UCSF

UC San Francisco Previously Published Works

Title

Determination of melphalan in human plasma by UPLC—UV method

Permalink

https://escholarship.org/uc/item/5n28m88d

Journal

Cancer Chemotherapy and Pharmacology, 83(5)

ISSN

0344-5704

Authors

Huang, Liusheng
Cheah, Vincent
Chan, Danna
et al.

Publication Date

2019-05-01

DOI

10.1007/s00280-019-03786-6

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Cancer Chemother Pharmacol. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:

Cancer Chemother Pharmacol. 2019 May ; 83(5): 905–910. doi:10.1007/s00280-019-03786-6.

Determination of melphalan in human plasma by UPLC-UV method

Liusheng Huang[1], **Vincent Cheah**[1], **Danna Chan**[1], **Florence Marzan**[1], **Christopher C. Dvorak**[2], **Francesca T Aweeka**[1], and **Janel Long-Boyle**[1]

[1.]Department of Clinical Pharmacy, University of California at San Francisco, San Francisco, CA 94143

[2.]Department of Pediatrics, University of California at San Francisco, San Francisco

Abstract

It is desirable to develop a fast method for quantification of melphalan due to its instability. Here we report a method for quantification of melphalan (MPL) in human plasma using a UPLC-PDA system. Briefly, 50 μL plasma sample was mixed with 25 μL internal standard (2500 ng/mL acetylmelphalan in methanol) and 25 μL 20% trichloroacetic acid, and centrifuged at 21,000 g (15,000 rpm) at 4 °C for 3 min. The supernatant (5 μL) was injected onto an Acquity™ BEH C18 LC column (2.1 × 50 mm, 1.7 μm) and eluted with 25 mM NH_4AC (pH 4.7) - acetonitrile in a gradient mode at a flow rate of 0.6mL/min. The column kept at 40±5 °C and the autosampler kept at 4±5 °C. The detector set at 261 nm, and sampling rate was 40points/sec. The retention times were typically 2.11 min for melphalan and 2.38 min for the internal standard. Total run time is 4 min per sample. Calibration range was 100–40,000ng/mL. The lower limit of quantification was 100ng/mL. The method was validated based on the FDA guidelines, and applied to a clinical pharmacokinetic study in pediatric patients.

Keywords

Melphalan; UPLC-UV; plasma; pediatric; pharmacokinetic

1. Introduction

The serendipitous observation, that victims of mustard gas in World War I often had leukopenia[1], lead to development of nitrogen mustards as anti-cancer drugs. As one of the early developed nitrogen mustards, melphalan (MPL) has been used in the treatment of haematological malignancies for over 60 years[2]. Lately, MPL has also been used as a conditioning treatment at a high-dose prior to both autologuous and allogeneic hematopoietic cell transplantation (HCT)[3]. Chemically, MPL is nitrogen mustard coupled with L-phenylalanine synthesized in early 1950's in the U.K.(Fig. 1)[4]. It is a bifunctional

Corresponding author: Liusheng Huang (ORCID, 0000-0003-2702-4486), Drug Research Unit, Department of Clinical Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94110, USA. Tel: 1-415-502-2594, fax: 1-415-476-0307, liusheng.huang@ucsf.edu.

Conflict of interest: The authors declare that they have no conflict of interest.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

alkylating agent targeting DNA. It is unstable in aqueous solution, mainly degraded to mono- and di-hydroxymelphalan with other minor products[5]. In plasma, 69% MPL is bound to proteins, contributing to a 3-fold higher stability than aqueous solution. The degradation half-life in human plasma is 12.8hr[6]. MPL is mainly eliminated by renal excretion[7].

To support a clinical study for the evaluation of systemic MPL exposure in pediatric HCT recipients, a method for quantification of MPL in low volumes of human plasma is needed. Based on the regimens of the intended clinical study and previous pharmacokinetic (PK) studies[3, 8–12], we expect minimal concentrations in plasma to be >100ng/mL. The maximum concentration reported in the PK studies is <20,000ng/mL. Therefore, we aim to develop a method with the calibration range at 100–40,000ng/mL. Since stability of MPL is a major concern but sensitivity requirement is not high, our goal is to develop a high throughput method with ultraviot (UV) spectrophotometry detection. Numerous methods using high performance liquid chromatography coupled with UV detector (HPLC-UV) have been published for measurement of MPL in human plasma[13–16]. The run times per sample are all over 10 min. Ultra performance liquid chromatography (UPLC) enables us to develop high throughput method with a run time of less than 5 min. So far, no UPLC-UV methods have been published. Here we reported a method using a UPLC system with a photo Diode Array (PDA) detector. The run time was only 4 min per sample.

