Poster

[P27-5] P27-5: Cardiovascular drugs (2)

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[P27-5-2] Simultaneous determination of metoprolol, amlodipine, canrenone and hydrochlorothiazide via LC-MS/MS in patients with therapy-refractory arterial hypertension

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Background

Therapy-refractory arterial hypertension is defined as a blood pressure (BP) in a subset of patients who fail to achieve BP control despite a three-drug regimen (including a diuretic). Various factors impact on loss of therapy response: drug-drug-interactions (DDIs) may cause altered pharmacokinetics (PK) of antihypertensive drugs. Upregulation of activity and expression of cytochrome P450 (CYP) enzymes can result in decreased plasma drug levels. Besides these PK considerations a significant problem could be nonadherence to drug therapy. In this regard Therapeutic drug monitoring (TDM) is a useful tool for detecting nonadherence. Therefore a LC-MS/MS-method for determination of Metoprolol(MET), Amlodipine(AML), Canrenone(CAN) and Hydrochlorothiazide(HCT) was developed.

Methods

An UHPLC-MS/MS method was developed and validated for simultaneous determination of MET, AML, CAN and HCT in serum and plasma matrix. Extraction of serum samples consisted of simple protein precipitation using acetonitrile. Stable isotope labeled analogues for each antihypertensive were obtained for internal standardization and quantitative analysis ($[^{2}H_{7}]$-MET, ($[^{13}C_{6}]$-AML, $[^{2}H_{4}]$-CAN, $[^{13}C_{6}]$-HCT,). Calibrators and quality controls were prepared in plasma matrix of normal individuals. Sample preparation: protein precipitation with acetonitrile and addition of internal standard-mix.

Results

All analytes were eluted within a runtime of 2.5 minutes. Linearity experiments were demonstrated in plasma over a concentration range from 1 - 750 g/l: MET: 5-750g/L, AML: 1-50g/l, CAN: 10-500g/l, HCT: 5-500g/L (R^2 >0.99).

Chromatographic separation was achieved using a C18 column (50x2.1mm, 1.9m particle size) and an isocratic elution. LC-MS/MS analyses were performed on a triple quadrupole mass spectrometer using positive and negative electrospray ionization in selected reaction monitoring (SRM) mode. Ion transitions monitored for quantitation were m/z 268 →74 for MET, m/z 409 →238 for AML, m/z 341 →91 for CAN and m/z 296 →205 for HCT. For all analytes, inter- and intra-day precisions (CV, %) varied between 1.7 and 14.0 and inter- and intra-day accuracy values ranged from -2.5 –7.1%. The lower limits of detection and quantification were: 0.08 and 0.23, 0.05 and 0.15, 2.82 and 8.54, and 0.02 and 0.05 g/L for MET, AML, CAN and HCT, respectively. Results of stability experiments were within the required range of +/- 15%.

Conclusions

Although the level of recommendation of TDM of antihypertensive drugs in patients with refractory hypertension is not yet established, the present LC-MS/MS-method can serve as an effective tool for detection of PK-alterations/nonadherence and may help to monitor antihypertensive pharmacotherapy.