A Sensitive LC-MS/MS Method for Determination of Melphalan in Human Plasma

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INTRODUCTION

High-Dose Melphalan administration with autologous stem cell transplantation (ASCT) is often used in eligible subjects with multiple myeloma (MM), however, this is considered as off-label use. Alkeran®, the marketed formulation of Melphalan, has limited chemical stability upon reconstitution. Further, Alkeran® uses propylene glycol as a co-solvent, which has been reported to lead to renal dysfunction, arrhythmias and a sepsis-like syndrome. CDX-353 is a reformulation of Alkeran® by the CyDex Pharmaceuticals, Inc. (A Ligand Company) which not only is propylene glycol free, but incorporates Captisol®, a specially modified cyclodextrin for enhanced stability and potentially allowing alternate, longer dosing regimens. The purpose of this work is to develop and validate a sensitive and reliable bioanalytical method for the determination of Melphalan in the presence of Captisol® in human plasma.

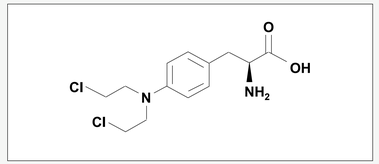

Figure 1. The Structure of Melphalan

METHOD

Plasma samples were combined with Mephalan-d_8, as an internal standard (I.S.), and extracted using protein precipitation. The resulting extracts were combined with an acid solvent and chromatographed on a C18 column with gradient elution using acetonitrile based mobile phase solutions. Melphalan and its internal standard were detected by API 3000 LC/MS/MS system under positive MRM mode.

Liquid Chromatography

HPLC : Shimadzu 10ADvp
Autosampler : Perkin Elmer 200
Column : Aquasil C18 (50 x 2.1 mm) 5µ
MP A : 0.1% Formic acid in DI H_2O
MP B : Acetonitrile
Rinse : MeOH/DI H_2O/Acetic acid (50/50/0.5)

Mass Spectrometry

AB Sciex API 3000 with Turbo-ion Spray interface
Polarity : Positive
Scan type : Multiple Reaction Monitoring
Melphalan: m/z 305 → 288
Melphalan-d_8: m/z 313 → 296 (I.S.)

RESULTS

The method was fully validated over a range of **25 to 11200 ng/mL** using a weighted linear regression ($1/x^2$). The correlation coefficients for precision/accuracy validation batches were 0.998 or better (**Figure 5**).

Figures 2 through 4 show typical chromatograms for selected samples. The S/N ratio for Melphalan peak at LLOQ level was greater than 40.

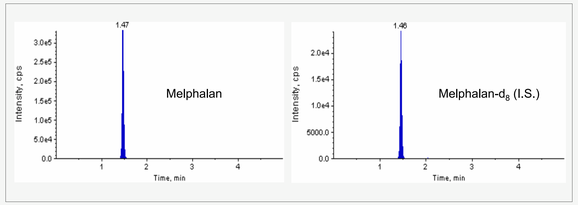

Figure 2. Extracted High Calibration Standard (11,200 ng/mL, ULOQ).

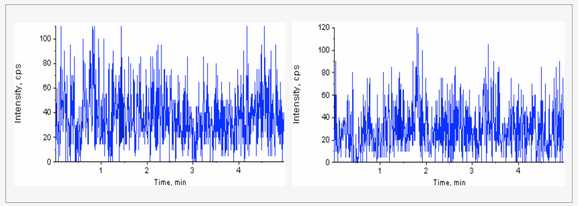

Figure 3. Matrix Blank (No I.S.)

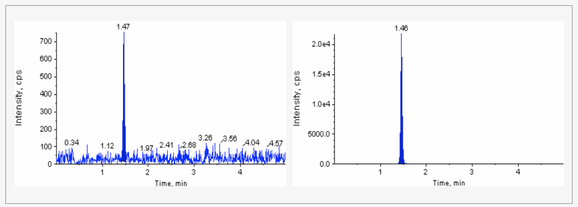

Figure 4. Extracted Low Calibration Standard (25 ng/mL, LLOQ).