2. Materials and Methods

2.1. Chemicals and reagents

Melphalan hydrochloride was purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA); the internal standard (IS) N-acetyl melphalan (Ac-MPL) was from ChemDiv Inc. (San Diego, CA, USA). Acetonitrile, methanol, water, and ammonium acetate, acetic acid, trichloroacetic acid (Certified ACS) were purchased from Thermo-Fisher Scientific (Fair Lawn, NJ, USA) as HPLC grade unless mentioned individually. Human plasma (sodium heparin as the anticoagulant) was purchased from Biological Specialty Co. (Colmar, PA, USA). Mobile phase A was prepared by dissolving 1.927g ammonium acetate (NH_4AC) and 1mL glacial acetic acid in 1L water followed by filtration before use; 20% trichloroacetic acid (TCA) was prepared by dissolving 4g TCA in 20mL water.

2.2. Instrumental

The work was performed on a Waters Acquity™ UPLC system consisted of Waters Acquity™ Binary Solvent Manager (two pumps), Sample Manager (Autosampler) and PDA detector, managed with Waters Empower® chromatography software version 3.0. LC separation was achieved on Acquity UPLC® BEH C_{18} column (50 × 2.1 mm, 1.7 μm) protected with VanGuard™ pre-column (5 × 2.1 mm, 1.7 μm) from Waters Inc, Milford, MA, USA. The LC setting was as follows: Mobile phase solvents were 25mM NH_4AC 0.1% HOAC (A) and acetonitrile (B). The flow rate was 0.6 mL/min. The gradient program consisted of 5%B (0–0.5min), 5–50%B (0.5–3.00min), 50–90%B (3.00–3.01min), 90%B (3.01–3.50min), 90–5%B (3.50–3.51min), and 5%B (3.51–4.00min). The column was heated at 40±5°C and autosampler was cooled to 4±5 °C. The PDA detector was set at

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

261nm with a sampling rate of 40 points/sec. Injection volume was 5 μL. The weak and strong needle wash solvents were 40% and 100% acetonitrile, respectively. The seal wash solvent was 20% methanol.

2.3. Preparation of calibrators and QC samples

Two aliquots of melphalan hydrochloride salt were weighed, converted to base form weight using a conversion factor of 0.893, and dissolved in methanol separately to prepare the MPL primary calibrator and QC stock solutions at 2 mg/mL (as base form).To confirm accuracy, the two stocks were diluted to 1μg/mL in water and immediately injected onto the UPLC system. The signal difference of the two solutions should be less than 5%. The stocks were diluted in methanol to prepare working solutions, which were spiked into heparinized human plasma to make the calibrators at 100, 250, 500, 1,000, 5,000, 10,000, and 40,000 ng/mL and QC samples at 300, 1,500, and 32,000 ng/mL. The content of methanol in the spiked plasma samples was ≤2.5%. Stock solution of Ac-MPL (the IS) was prepared in methanol, which was diluted in methanol to make 2.5 μg/mL IS working solution. The prepared solutions and plasma samples were stored at −70 °C until use.

2.4. Sample preparation

Plasma sample was pre-treated based on a previous method[17]: Plasma samples (50 μL) were pipetted into 1.5 mL polypropylene eppendorf tubes, to which were added 25 μL IS (2.5 μg/mL AC-MPL) and 25 μL 20% TCA. After vortex mixed, the samples were centrifuged at 25,000g at 4 °C for 3min and 60 μL of the supernatant was transferred to an autosampler vial. The processed samples were immediately placed in the autosampler maintained at 4 °C for injection. Injection volume was 5 μL. Samples were processed and run by a batch of 48 samples to avoid significant degradation.

