Development of a LC-MS/MS-Method for the Determination of Colistin and Colistin methanesulfonate and Application for Plasma-Samples of Critically Ill patients

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Introduction
The extent of multidrug-resistant (MDR) strains of Pseudomonas aeruginosa and Acinetobacter baumannii has initiated a concern of basically losing second line antibiotics. Colistin, a cyclic lipopeptide, is a last resort antibiotic to be used in critically ill patients. Since the availability of suitable methods is crucial for clinical and research purposes, the objective is to develop a method suitable for determination of Colistin and Colistin methanesulfonate (CMS) in human plasma.

Materials and Methods

Stock Solutions
Colistin stock solution was made in the following: 0.01 mg/mL in 0.9% saline solution 1:10 dilution. Stock solutions were stored at -20°C.

Sample Preparation
Blood samples were collected in K2EDTA vacutainer tubes and centrifuged at 1610 g, 10 min at RT, to obtain plasma. The samples were stored at -20°C until analysis.

Results

Sample Stability

The obtained results showed no detectable carryover for the isotope dilution method (ISDM) for both Colistin (A) and Colistin methanesulfonate (B) in the absence or presence of patient plasma at 0°C for 24 h and at 37°C for 30 min. This can be attributed to the stability of the analytes during the described method conditions.

Extraction Recovery

Extraction recovery studies were performed using matrix-matched ISDM solutions at concentration levels ranging from 1 to 20 ng/mL. The results showed an extraction recovery of 76.7% at the lower level and 87.6% at the higher level, which are well within the acceptable range of 70-110%.

Carryover

Carryover was not observed in 100% and 10% dilutions of ISDM solution for both analytes.

Matrix Effects

Matrix effects were determined by adding colistin to human plasma before and after protein precipitation. As a reference, the matrix effect of Colistin A is the same concentration was prepared in 0.1% TFA at 3 mg/mL methanesulfonate in water and treated the same way as the plasma samples have been prepared. Human plasma without colistin was precipitated and after precipitation spiked with colistin and precipitation of the same concentration was made to assess the matrix suppression.

Carryover

The for the auto sampler type used, with the use of 10% methanol and 0.05% TFA in water was possible to reduce the carryover below 1% which was acceptable.

Patient Samples

Colistin-N plasma samples of critically ill patients were obtained in a 2528-hydrophilic interaction column. An isocratic mobile phase consisting of 0.1% TFA in water:acetonitrile (95:5) was used. The ion spray voltage was set at 4000 V, the temperature at 700°C, the curtain gas at 0.8 L/min and the nebulizer gas at 20 L/min. The analysis was performed using an API 4000 QSTAR triple quadrupole mass spectrometer equipped with an ESI source. The multiple reaction monitoring (MRM) method was used for screening and confirmation of all analytes. The software used for the analysis was Analyst 1.5.2.

Results in Critically Ill patients

Five patients have been already under treatment with CMS when the titr was started, with two of them having a colistin levels below the recommended minimum concentration of 2 mg/L. It is found, that the measured colistin levels in general showed a good variability depending on the actual treatment situation with a mean value of 3.8 ± 1.10 mg/L. There were no significant differences between trough and peak levels of colistins measured.

Due to the short half-life of CMS the trough levels are low compared to the peak levels. In patients with BMN symptoms we have been able to eliminate CMS from plasma and calculated half-lives of 107 ± 3 h. However, in 3 patients a two-compartment model seems better to describe the elimination of colistin with half-lives of 58 ± 8 h and 8 ± 5 h.

Discussion

The obtained results showed no detectable carryover for both analytes. This can be attributed to the stability of the analytes during the described method conditions.

Matrix effects were determined by adding colistin to human plasma before and after protein precipitation. As a reference, the matrix effect of Colistin A is the same concentration was prepared in 0.1% TFA at 3 mg/mL methanesulfonate in water and treated the same way as the plasma samples have been prepared. Human plasma without colistin was prepared and after precipitation spiked with colistin and precipitation of the same concentration was made to assess the matrix suppression.

The analysis of plasma samples was performed using the multiple reaction monitoring (MRM) method with a flow of 10 µL/min via a 10-cm PEEK tube and the caliper flow. The signal for the MRM transitions was integrated and the signal on the mass spectrometer was used for quantification.

For the auto sampler type used, with the use of 10% methanol and 0.05% TFA in water was possible to reduce the carryover below 1% which was acceptable.

Patient Samples

Because of patients under treatment with CMS, colistin-N plasma samples of critically ill patients were obtained in a 2528-hydrophilic interaction column. An isocratic mobile phase consisting of 0.1% TFA in water:acetonitrile (95:5) was used. The ion spray voltage was set at 4000 V, the temperature at 700°C, the curtain gas at 0.8 L/min and the nebulizer gas at 20 L/min. The analysis was performed using an API 4000 QSTAR triple quadrupole mass spectrometer equipped with an ESI source. The multiple reaction monitoring (MRM) method was used for screening and confirmation of all analytes. The software used for the analysis was Analyst 1.5.2.

Several patient plasma samples were found to have CMS at concentrations above the recommended minimum concentration of 2 mg/L. The measured CMS levels in general showed a good variability depending on the actual treatment situation with a mean value of 3.8 ± 1.10 mg/L. There were no significant differences between trough and peak levels of CMS measured.

Due to the short half-life of CMS the trough levels are low compared to the peak levels. In patients with BMN symptoms we have been able to eliminate CMS from plasma and calculated half-lives of 107 ± 3 h. However, in 3 patients a two-compartment model seems better to describe the elimination of colistin with half-lives of 58 ± 8 h and 8 ± 5 h.