Supplemental Materials for the Methods paper:

**A New LC-MS Assay for the Quantitative Analysis of Vitamin K Metabolites in Human Urine**

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Supplemental Figure S1. LC-MS/MS APCI negative daughter ion spectra of derivatized K Acid I (High and Low CE)
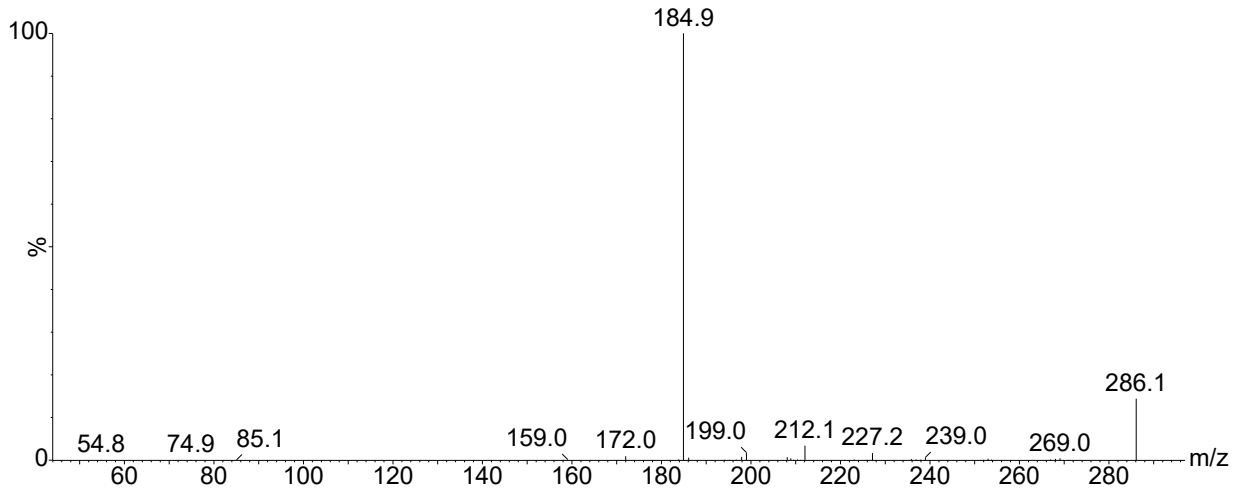
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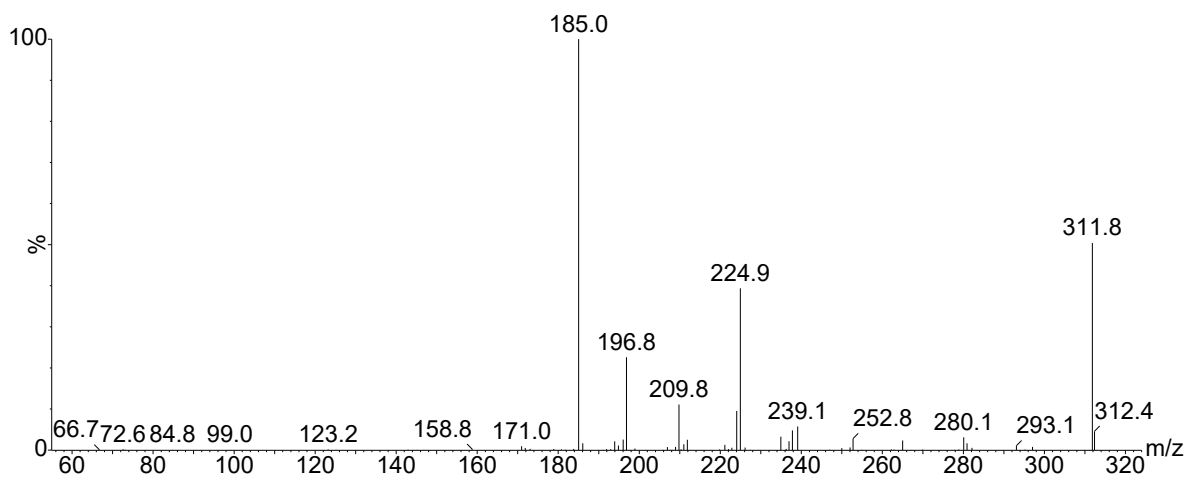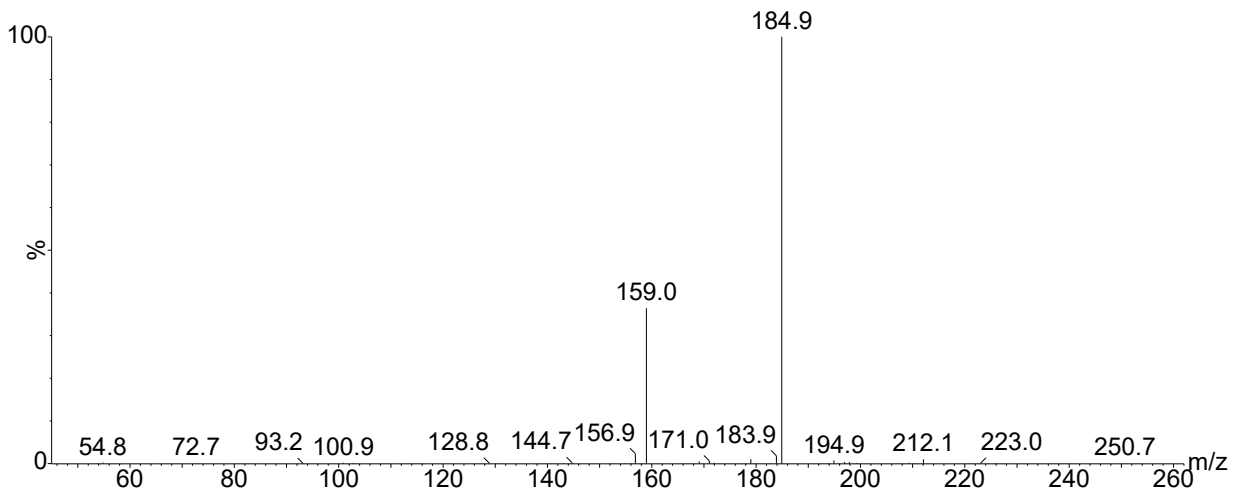