2.5. Validation

The method was validated according to the guidelines of NIH-sponsored AIDS Clinical Trial Group Network[18], which was based on FDA guidelines[19]. One set of calibrators were processed for each run and injected in the beginning. Calibration curves were constructed by linear regression of the peak area ratio of analyte to internal standard (Y-axis) versus the nominal analyte concentrations (X-axis) with a weighting factor of 1/x. The lower limit of quantification (LLOQ) was established with precision and accuracy <20%. Intra-day precision and accuracy were determined by analysis of at least five replicates of each QC sample at low (300 ng/mL), medium (1,500 ng/mL), and high (32,000 ng/mL) concentration levels extracted with a set of calibrators in one batch. The same procedure was repeated on at least 2 different days with new samples to determine inter-day precision and accuracy (total: n ≥ 15 per concentration level). Precision was reported as relative standard deviation (RSD) and accuracy as percent deviation of the nominal concentration (% dev).

Recovery was evaluated by spiking MPL working solution in plasma and water, respectively, at the low and high concentrations, processed and analyzed in triplicate. Matrix effect was evaluated by spiking the MPL working solution in 6 different lots of heparin plasma and two lots of K_3EDTA plasma at low and high concentration, processed and analyzed in triplicate along with a set of calibrators. Dilution integrity was evaluated by diluting an extra-high QC

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

at 100 μg/mL by 3-, 5- and 10-fold with blank plasma, processed and analyzed in triplicate along with a set of calibrators and 2 sets of QCs. The stability of MPL was evaluated in the following conditions: 3 freeze-thaw cycles, freezer (−70 ºC) for 2 years, 4 °C and room temperature (22–25 ºC) in plasma and injection solvent (in autosampler 4 °C) for 24hr. Stock solution of MPL was tested for 2 years at −70 ºC. MPL working solution was tested in methanol for 4 days and water and 50% acetonitrile for 24 hr. IS working solution was tested at room temperature (22–25 ºC) for 6 days. The treated plasma samples were measured in triplicate at low and high concentrations and compared to the corresponding untreated samples. The treated neat solutions were diluted to 1μg/mL in water and immediately measured in triplicate. Stability was expressed as % degradation and calculated as follows:

$$\%\ \text{degradation} = 100 \times (C_{treated} - C_{untreated})/C_{untreated},$$

where C represents the measured mean concentration.

2.6. Application

The assay has been used in an ongoing clinical PK study on pediatric HCT patients at the University of California Benioff's Children's Hospital. The Institutional Review Board (IRB) at the University of California San Francisco approved this study and all patients and/or guardians provided written informed consent to participate in PK sampling. MPL was infused at variable doses per protocol over 15–30 minutes. Blood specimens (~0.5 mL) were collected in 2mL lithium heparin tubes at 5min, 15min, 30min, 1hr, and 2hr post end-of-infusion and centrifuged at 3000–4000rpm for 10min at 4°C within 30 minutes of drawing. After centrifugation, the plasma fraction was collected and frozen at −70 °C until analysis.

3. Results and discussion

3.1. UPLC optimization

MPL is acidic with predicted pKa1 = 2.1 and pKa2=9.5, calculated with advanced chemistry development (ACD/labs) software V11.02. We chose 25mM NH_4AC 0.1% HOAC in water (pH4.7±0.2) as mobile phase solvent A and acetonitrile as mobile phase B. Initially we tried to detect MPL and its hydrolyzed products monohydroxy MPL and dihydroxy MPL on an Acquity BEH C_{18} column (50×2.1mm, 1.7μm), the retention time was 0.40min, 1.34min, and 2.11min for dihydroxy-, monohydroxy- and parent MPL. However, interfering peaks were observed from plasma samples. We changed to an Acquity HSS T_3 column (75× 2.1mm, 1.8μm), with retention times for the 3 analytes at 0.75min, 1.64 min, and 2.44 min, respectively. Interfering peaks for dihydroxy MPL were still observed. Since our primary goal is to measure MPL, the method was validated for quantification of MPL only, while the hydrolyzed products could be qualitatively profiled if needed.

3.2. Sample preparation

To minimize the time of analysis, simple protein precipitation was chosen for sample preparation. A published method for MPL utilized MeCN to precipitate plasma protein, which required further dilution to match the low initial organic mobile phase[20].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Precipitation with cold methanol followed by direct injection was also reported[13], but distorted MPL peak was observed in our case. To simplify the procedure and match the initial LC mobile phase, here we used aqueous TCA to precipitate plasma protein as we described before[17]. The supernatant could be directly injected onto UPLC system without compromising the peak shape. In addition, sample was only diluted by 2-fold after TCA protein precipitation, while protein precipitation with organic solvents resulted in more diluted samples[21]. The following procedure was used in this assay: 25 μL 20%TCA and 25 μL IS solutions were added into a 50uL plasma sample. After vortex-mixing and centrifugation, 70 μL of the supernatant was transferred into an autosampler vial maintained at 4±5 °C and 5 μL was injected onto the UPLC system.

