

## Biomarker for oxidative stress

### Background:

Quantification of F<sub>2</sub>-Isoprostanes is considered a reliable index of the oxidative stress status in vivo and is valuable in the diagnosis and monitoring of a variety of diseases. Because of complex and lengthy sample preparation procedures, current chromatography/mass spectrometry and immunoassays are impractical for measuring larger numbers of samples. Thus, we developed and validated a semi-automated high-throughput HPLC tandem mass spectrometry assay for the quantification of F<sub>2</sub>-Isoprostane F<sub>2t</sub> in human urine and plasma.

### Methods:

After protein precipitation (500 µL methanol/zinc sulfate added to 500 µL plasma), samples were injected into the HPLC system and extracted online. The extracts were then back-flushed onto the analytical column and detected with an atmospheric pressure chemical ionization-triple quadrupole mass spectrometer monitoring the deprotonated molecular ions [M-H]<sup>-</sup> of 15-F<sub>2t</sub>-IsoP ( $m/z= 353 \rightarrow 193$ ) and the internal standard 15-F<sub>2t</sub>-IsoP-d<sub>4</sub> ( $m/z= 357 \rightarrow 197$ )

### Results:

In human urine, the assay was linear from 0.025 to 80 µL/L and in human plasma from 0.0025 to 80 µL/L ( $r^2 > 0.99$ ). Interday accuracy and precision for concentrations above the lower limit of quantification were <10%. Autosampler stability at +4°C was established for 24 hours and frozen samples could undergo 3 freeze-thaw cycles. A potential effect of ion suppression on the results was excluded. Concentrations of 15-F<sub>2t</sub>-IsoP in urine of 16 healthy individuals ranged from 55–348 ng/g creatinine. In 16 plasma samples from healthy individuals, free 15-F<sub>2t</sub>-IsoP was detectable in all samples and concentrations were 3–25 ng/L.

### Conclusions:

This assay was fully validated, met all predefined method performance criteria, allows for analysis of >80 samples/day, and has sufficient sensitivity for quantifying 15-F<sub>2t</sub>-IsoP concentrations in plasma and urine from healthy individuals. It is, thus, suitable for clinical routine monitoring and has been used for the analysis of samples from large clinical trials.

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HPLC–Atmospheric Pressure Chemical Ionization MS/MS for Quantification of 15-F<sub>2t</sub>-Isoprostane in Human Urine and Plasma Clin. Chem., Mar 2007; 53: 489 - 497.

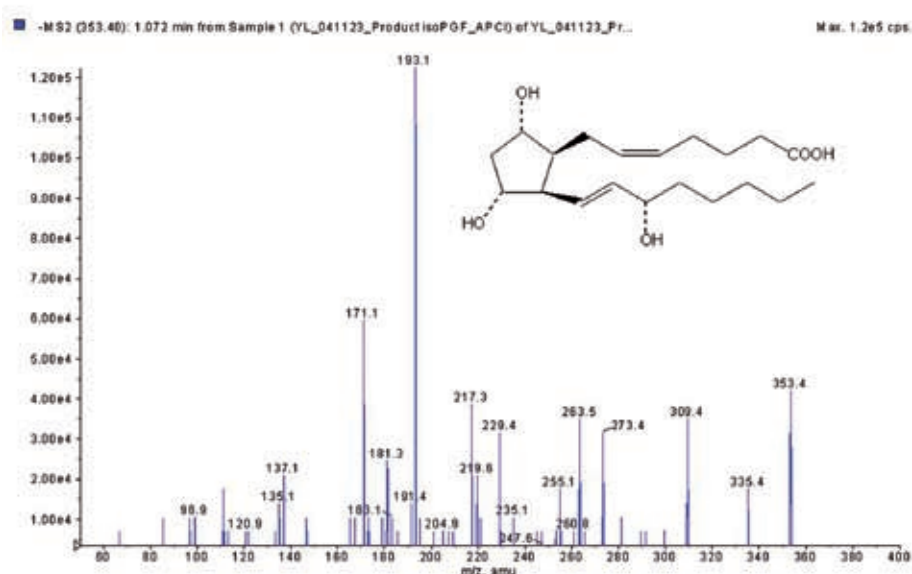


Fig. 1. MS/MS spectrum of 15-F<sub>2t</sub>-isoprostane and structure of fragment used for MRM. cps, counts per second. amu, atomic mass units.

