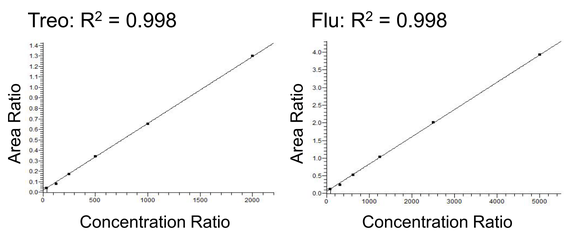
Accuracy was evaluated by calculating the percent recovery of Treo and Flu spiked into blank plasma at concentrations spanning the analytical measurement range (AMR) for each drug.

	Nominal Value	Recovery (%)	Within-Day CV (%)	Between-Day CV (%)
Treo QC 1	200 µg/mL	101.3	2.0	2.8
Treo QC 2	750 µg/mL	99.3	2.6	3.5
Treo QC 3	1,500 µg/mL	104.5	2.9	3.9
Flu QC 1	250 ng/mL	92.5	2.0	2.9
Flu QC 2	1,500 ng/mL	94.6	3.3	4.0
Flu QC 3	3,500 ng/mL	100.7	6.1	6.4

Reportable Range

- Treo AMR: 31 – 2,000 µg/mL
- Treo clinical reportable range (CRR): 31 – 10,000 µg/mL
- Flu AMR: 78 – 5,000 ng/mL
- Flu CRR: 78 – 50,000 ng/mL
- Typical R^2 values > 0.995





Interferences

To test for the presence of matrix effects, calibration curves prepared by spiking Treo and Flu into methanol were compared to calibration curves prepared by spiking the drugs into blank plasma extract. Matrix effects were < 10% across all mass transitions.

To test for other potential interferences, Treo and Flu were spiked into blank plasma containing hemolysate, triglycerides, conjugated bilirubin, or unconjugated bilirubin. Drugs were also spiked into a Biorad TDM Liquichek level 3 control containing a variety of common therapeutic drugs. Calculated Treo and Flu concentrations obtained in the presence of these interferents were compared to calculated concentrations obtained by spiking Treo and Flu into blank plasma.

	[Interferent]	Treo % Difference*	Flu % Difference*
Hemolysate	2,000 mg/dL	2.1	4.4
Triglycerides	250 mg/dL	0.2	1.8
Conj. Bilirubin	2 mg/dL	-2.9	3.0
Unconj. Bilirubin	15 mg/dL	-10.0	-5.6
Biorad TDM Control	---	1.0	9.1

*Relative to blank plasma

Carryover

Carryover was assessed by repetitive analysis (N=10) of blank plasma tested immediately after calibrator 6, which contains the highest concentrations of Treo and Flu. The blank plasma response was compared to the calibrator 6 response. No significant carryover was observed at any mass transition.

Conclusions

Our TFLC-MS/MS method measures Treo and Flu simultaneously in plasma with acceptable precision, accuracy, and linearity, and it is not impacted by matrix effects, carryover, or other common interferents. This assay will facilitate clinical TDM of Treo and Flu.