3.3. Validation

3.3.1. Calibration range: At 100 ng/mL, the peak area of MPL was 900, and the ratio of signal/noise was 13. Therefore, the LLOQ in this assay was set at 100ng/mL. The calibration curve was constructed with least square linear regression weighted by 1/x. In the 3 intra-/inter- day precision/accuracy experiments, the coefficient of determination (R^2) of calibration curve was 0.9992 or higher. The inter-day back-calculated concentrations of calibrators over 3 days were listed in Table 1. Representative chromatograms of MPL at LLOQ level along with blank plasma (double blank) and blank plasma spiked with IS were shown in Fig. 2.

3.3.2. Precision and accuracy: The intra-day precision (n = 6) was within 5% at low, medium, and high concentrations. The inter-day precision, calculated with the individual mean concentration from 3 days, was within 6% at all three concentration levels. The intra- and inter-day accuracy was all within 11%. At the LLOQ levels, the precision and accuracy met the criteria of <20% (Table 2).

3.3.3. Recovery and matrix effect—Recovery was evaluated by comparing the peak area of MPL or IS from plasma to the peak area of MPL or IS in injection solvent at the same concentration as the plasma samples. The recovery of MPL at low (300 ng/mL) and high (32000 ng/mL) was 81.8%and 87.8%, respectively, and the recovery for the IS was 95%. Matrix effect was evaluated using 6 different lots of sodium heparin plasma and 2 lots of K_3EDTA plasma. The deviation from nominal values at 300 ng/mL and 32,000 ng/mL was from −3.7 to 11.3%, for all lots of plasma, suggesting no significant matrix effect in the assay. In addition, no significant peak was found at the retention times of MPL and IS from the 6 lots of blank heparin plasma and 2 lots of K_3EDTA plasma (supplementary material Fig. S1). Although residual peak was co-eluted with the IS in some cases, the intensity was <5% of IS peak area.

3.3.4. Dilution integrity.—Plasma sample at 100μg/mL was diluted by 3-, 5- and 10-fold and analyzed in triplicate. The precision was within 5% and the deviation from nominal values was within 15%(Table 3). The results demonstrated clinical samples above the ULOQ level could be diluted by up to 10-fold without compromising the integrity of samples.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3.3.5. Stability.—In plasma, MPL was stable at room temperature (23±2 °C) for 4hr, but over 50% degradation observed after 26hr; MPL was stable for up to 24 hr at 4 °C and 2 years at −70 °C. The plasma samples were also stable after 3 freeze-thaw cycles if the thaw time was less than 1hr. In autosampler maintained at 4±5 °C, the processed samples were stable for 4hr but not stable overnight (21hr). We found the IS was degraded over 15% after 20hr in the autosampler. Therefore, analysis of samples on UPLC should be completed in a few hours after processed. MPL stock in methanol was stable for at least 12 days at room temperature and 2 years at −70 °C. MPL working solution (10 μg/mL) was not stable in water with 21.9% degradation observed after 1.5 hr and over 60% degradation after 8hr; it was stable in 50% acetonitrile for at least 8hr but 16.6% degradation was observed after 24hr (Table 4). Further investigation is ongoing to define long term stability in −70 ºC freezer.

3.3.6. Application: Using this method, 110 plasma samples were tested with the measured concentrations ranging from 105 to 7674 ng/mL, all within the calibration range. During the analysis, the precision of QCs at low, medium, and high levels were 8.4% (n=14), 9.9% (n=10), and 7.3% (n=14), respectively. All QCs were within the accuracy range (85–115%). The results demonstrate the sensitivity of the method met the requirement of the intended study.