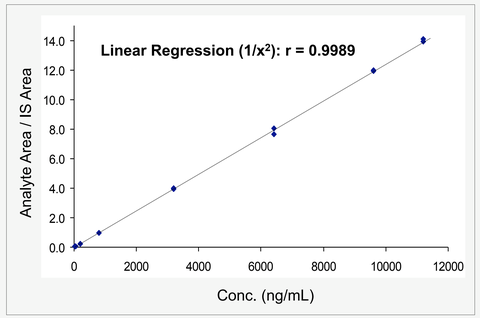

Figure 5. A Typical Calibration Curve for Melphalan

The inter- and intra-batch results for QC samples are shown in **Table 1**. Matrix effect was evaluated for Melphalan in six different lots of blank plasma at concentrations of 75 ng/mL (**Table 2**).

Batch		25.0 ng/mL (LLOQ)	75 ng/mL (Low)	1600 ng/mL (Mid)	8000 ng/mL (High)
1	**Mean**	26.8	72.0	1600	7940
	% CV	5.2	5.7	1.1	0.5
	% Bias	7.2	-4.1	0.0	-0.8
2	**Mean**	24.6	72.7	1600	8090
	% CV	8.9	6.1	2.3	1.3
	% Bias	-1.7	-3.1	0.1	1.1
3	**Mean**	26.9	76.2	1610	7980
	% CV	4.6	2.8	1.4	1.8
	% Bias	7.4	1.6	0.9	-0.3
Overall	**Mean**	26.1	73.6	1610	8000
	% CV	7.3	5.4	1.6	1.5
	% Bias	4.3	-1.8	0.3	0.0

Table 1. Precision and Accuracy Results for Melphalan Quality Control Samples (n = 6 replicates per batch)

Plasma lot	Avg drug area	Avg I.S. area	Avg area ratio
Lot 1	29495	307712	0.09585
Lot 2	30769	314457	0.09785
Lot 3	30997	316709	0.09787
Lot 4	31125	320286	0.09718
Lot 5	32029	322400	0.09935
Lot 6	31338	317665	0.09865
mean	**30959**	**316538**	**0.09779**
n	**6**	**6**	**6**
SD	**837**	**5137**	**0.00121**
% CV	**2.7**	**1.6**	**1.2**

Table 2. Matrix Effect for Melphalan in Human Plasma

Hemolytic and lipemic plasma matrix testing and the presence of 560 µg/mL of Captisol® did not indicate any measurable effect on the assay (data not shown). Incurred sample reanalysis (ISR) from clinical trials indicated that all ISR samples were within 12% of their original values (61 out of 528 tested). Melphalan has been determined as stable in human K_2EDTA plasma for up to 603 days storage in polypropylene tubes at -70 °C.

This method was subsequently used for sample analysis from a Phase IIa clinical trial entitled "Open-Label, Randomized, Pharmacokinetic Comparative, Cross-Over Study of CDX-353 and Alkeran® for Injection in MM Patients Undergoing ASCT." Representative data is presented in **Figure 6** with pharmacokinetic sampling from 10 min to 8 hrs post infusion. The C_{max} following CDX-353 administration, ranged from 2,700 to 6,900 ng/mL, with the majority of samples below the quantitation limit at 8 hrs.

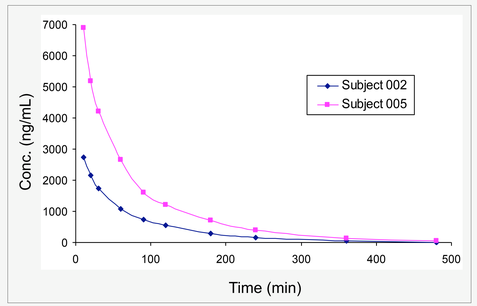

Figure 6. Melphalan Plasma Concentration Profiles (Min – Max) post 30 minute central-line infusion of CDX-353 at 100 mg/M²

CONCLUSION

This bioanalytical method showed acceptable accuracy, precision, selectivity, stability, and reproducibility. The validated bioanalytical method by KCAS has been demonstrated to be simple and reliable in supporting formulation development and clinical studies.

ACKNOWLEDGEMENTS

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