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CONFERENCE

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TITLE

Quantitative LC-MS/MS Determination of Melphalan in Human Plasma

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ABSTRACT

Novel Aspect: A simple and reliable LC/MS/MS method for a compound that shows instability in human plasma.

Introduction

Melphalan is a chemotherapy drug belonging to the class of nitrogen mustard alkylating agents. As a wide spectrum antitumor agent it is indicated for the palliative treatment of multiple myeloma, and non-resectable epithelial carcinoma of the ovary. Melphalan shows to be unstable in human plasma and degrades significantly at room temperature and in ice bath. We report here the procedure to stabilize the compound in human plasma and the development and validation of a protein precipitation LC/MS/MS method for melphalan in human plasma.

Methods

Human plasma samples were treated with acetic acid upon collection for stabilization of melphalan. The analyte and its stable isotope labeled internal standard were extracted from 100µL of human plasma by protein precipitation using 200µL of methanol. Following vortexing and centrifugation, the supernatant was transferred to autosampler vials and injected directly for LC/MS/MS analysis. Liquid chromatography was performed using a Luna Phenol-Hexyl column (50x2 mm, 5µ) with gradient elution on Shimadru LC-10A pumps and CTC autosampler. The MS/MS detection of the analyte was performed on a Sciex API4000 tandem mass spectrometer under positive TurboIonSpray mode. The retention time for the analyte was ~1.5 min. The analytical run time was ~5 min.

Preliminary results

Results of evaluation on stability of melphalan in K_2EDTA human plasma showed that melphalan degraded ~30% after 6-hour storage at room temperature. The degradation on ice-bath is reduced but noticeable. To ensure sample integrity during sample storage and handling process, stabilization of the analyte in plasma was evaluated. Knowing the nature of instability for melphalan, stabilization using acidic condition was tested and proven that the degradation can be prevented. With the plasma treated with acetic acid as the stabilizer, the method was validated over a linear calibration range of 0.2 to 200 ng/mL. The validation data showed that through 3 consecutive core validation runs on LLOQ, low, middle, and high QC concentrations, the assay shows intra- and inter-day precision of ≤7.1 and ≤3.5 %CV, respectively, and intra- and inter-day accuracy of -8.0 to 5.0 %RE and -7.6 to 2.7 %RE, respectively. The method was selective, no interference peaks were found at the retention times of the analytes and the internal standards. Matrix effect evaluation showed a moderate ion suppression. Stability of the analyte was evaluated in acetic acid treated human plasma under conditions of room temperature bench-top storage, freeze/thaw cycles, and freezer (-20°C and -70°C) storage. Melphalan was found to be stable under all conditions evaluated. In brief, the method showed to be simple, sensitive, reproducible, and reliable.