4. Conclusion

Using a simplified protein precipitation for sample preparation, a fast UPLC-UV method was developed and validated for determination of MPL in low volume (50 uL) human plasma. With a short run time, 48 samples could be analyzed in 4hr that is in accordance with instability of MPL. The sensitivity of the assay met the requirement of the intended clinical study in pediatric patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

This work was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI Grant Number UL1 RR024131 (a shared instrument award to Dr. Huang) and UCSF-CTSI Grant Number KL2 TR000143 (Dr. Long-Boyle). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Funding support: This work was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI Grant Number UL1 RR024131 (a shared instrument award to Dr. Huang) and UCSF-CTSI Grant Number KL2 TR000143 (Dr. Long-Boyle). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

References

1. Krumbhaar EB, Krumbhaar HD. The Blood and Bone Marrow in Yelloe Cross Gas (Mustard Gas) Poisoning: Changes produced in the Bone Marrow of Fatal Cases. J Med Res 40(3), 497–508 493 (1919). [PubMed: 19972497]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2. Hall AG, Tilby MJ. Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies. Blood Rev 6(3), 163–173 (1992). [PubMed: 1422285]
3. Mizuno K, Dong M, Fukuda T et al. Population Pharmacokinetics and Optimal Sampling Strategy for Model-Based Precision Dosing of Melphalan in Patients Undergoing Hematopoietic Stem Cell Transplantation. Clin Pharmacokinet 57(5), 625–636 (2018). [PubMed: 28918602]
4. Bergel F, Stock JA. Cyto-active amino-acid and peptide derivatives. Part I. Substituted phenylalanines. J Chem. Soc 0(0), 2409–2417 (1954).
5. Boschmans J, De Bruijn E, Van Schil P, Lemiere F. Analysis of novel melphalan hydrolysis products formed under isolated lung perfusion conditions using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 27(7), 835–841 (2013). [PubMed: 23495030]
6. Ehrsson H, Lonroth U. Degradation of melphalan in aqueous solutions--influence of human albumin binding. J Pharm Sci 71(7), 826–827 (1982). [PubMed: 7120075]
7. Reece PA, Hill HS, Green RM et al. Renal clearance and protein binding of melphalan in patients with cancer. Cancer Chemother Pharmacol 22(4), 348–352 (1988). [PubMed: 3168148]
8. Nath CE, Shaw PJ, Montgomery K, Earl JW. Population pharmacokinetics of melphalan in paediatric blood or marrow transplant recipients. Br J Clin Pharmacol 64(2), 151–164 (2007). [PubMed: 17324241]
9. Gouyette A, Hartmann O, Pico JL. Pharmacokinetics of high-dose melphalan in children and adults. Cancer Chemother Pharmacol 16(2), 184–189 (1986). [PubMed: 3948304]
10. Nath CE, Trotman J, Tiley C et al. High melphalan exposure is associated with improved overall survival in myeloma patients receiving high dose melphalan and autologous transplantation. Br J Clin Pharmacol 82(1), 149–159 (2016). [PubMed: 26879446]
11. Nath CE, Shaw PJ, Trotman J et al. Population pharmacokinetics of melphalan in patients with multiple myeloma undergoing high dose therapy. Br J Clin Pharmacol 69(5), 484–497 (2010). [PubMed: 20573084]
12. Nath CE, Shaw PJ, Montgomery K, Earl JW. Melphalan pharmacokinetics in children with malignant disease: influence of body weight, renal function, carboplatin therapy and total body irradiation. Br J Clin Pharmacol 59(3), 314–324 (2005). [PubMed: 15752378]
13. Pinguet F, Joulia JM, Martel P, Grosse PY, Astre C, Bressolle F. High-performance liquid chromatographic assay for melphalan in human plasma. Application to pharmacokinetic studies. J Chromatogr B Biomed Appl 686(1), 43–49 (1996). [PubMed: 8953191]
14. Poujol S, Pinguet F, Mougenot P et al. Quantitation of melphalan in human plasma by liquid chromatography and solid phase extraction: Application to pharmacokinetic study. J Liq Chromatogr R T 27(2), 301–313 (2004).
15. Springolo V, Borella F, Finardi GP, Gatti MT, Coppi G. High-performance liquid chromatographic determination of m-bis(chloroethyl)aminophenyl-L-alanine in plasma. J Chromatogr 490(1), 224–229 (1989). [PubMed: 2760154]
16. Chang SY, Alberts DS, Melnick LR, Walson PD, Salmon SE. High-pressure liquid chromatographic analysis of melphalan in plasma. J Pharm Sci 67(5), 679–682 (1978). [PubMed: 641809]
17. Huang L, Lizak P, Dvorak CC, Aweeka F, Long-Boyle J. Simultaneous determination of fludarabine and clofarabine in human plasma by LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 960 194–199 (2014).
18. Clinical Pharmacology Quanlity Assurance (Cpqa) Program. CPQA Guidelines for chromatographic method development and validation based on (and including) FDA guidelines dated May 2001, Version 4.0 (2012) 1–52 (2012).
19. Us Department of Health and Human Services, Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation 5 2001.
20. Sparidans RW, Martens I, Valkenburg-Van Iersel LB, Den Hartigh J, Schellens JH, Beijnen JH. Liquid chromatography-tandem mass spectrometric assay for the PARP-1 inhibitor olaparib in combination with the nitrogen mustard melphalan in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 879(21), 1851–1856 (2011).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Huang L, Marzan F, Jayewardene AL, Lizak PS, Li X, Aweeka FT. Development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometry method for determination of isoniazid in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 877(3), 285–290 (2009).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

