A candidate reference measurement procedure for aldosterone measurement in human serum and urine on the basis of isotope dilution liquid chromatography–tandem mass spectrometry

Supplementary Information

Contents

1. Equation

1.1 Eq. S1 Equation for calculating sample concentration

$$c = \frac{I_S - m}{b} \times c_I \times V_1 \times \frac{1}{V_S} \times \frac{1000}{M} \times P \qquad (1)$$

Here, c represents the actual concentration of aldosterone in the sample (nmol/L), I_S is the peak area ratio of aldosterone/internal standard peaks in the sample, c_I is the concentration of the internal standard solution, V_1 is the volume of added internal standard solution, V_S is the volume of samples, M is the molecular weight of aldosterone, P is the purity value of the standard (provided by the certificate of the standard substance), m is the intercept of the linear regression equation of the standard curve, b is the slope of the linear regression equation of the standard curve.

1.2 Eq. S2 Equation for testing the significance of trends in slope stability

Residual standard deviation, Syx:

$$s_{yx}^2 = \frac{\sum_{i=1}^{n}(Y_i - \widehat{Y_i})^2}{n - 2}$$

Standard error of the slope, s(b1):

$$s(b_1) = \frac{s_{yx}}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}} \qquad (2)$$

Here, Y_i is the measured concentration at time point i, $\widehat{Y_i}$ is the fitted value estimated from the regression model, n is the total number of observations, X_i is the time (h) at observation i, and $\bar{X}$ is the mean of all time points.

1.3 Eq. S3 Equation for the rate of carrying contamination

$$K_i = \frac{A_{i4} - A_{i6}}{A_{i3} - A_{i6}} \times 100\% \qquad (3)$$

Here, A_{i3} is the measured value of the 3rd sample of each group, A_{i4} is the measured value of the 4th sample of each group, A_{i6} is the measured value of the 6th sample of each group, and i is the serial number of the group.

1.4 Eq. S4 Equation for measuring sample concentration

$$c = \frac{W_s \times Q_I}{V_S} \times \frac{1000}{M} \times P = \frac{I_S - m}{b} \times C_I \times V_I \times \frac{1}{V_S} \times \frac{1000}{M} \times P \qquad (4)$$

Here, c is the concentration of aldosterone in the actual sample, V_S is the volume in the sample (mL), W_s is the mass ratio of aldosterone/internal standard in the sample, Q_I is the content of the internal standard in the sample, I_s is the ratio of the peak area of the aldosterone/internal standard peaks in the sample, V_I is the volume of the internal standard solution added (mL), C_I is the concentration of the internal standard solution, P is the purity value of the standard (provided by the certificate of the standard substance), M is the molecular weight of aldosterone, and m is the intercept of the linear regression equation of the standard curve

1.5 Eq. S5 Equation for testing the significance of the trend in relative expanded uncertainty

Residual standard deviation, Syx:

$$s_{yx}^2 = \frac{\sum_{i=1}^{n}(Y_i - \widehat{Y_i})^2}{n - 2}$$

Standard error of the slope, s(b1):

$$s(b_1) = \frac{s_{yx}}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}} \qquad (5)$$

Here, Y_i represents the relative expanded uncertainty of the i-th sample, $\widehat{Y_i}$ is the predicted

relative expanded uncertainty for the i-th sample, n is the number of sample concentration points, X_i is the concentration of the i-th sample and $\bar{X}$ is the mean concentration of all samples.
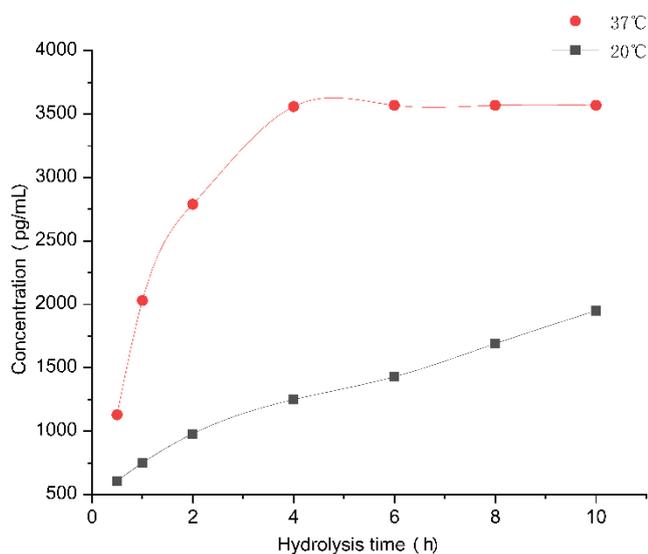
2. Figure

