Poster

[P27-1] P27-1: Anti-infective drugs (6): Anti-MRSA and antifungals

Chair: Yasuhiro Tsuji, Japan

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[P27-1-10] A validated easy HPLC-MS method for quantification of isavuconazole and other four triazole antifungal drugs in human plasma

Antonio D'Avolio[1], Giovanna Fatiguso[2], Fabio Favata[3], Ilaria Zedda[4], Amedeo De Nicolo'[5], Giovanni Di Perri[6]
(1.University of Turin, 2.University of Turin, 3.University of Turin, 4.University of Turin, 5.University of Turin, 6.University of Turin)

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Background

Azoles compounds are able to inhibit key enzyme in fungal ergosterol synthesis, and remain a primary necessity for treatment of serious systemic infections in critically ill patients. This class includes first generation triazoles as *fluconazole (FLU)* and *itraconazole (ITC)*, second generation *voriconazole (VRC)* and *posaconazole (PSC)*, and the recently approved *isavuconazole (ISC)*[1].
Therapeutic drug monitoring (TDM) seems to be the best approach to optimize dosing for individual patients, who showed altered pharmacokinetics and pharmacodynamics for these drugs[2]. We aimed to develop and validate a HPLC-single mass spectrometry method for quantification of FLU, ITC, VRC, PSC and ISC in human plasma.

Method:

Sample preparation steps consisted in protein precipitation and dilution with water. [^{13}C, $^{2}H_4$]-isavuconazole, [$^{2}H_4$]-fluconazole and 6,7-dimethyl-2,3-di(2-pyridyl)quinaxoline have been used as internal standards. Chromatographic separation was performed on Atlantis(®)T3-5μm 4.6×150mm column, with a gradient of water and acetonitrile, both added with 0.05% formic acid. The instrument (HPLC-single mass detector) was settled in positive electrospray ionization mode (ESI+) for all drugs. The method was fully validated following the FDA and EMA guidelines.

Results

Run time was of 13 minutes and chromatographic peaks resulted well separated. Accuracy and precision inter/intra-day were below 15% for all drugs, and both recoveries and matrix effects resulted within the guidelines acceptance criteria. Calibration range was 15000-52 ng/mL for FLU and VRC, and 8000-31 ng/mL for the other drugs, basing on previously clinical literature data; a good sensibility resulted for lower standards. Integration was performed considering peak areas for each analyte. The performed stability investigations of stock solutions, standards and plasmatic quality controls in four different conditions (-80˚C;-20˚C;4˚C and room temperature) give reliable results. The selectivity evaluation with more than 40 other possible concomitant drugs showed no interferences. Moreover, the method has been evaluated with excellent results to an External-Quality Assessment and in real patients.

Conclusions

Monitoring antifungal levels could optimize efficacy and/or decrease toxicity for these drugs with variable plasma concentrations. Our method resulted precise and accurate, very simple, fast and feasible in clinical

routine analysis with a simple HPLC-MS instrument and it could be also used for isavuconazole TDM, the newest antifungal drug.

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