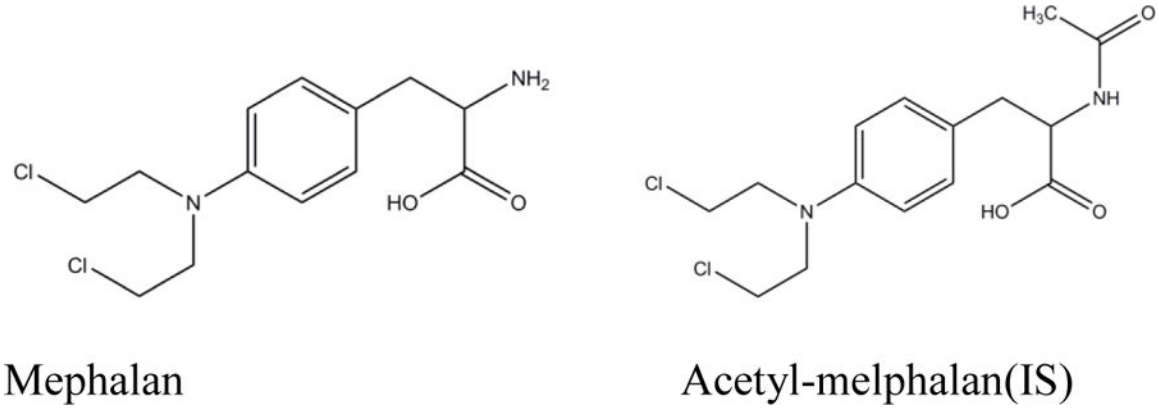

Fig. 1.
Chemical structures of melphalan and acetyl-melphalan (IS).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