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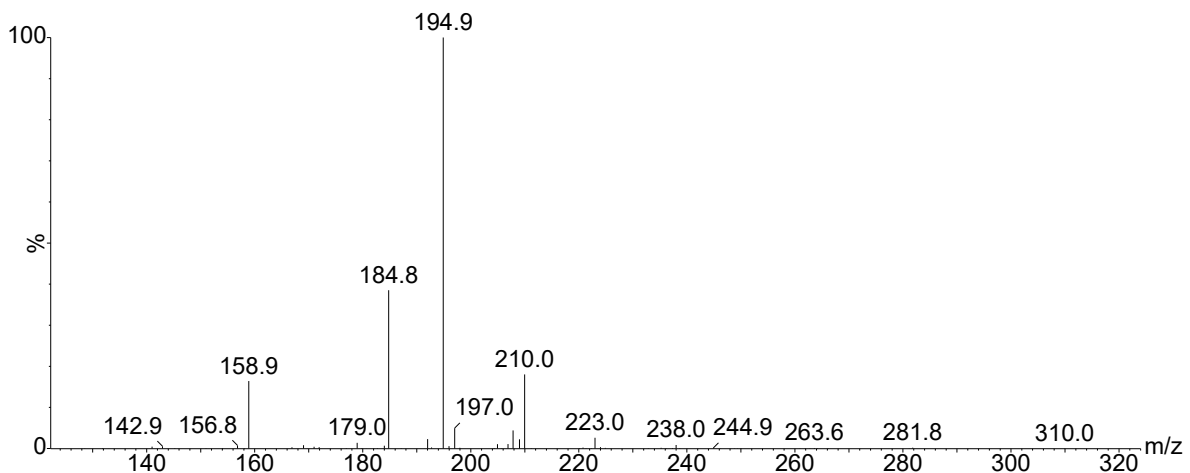
Supplemental Figure S2. LC-MS/MS APCI negative daughter ion spectra of derivatized K Acid II (High and Low CE).

