Poster

[P27-7] P27-7: Assay

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[P27-7-5] Method development matrix effects: a case study

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Background

When developing HPLC-MS/MS methods for therapeutic drug monitoring or toxicology, it is important to work with a sample matrix which closely matches the matrix of unknown samples. Generally, this involves the use of pooled materials for which no demographic information is available. In this session, we discuss the discovery of age-specific matrix interference during the development of an assay for quantification of chlorhexidine, an antiseptic agent used for washing neonatal patients. We investigate the source of these matrix age-related effects for chlorhexidine and additionally investigate whether these age-specific effects also occur in serum spiked with two therapeutic drugs which are monitored in our laboratory.

Methods

We generated pools of serum from either neonatal patients (0-2 years), adults (>18 years), and commercially-available drug-free serum with chlorhexidine and two additional compounds, nortriptyline and voriconazole, at concentrations across their measurement intervals. We analyzed these materials and compared analyte recovery in all of the serum pools tested for each compound, when quantified with calibration standards prepared from matching and non-matching serum types, i.e. neonatal spikes quantified with drug-free commercially-available serum, etc. All analysis was performed with LC-MS/MS methods which were validated using either US FDA (chlorhexidine) or CLSI (nortriptyline and voriconazole) guidelines for method verification.

We also infused directly into a high-resolution mass spectrometer chlorhexidine standard solution, blank neonatal serum to determine whether the material causing chlorhexidine interference in neonatal serum had the same exact mass (5 ppm mass tolerance) as chlorhexidine.

Results

We found that the age-specific matrix effects dramatically affected chlorhexidine concentrations, resulting in >300% inaccuracy, relative to spiked concentrations, when neonatal serum spikes were quantified with non-neonatal serum calibrators. This inaccuracy was not observed when matrices were matched for spiked and calibration standards. The effect of calibrating with non-demographically matched serum has a less significant effect (<30%) on the accuracy of voriconazole and nortriptyline concentrations.

Conclusions

In this case of chlorhexidine analysis in neonatal serum, it was inappropriate to prepare calibration standards as per our typical procedure, with commercially-available drug-free serum. Additional care should be taken when developing methods which are intended to serve specific patient population, such as neonates.