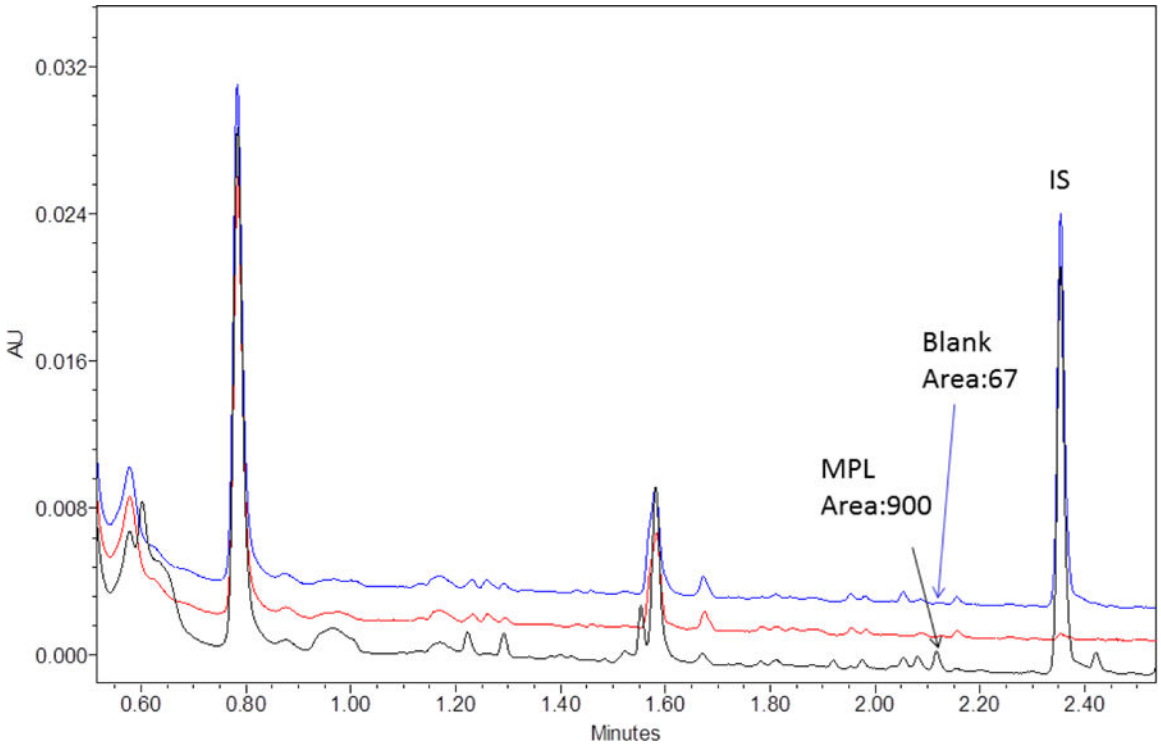

Fig 2.

Representative chromatograms of blank plasma (red), blank plasma spiked with IS (blue), and MPL at LLOQ level (black).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table.1.

Inter-day average back-calculated calibrataor concentrations (n = 3)

Nominal conc, ng/mL	100	250	500	1000	5000	10000	40000
Mean	97.9	243	541	962	5082	9833	40091
%CV	12.5	2.1	2.1	7.0	4.3	2.0	1.1
% dev	−2.13	−2.67	8.27	−3.83	1.65	−1.67	0.23
n	3	3	3	3	3	3	3

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2.

Intra- and inter-day precision and accuracy.

	Intra-day				Inter-day			
nominal, ng/mL	100	300	1500	32000	100	300	1500	32000
Mean	96–120	283–298	1344–1485	31492–33488	108	292	1425	32250
%CV	2.3 – 6.9	1.6 – 2.4	1.8 – 3.8	1.2 – 3.1	11.1	2.9	5.1	3.4
%dev	−3.8 – 20	−5.8 – (−0.7)	−10.4 – (−1.0)	−1.6 – 4.6	7.7	−2.5	−5.0	0.8
n	6	6	6	6	3	3	3	3

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3.

Dilution Integrity (n=3)

Dilution (fold)	**3**	**5**	**10**
Nominal, ng/mL	**33333**	**20000**	**10000**
Mean	35965	21216	11193
CV %	1.8	1.0	0.5
% Dev	7.90	6.08	11.9
n	3	3	3

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 4.

Stability of melphalan. Data represents mean ±SD (n=3) of concentration for plasma samples or peak area for solution.

conditions	untreated	treated	degradation, %
Plasma, 23±2 °C			
2hr 300 ng/mL	312±5	287±8	−8.0
32,000ng/mL	32459±367	30091±413	−7.3
4hr 300 ng/mL		277±1	−11
32,000ng/mL		28418±165	−12.4
26hr 300 ng/mL		108±1	−65.4
32,000ng/mL		15333±216	−52.8
Plasma, 4 °C			
24hr 300 ng/mL	312±5	317±22	1.6
32,000ng/mL	32459±367	30216±224	−6.9
Plasma, −70 °C			
2yr 300 ng/mL	281±3	241±2	−14
32,000ng/mL	35804±311	34916±266	−2.5
Plasma 3 freeze-thaw cycles			
300 ng/mL	309±5	293±6	−5.2
32,000ng/mL	31555±1012	30260±341	−4.1
In autosammpler, 4 °C			
4hr 300 ng/mL	306±10	308±12	0.7
32,000ng/mL	29554±1166	30085±1188	1.8
21hr 300 ng/mL	282±4	327±7	16
32,000ng/mL	32178±176	36646±326	14
IS in 5%TCA 25%MeOH, 4 °C			
3hr	21782±82	21347±458	−2.0
20hr		17946±479	−18
IS in methanol, 23±2 °C, 6 days	22863±103	22829±333	−0.1
Stock MPL in methanol			
23±2 °C, 12 days	23289±1468	22594±426	−3.0
−70 °C, 2 years	24356±73	24552±286	0.8
10 μg/mL MPL 23±2 °C			
in methanol 24hr	19496±673	19187±233	−1.6
4 days		20328±215	4.3
in water 1.5hr	19555±1686	15278±127	−21.9
3hr		12601±419	−35.6
8hr		7478±90	−61.8
24hr		1892±75	−90.3
in 50% acetonitrile 3hr	20024±1440	19915±22	−0.54
8hr		20203±70	0.90
24hr		16695±125	−16.6