Title: Bioanalytical Method Validation for the Quantitation of Perfluorooctanoic Acid in Human Serum

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Novel Aspect: A rigorous bioanalytical method validation of a representative, ubiquitous perfluorinated compound.

Introduction

Perfluorinated surfactants have received increased attention in the environmental and human health communities due to their ubiquitous presence, as well as their environmental persistence and possibly long half-lives in the human bloodstream. Advances in analytical methodology have facilitated the detection of these compounds at background levels in the low parts-per-billion range in human blood plasma and serum. The analytical uncertainty associated with these measurements is open to scientific criticism, and the errors are often unacceptable when compared to typical bioanalytical acceptance criteria. Application of the bioanalytical approach to method validation will be presented for the quantitation of perfluorooctanoic acid (PFOA) in human serum for two LC/MS/MS methods.

Methods

The bioanalytical methods both utilized ion-pairing, liquid-liquid extraction followed by gradient elution liquid chromatography and tandem mass spectrometry. Shimadzu LC 10AD pumps, a PE200 autosampler, and ionspray SRM MS/MS (positive ion) on a PE Sciex API 3000 were used in both methods. The target ranges of quantitation for the two methods were 1 ng/mL to 100 ng/mL and 0.05 µg/mL to 10 µg/mL. A structural analog, 9H-hexadecafluorononanoic acid, was used as the internal standard (IS). Due to the presence of PFOA in control human serum, the standard curves for the lower-level method were prepared in rabbit serum. Quality control (QC) samples were assayed in both human and rabbit serum to validate the use of rabbit serum standards for the quantitation of PFOA in human serum. Background subtraction was used to correct for the presence of PFOA in the control serum in both methods. In addition, extensive measures were taken to eliminate potential sources of PFOA from the extraction and the chromatographic system.

Preliminary Data

Higher-Level Method: The assay demonstrated a lower limit of quantitation of 50 ng/mL based upon a 25 µL aliquot of human serum. The calibration curves were fitted with a $1/x^x$ weighted quadratic regression, and the coefficients of determination (r^2) were ≥ 0.9950. The intra- and inter-assay accuracy (RE, -12.3 to 9.00%) and precision (CV, 2.07 to 7.70%), based upon the QC samples, were within the acceptance criteria (± 15%). The extraction recoveries of PFOA and the IS were 81.8 and 90.9%, respectively.

Lower-Level Method: The assay demonstrated a lower limit of quantitation of 1 ng/mL based upon a 50 µL aliquot of human serum. The calibration curves were fitted with a $1/x^x$ weighted quadratic regression, and the coefficients of determination (r^2) were ≥ 0.9929. The intra- and

inter-assay accuracy (RE, -0.567 to 5.57%) and precision (CV, 1.77 to 4.66%), based upon the human serum QC samples, were within the acceptance criteria (± 15%).

Keywords: Ionspray, MS/MS, Liquid Chromatography; Quantitative Analysis; Selected Reaction Monitoring

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