Quantification of ceftazidime in vitreous humor using ultra-performance convergence chromatography-tandem mass spectrometry

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Background: Patients with suspected postoperative bacterial endophthalmitis are at high risk of rapidly progressing towards blindness if not treated immediately. The initial approach to treatment frequently involves the administration of intravitreal antibiotics. Ceftazidime, in conjunction with vancomycin, is commonly administered as the primary therapeutic intervention for such cases.

Ceftazidime exposure should exceed the minimal inhibitory concentration (MIC) at the actual site of infection. In order to investigate the disposition of ceftazidime following an intravitreal injection, we developed and validated a quantification method for ceftazidime in vitreous humor using ultra-performance convergence chromatography coupled to tandem mass spectrometry.

Methods: Sample preparation was performed by protein precipitation with the internal standard solution (2 mg/L of meropenem-D_6 in methanol) to 20 µL of each sample. The analysis was performed on a Waters Acquity UPC^2-system which was coupled to a Waters Xevo TQ-S micro triple quadrupole mass spectrometer (Waters Corp, Milford, MA, USA). For the chromatographic separation, a gradient elution program was applied with a total run time of 5.0 min. MS/MS detection was performed in positive ion mode using electrospray ionization. Analytical method validation was performed according to the European Medicines Agency (EMA) and Food and Drug Administration (FDA) guidelines.

Results: Validation included the following parameters: linearity, limits of quantification, accuracy, inter-day and intra-day precision, carry-over, autosampler stability, short-term and long-term stability. The method was found linear ($r^2 > 0.990$) in the range from 1.3 mg/L to 99.6 mg/L and the inaccuracies and imprecisions were <15%. A significant carryover effect was observed (53% of the LLOQ) when injecting a blank sample after an ULOQ sample. However, after including the injection of one extra blank sample, no carryover effect was observed. Therefore, after each patient sample, one blank sample should be injected.

Conclusions: We developed and validated an ultra-performance convergence chromatography-tandem mass spectrometry method for the fast and reliable determination of ceftazidime in vitreous humor. The method was already successfully applied to 22 clinical patient samples. The fast and efficient sample preparation and short analysis run time make this method highly suitable for clinical settings and research purposes.