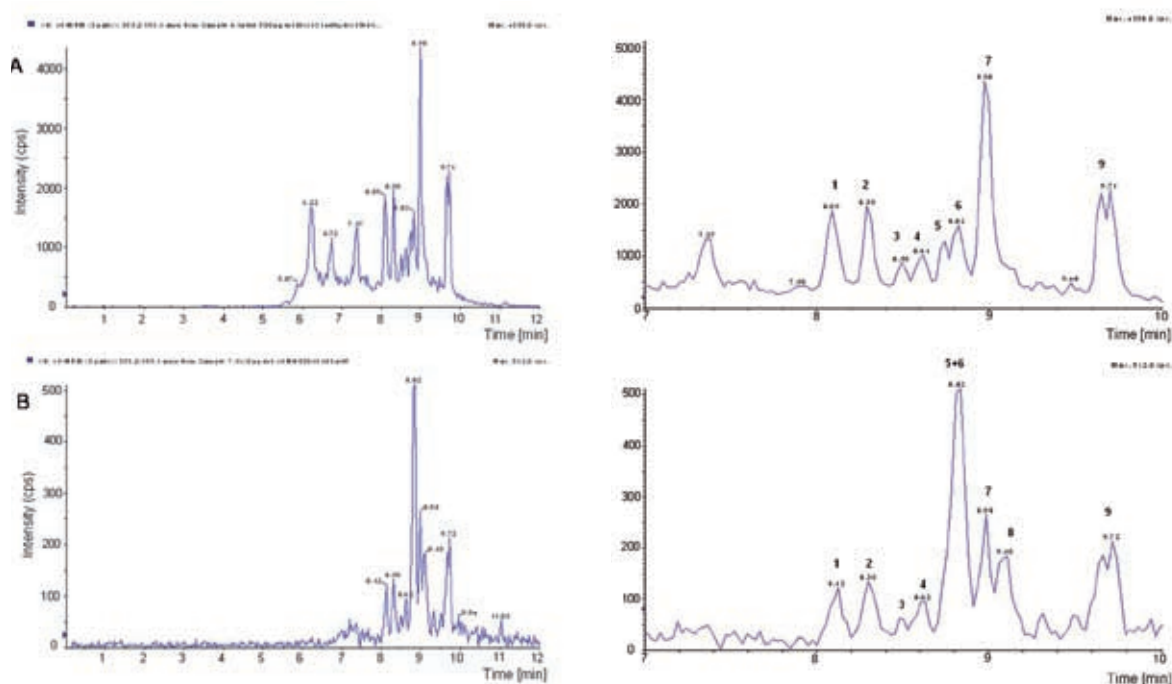


Fig. 2. Representative ion chromatograms of extracted urine and plasma samples.

Representative ion chromatograms of urine (A) and plasma from healthy subjects (B) with endogenous 15-F<sub>2t</sub>-isoprostane peak (arrow). Enlarged section (7 to 10-min) of representative ion chromatograms of urine (C) and plasma from healthy persons (D). Peak 1: 15(R)-F<sub>2t</sub>-isoprostane, peak 2: 15-F<sub>2t</sub>-isoprostane, peak 3: 9 $\alpha$ ,11 $\beta$ -prostaglandin F<sub>2</sub>, peak 4: unidentified, peak 5: 15(R)-prostaglandin F<sub>2 $\alpha$</sub> , peak 6: 5-trans prostaglandin F<sub>2 $\alpha$</sub> , peak 7: prostaglandin F<sub>2 $\alpha$</sub> , peaks 8 and 9: unidentified. cps, counts per second.