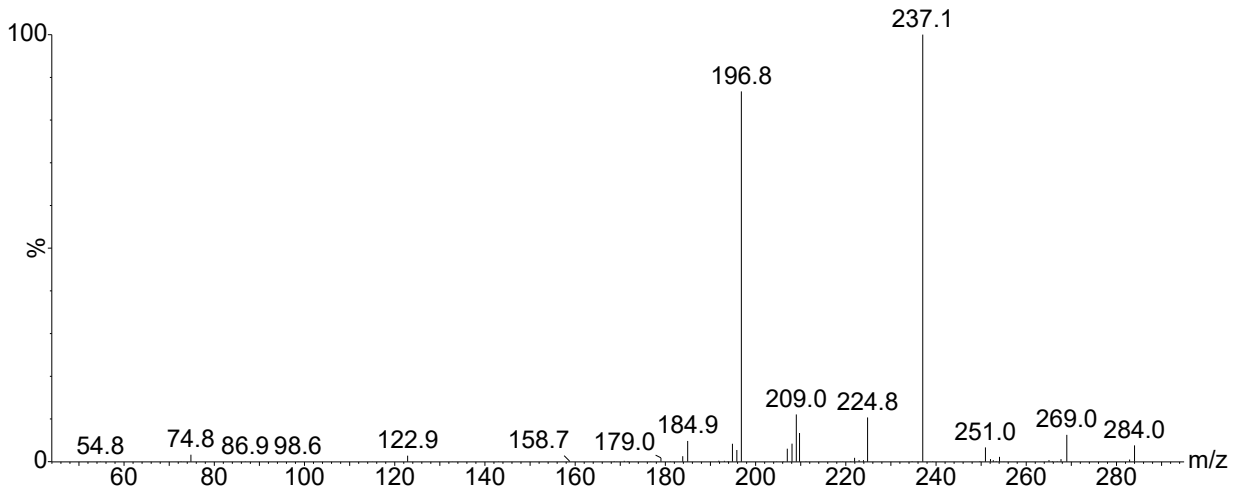**50_uM_VK_ester_mix_2_CE=20**

50_uM_VK_ester_mix_2_CE=40

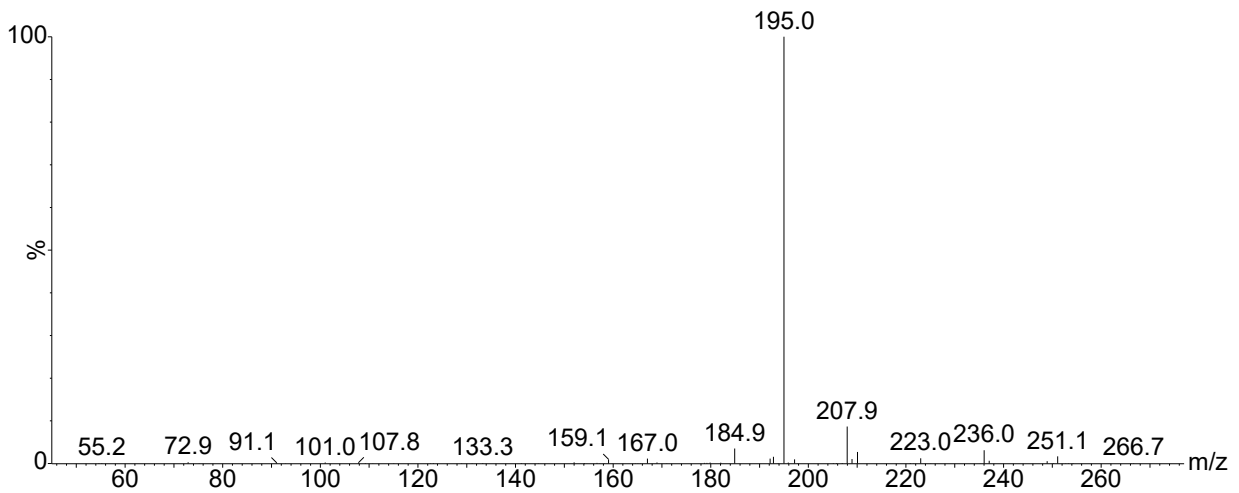**Supplemental Figure S3.** LC-MS/MS APCI negative daughter ion spectra of derivatized MK1 ω-COOH (High and Low CE).
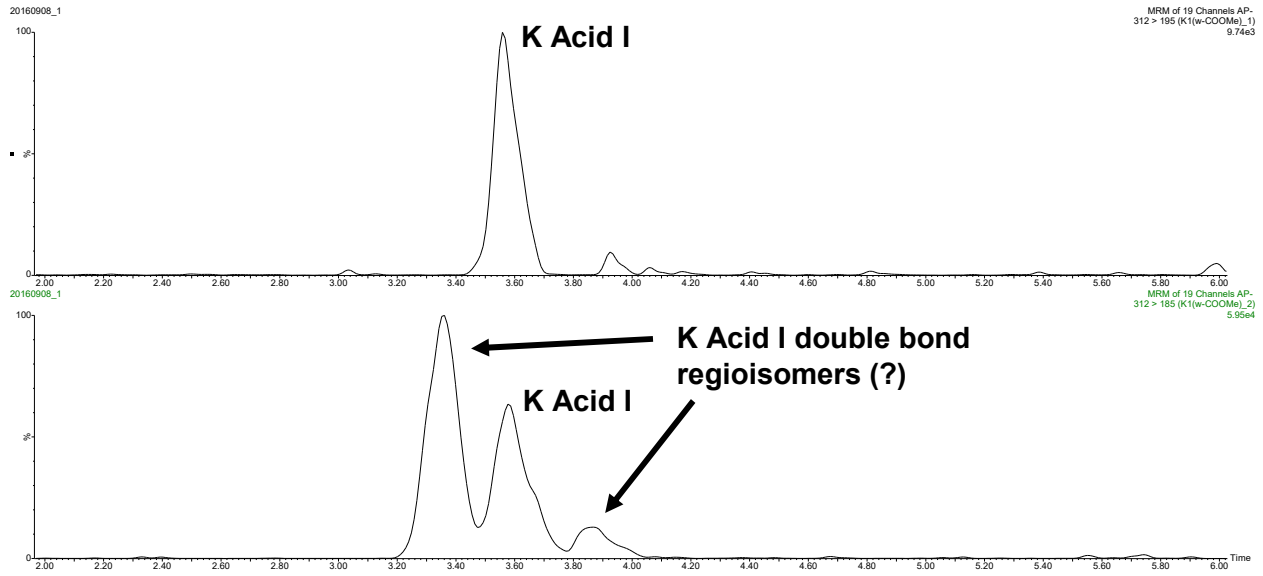
**50_uM_VK_ester_mix_2_CE=20**

50_uM_VK_ester_mix_2_CE=40

Supplemental Figure S4. LC-MS/MS analysis of a baseline human urine sample showing the K Acid I methyl ester mass transition channels *m/z* 312 > 195 (top trace) and 312 > 185 (bottom trace):

Supplemental Figure S5. Combined APCI⁻ LC-MS/MS (SRM) chromatograms showing vitamin K catabolites K Acid II (*m/z* 286 > 185), K Acid I (*m/z* 312 > 195), MK1 ω-COOH (*m/z* 284 > 195) and the internal standard, MK1 (*m/z* 240 > 185). **A**. Chromatogram obtained from a urine sample showing basal (i.e. pre-supplement) levels of vitamin K catabolites. **B**. Chromatogram of the urinary extract, obtained from the same subject as in chromatogram **A**, collected 0-24 hours after the subject ingested two capsules of Koncentrated K. The MK1 ω-COOH peak is obscured by the much larger K Acid II peak in chromatogram **A**.

