Poster

[P27-3] P27-3: Anti-infective drugs (8): Antiviral

Chair: Birgit C. P. Koch, The Netherlands

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[P27-3-2] A simple, fast and high throughput quantitative method for the simultaneous determination of dolutegravir, atazanavir, darunavir, efavirenz, lopinavir and ritonavir in human plasma using ESI+ LC-MS/MS

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Background

Since HIV discovery in 1983, FDA has approved 40 products for HIV treatment. Assuring the correct plasma concentration is fundamental for the prevention of HIV transmission and toxicity effects of antiretroviral drugs (ARV), as well as avoiding virus resistance and to ensure the patients quality of life[1,2]. Dolutegravir, an integrase inhibitor, is a promising ARV[3] and thus a fast bioanalytical method was developed for quantification. Five other common ARVs were also added, based on present and future ARV TDM relevance for the Swedish population.

Methods

50 L of patient plasma was deproteinised using acetonitrile containing deuterated internal standards followed by centrifugation. Five microliters of supernatant was injected onto a Waters Acquity Ultra Performance LC-system. Total chromatographic time was 2.5 min using an Acquity UPLC BEH C18 1.7M, 2.1 X 50 column, with a gradient using 0.1% formic acid in MQ water and methanol as mobile phases, at a flow rate 0.4 mL/min. The mass spectrometric detection was performed in positive mode (ESI+) for all analytes, on a Waters Quattro Premier XE triple quadrupole mass spectrometer.

Results

The bioanalytical method was successfully validated according to European Medicines Agency (EMA)[4] for the range 50 –25’000 ng/mL for atazanavir, darunavir, dolutegravir, lopinavir and ritonavir and 100 –25’000 ng/mL for efavirenz. The accuracy, inter- and intra-assay precision was within EMA criteria for all tested QC levels. Samples were stable up to one week at 4° and -20˚C, and at least 4 months in -80˚C.

Conclusion

This simple chromatographic method features the simultaneous quantification of dolutegravir and 5 other commonly used ARVs. This combination covers the main TDM needs of the Swedish patient population, including new first-choice HIV treatments with dolutegravir. The method was optimized for a clinical environment and the short chromatographic run time is compatible with high throughput. The detection of all drugs in positive mode allows good method performance even using basic tandem mass spectrometric hardware. In conclusion, a flexible TDM method was developed and successfully validated according to international standards.

References:

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