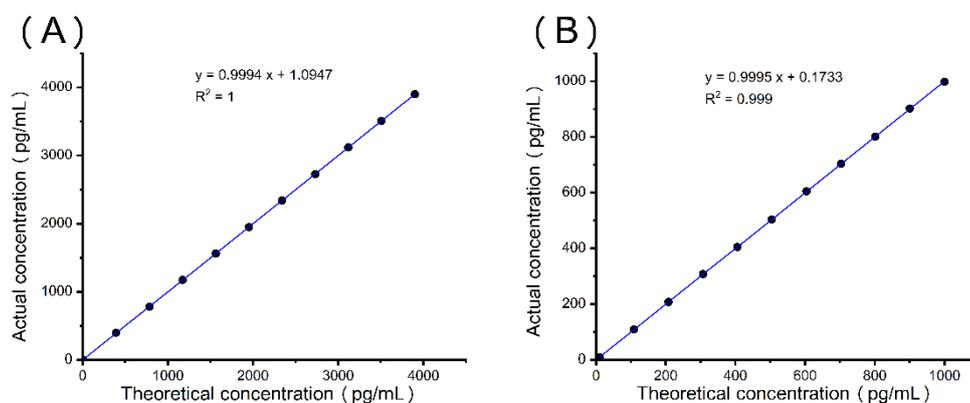**Figure S1** Hydrolysis efficiency at different temperatures and times.

Figure S2 Linear Curve of Theoretical and Mean Measured Concentrations of Aldosterone.
(**A**) Linear curve of serum aldosterone theoretical concentration versus mean measured concentration.
(**B**) Linear curve of urine aldosterone theoretical concentration versus mean measured concentration

3. Table

Table S1 Sample ion ratio statistics

Sample	Ion ratio	Relative deviation (%)
Aldosterone standard solution (serum)	0.6393	/
Clinical serum samples	0.6427	0.5
Add samples of clinically common interfering serum	0.6001	-6.1
Add samples of specific interfering serum	0.7006	9.6
Aldosterone standard solution (urine)	0.7098	/
Clinical urine samples	0.7127	0.4
Addition of interfering substances to urine samples	0.6805	-4.1

Table S2 Sample precision data

Sample		Average value (pg/mL)	Standard deviation within lot (n=9)	Interday variance (n=45)	Standard deviation of intermediate precision in the laboratory (n=45)	CV in the laboratory (n=45)
Serum	Low concentration	5.1	0.00012	1.5×10^{-8}	0.00017	1.21
	Medium concentration	991.2	0.014	0.00019	0.019	0.68
	High concentration	3038.5	0.027	0.027	0.038	0.45
Urine	Low concentration	25.4	0.2630	0.06724	0.3588	1.41
	Medium concentration	247.0	1.8842	3.6169	2.6024	1.05
	High concentration	1016.8	8.8141	78.2798	12.1382	1.19

Table S3 Sample Stability Experimental Results

Sample	Syx	s(b1)	$t_{(0.05, n-2)}$	Slope b1	Conclusions
Serum	0.00017	0.00035	3.18	-0.0010	The trend is not significant
Urine	3.10E-02	2.66E-03	3.18	-1.10E-03	The trend is not significant

Table S4 Experimental results of carrying contamination rate

Sample		Measured concentration (pg/mL)			Ki (%)
		Ai3	Ai4	Ai6	
Serum	Group 1	3370.11	0.0088	0.0089	-0.0012
	Group 2	3366.51	0.0088	0.0089	-0.0014
	Group 3	3366.51	0.0089	0.0090	-0.0005
	Group 4	3366.51	0.0091	0.0091	0.0001
	Group 5	3370.11	0.0090	0.0088	0.0020
Urine	Group 1	497.80	0.00	0.00	0.00
	Group 2	493.80	0.00	0.00	0.00
	Group 3	498.90	0.00	0.00	0.00
	Group 4	498.60	0.00	0.00	0.00
	Group 5	491.80	0.00	0.00	0.00

Table S5 t-test results for assessing the significance of uncertainty trends

Sample	Syx	s(b1)	$t_{(0.05, n-2)}$	Slope b1	Conclusions
Serum	3.1E-6	1.5 E-5	2.31	3.4 E-4	The trend is not significant
Urine	4.33E-06	2.15 E-06	2.31	-1.85 E-06	The trend is not significant