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CONFERENCE

57th ASMS Conference on Mass Spectrometry

TITLE

Quantitative Determination of Temozolomide In Human Plasma by LC-MS/MS

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ABSTRACT

Novel Aspect: Stabilization procedure was established and a quick and reliable LC-MS/MS method was validated and applied for temozolomide in human plasma.

Introduction

Temozolomide is an oral alkylating agent used for the treatment of Grade IV astrocytoma which is an aggressive brain tumor, also known as glioblastoma multiforme. Temozolomide is known to be unstable under physiological conditions and is converted to 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) by a nonenzymatic, chemical degradation process. To support clinical studies of temozolomide, we report here the evaluation of stabilization of the analyte, and the development and validation of a solid-supported liquid-liquid extraction LC/MS/MS method for the determination of temozolomide in human plasma.

Methods

Blank plasma was fortified with 1N HCL to stabilize Temozolomide. The method utilized solid-support liquid-liquid extraction of the analyte and internal standard from human plasma using ethyl acetate:methyl tert-butyl ether/3:1 (v:v) and analysis using reversed phase HPLC with Turbo Ion Spray® MS/MS detection. Positive $(M+H)^+$ ions for temozolomide and temozolomide-d_3 were monitored in MRM mode. Analyte-to-IS peak area ratios for the standards were used to create a linear calibration curve using $1/x^2$ weighted least-squares regression analysis. The analysis time was 6 minutes and flow rate was 0.4 mL/min. Interday and intraday accuracy and precision, sample stability at ambient temperature, freeze/thaw stabilities, autosampler stability, matrix effect, dilution and recovery were determined.

Preliminary results

An LC/MS/MS assay was validated in the range of 0.1 to 20 mg/mL. Intra day and Interday precision and accuracy were determined by analyzing six replicates of each QC sample from three consecutive core validation runs. Intraday precision and accuracy ranged from 1.5 to 5.1 %CV and -10.0 to 7.7 %RE (relative error), respectively. Interday precision and accuracy ranged from 4.5 to 5.3%CV and -5.0 to 3.3 %RE, respectively. The analyte is stable in fortified plasma (with 1N HCL to stabilize) for 19 hours at room temperature, and after 5 freeze/thaw cycles at -70ºC. The extraction efficiency at low, middle and high concentrations using five replicates was 53.8 – 63.2%. Specificity of the method was proven by extracting and analyzing six individual lots of blank human plasma and six individual lots of blank plasma spiked with drug and appropriate internal standard. No significant interference was observed at the retention time of the Temozolomide peak. The relative error for the analyte at 0.1 µg/mL was -3.0 %, while the coefficient of variation for the analyte was 5.2%. Human plasma samples were prepared at one concentration (30 mg/mL) and diluted with pooled blank human plasma at a dilution factor of 20 in five replicates. The test results show that after the dilution factor was applied, the mean of the determined concentrations of the diluted samples were within -14.0% of the nominal value before dilution, and the %CV was 5.2%. Experiments were also conducted to evaluate the matrix effect, reinjection reproducibility and injection carryover and the results will be presented. The assay was shown to be rugged, sensitive, selective, accurate and reproducible. This method has been successfully applied to

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support clinical study.