Resolution of Unexpected Reference Standard Components of (5R,S)-isoprostane F2 Type VI (iPF2 -VI) Using a Shallow UHPLC-MS/MS Gradient

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OVERVIEW
- Isomers of Prostaglandin F₂ (PGF₂α) are used as a measure of oxidative stress
- Ultra-High Performance Liquid Chromatography (UHPLC) has unprecedented resolving power
- Accurate quantification depends on well characterized reference standards

INTRODUCTION
A method improvement exercise for unconjugated iP2OAc acid, which incorporated an upgrade from conventional HPLC to UHPLC equipment, revealed a weakness in the certified characterization of the procured reference standard. The iP2OAc mixed diastereomer reference material used in the conventional method eluted as a single peak. However, the UHPLC method separated these known isomers as well as two additional unidentified and unexpected components in the reference material.

METHODS

Figure 1. Structures

Figure 2a. Reference Solutions
Figure 2b. Ultra Richard

RESULTS
- The IS was described as a pure 5S enantiomer, and only one peak was observed. Therefore the 1st analysis isotopic peak eluting closest to the retention time of the IS peak was assumed to be the 5S isomer (Figure 2a). The IS solution was considered as a working solution for all unknown species. The unknown peaks observed in the reference material were also present in urine lots that were assayed, as shown in Figure 2b.

CONCLUSIONS
No connection was verified between observed peaks in the reference material and potential late-eluting ethyl ester lactone components. The consistency of product ion spectra would suggest that the contaminants were other diastereomers. The project is on hold while the investigation of late-eluting ethyl ester lactone or ester M-H ion or simple adduct m/z that would generate a potential scans with both ESI and APCI ionization yielded no observed determination of the material.

REFERENCES