B.
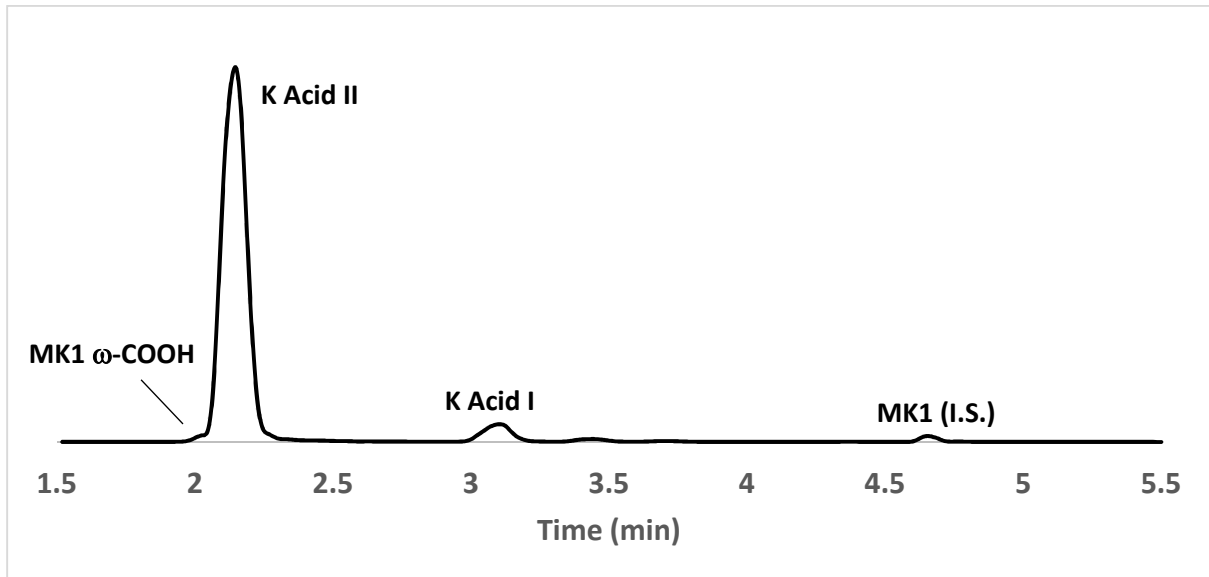
A.
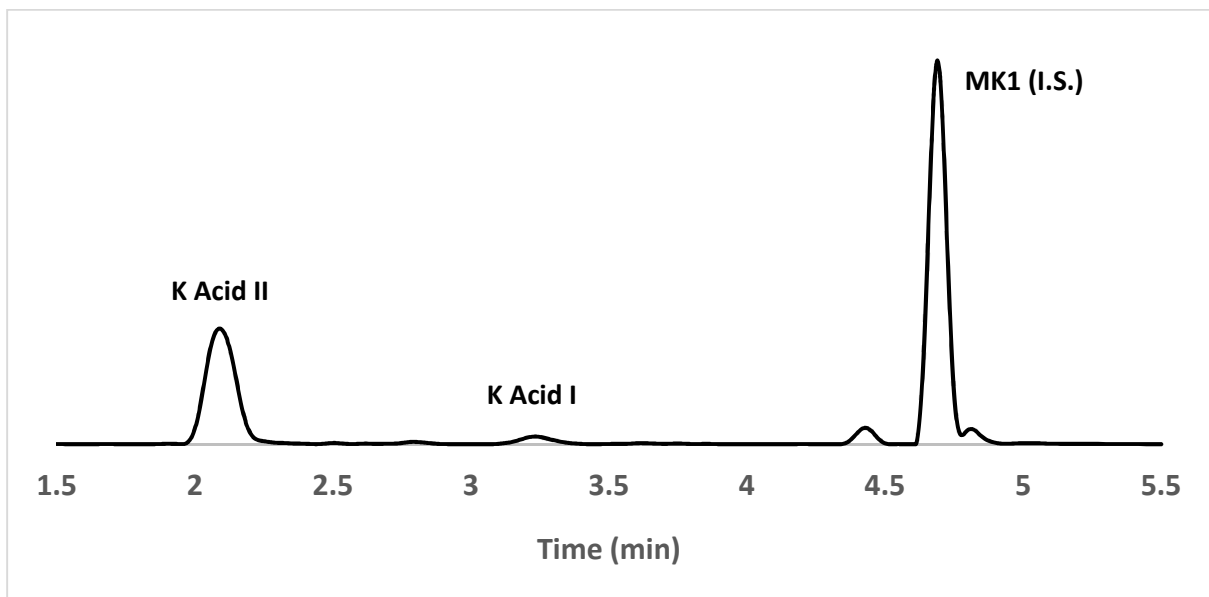
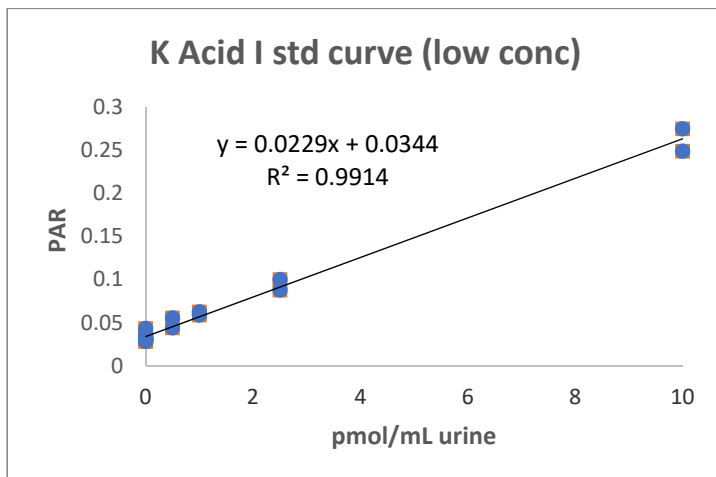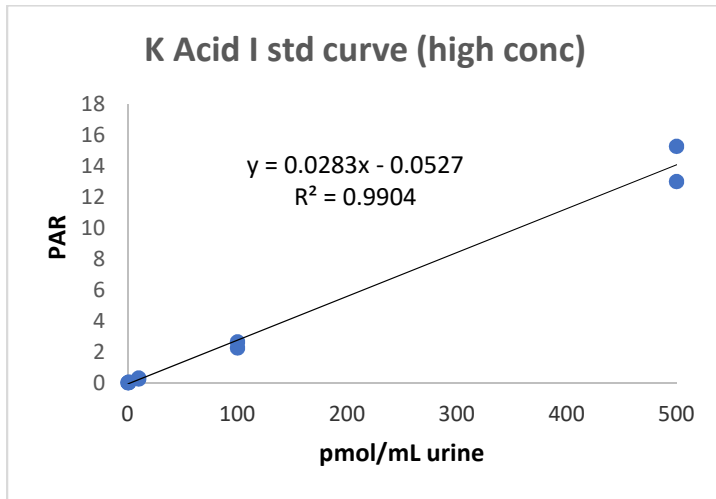**Supplemental Figure S6.** Low ($\leq$ 10 pmol/mL urine) and high concentration standard curves for K Acid I spiked into human urine and worked up as described in the Methods section (shown with linear regression analysis). The metabolite was analyzed as its methyl ester derivative by APCI$^-$ LC-MS/MS SRM (m/z 312 > 195) and is quantified as the Peak Area Ratio relative to the MK1 internal standard peak area (m/z 240 > 185). The y-intercept value for the low concentration standard curve, divided by the slope, reflects the amount of endogenous K Acid I present in the urine sample.

K Acid I std curve (high conc)

$y = 0.0283x - 0.0527$
$R^2 = 0.9904$

PAR vs pmol/mL urine

K Acid I std curve (low conc)

$y = 0.0229x + 0.0344$
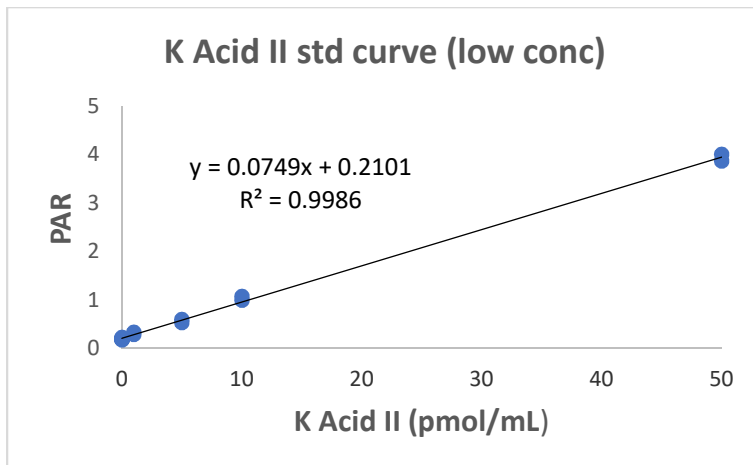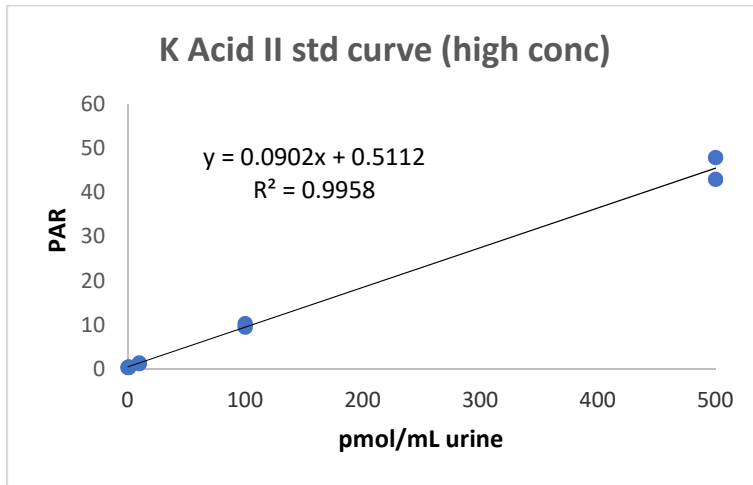$R^2 = 0.9914$

PAR vs pmol/mL urine

Supplemental Figure S7. Low (≤ 50 pmol/mL urine) and high concentration standard curves for K Acid II spiked into human urine and worked up as described in the Methods section (shown with linear regression analysis). The metabolite was analyzed as its methyl ester derivative by APCI⁻ LC-MS/MS SRM (m/z 286 > 185) and is quantified as the Peak Area Ratio relative to the MK1 internal standard peak area (m/z 240 > 185). The y-intercept value for the low concentration standard curve, divided by the slope, reflects the amount of endogenous K Acid II present in the urine sample.

K Acid II std curve (high conc)

$y = 0.0902x + 0.5112$
$R^2 = 0.9958$

K Acid II std curve (low conc)

$y = 0.0749x + 0.2101$
$R^2 = 0.9986$

Supplemental Figure S8. Low ($\leq$ 1 pmol/mL urine) and high concentration standard curves for MK1 ω-COOH spiked into human urine and worked up as described in the Methods section (shown with linear regression analysis). The metabolite was analyzed as its methyl ester derivative by APCI$^-$ LC-MS/MS SRM (*m/z* 284 > 195) and is quantified as the Peak Area Ratio relative to the MK1 internal standard peak area (*m/z* 240 > 185). The y-intercept value for the low concentration standard curve, divided by the slope, reflects the amount of endogenous MK1 ω-COOH present in the urine sample.

MK1 ω-COOH std curve (high conc)

$y = 0.1283x + 0.5938$
$R^2 = 0.9925$

PAR vs pmol/mL urine

MK1 ω-COOH std curve (low conc)

$y = 0.1713x + 0.0066$
$R^2 = 0.9951$

PAR vs pmol/mL urine