

Figure S1 Endogenous substances subtracted calibration curve of L-carnitine in the plasma.

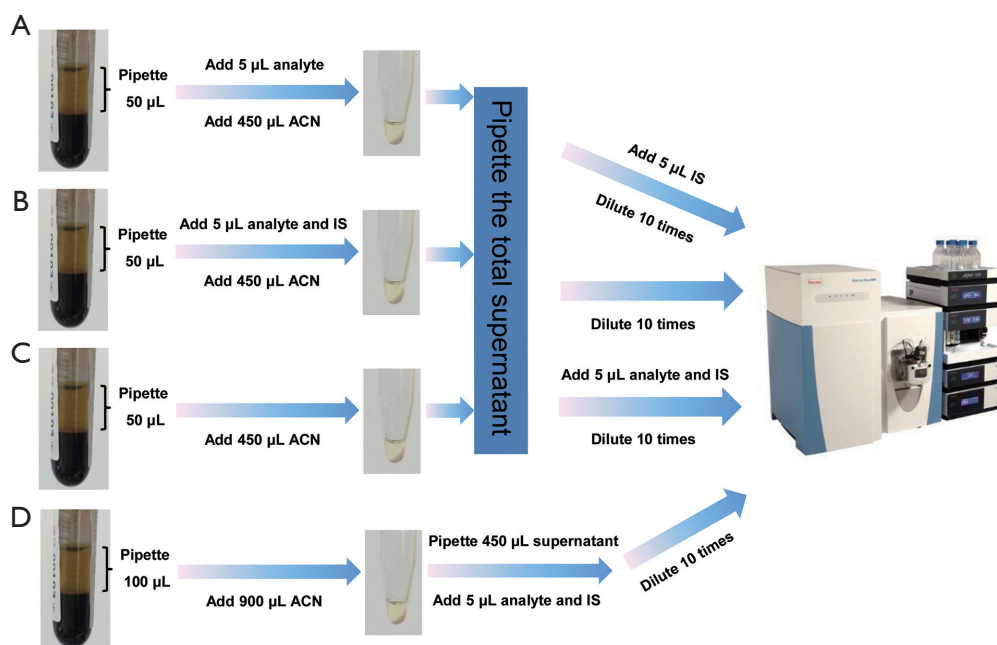


Figure S2 Different sample processing methods. IS, internal standard; ACN, Acetonitrile.

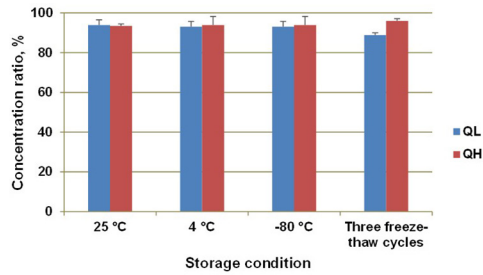


Figure S3 Assessment of stability of the analyte L-carnitine. QL, low concentration quality control sample; QH, high concentration quality control sample.

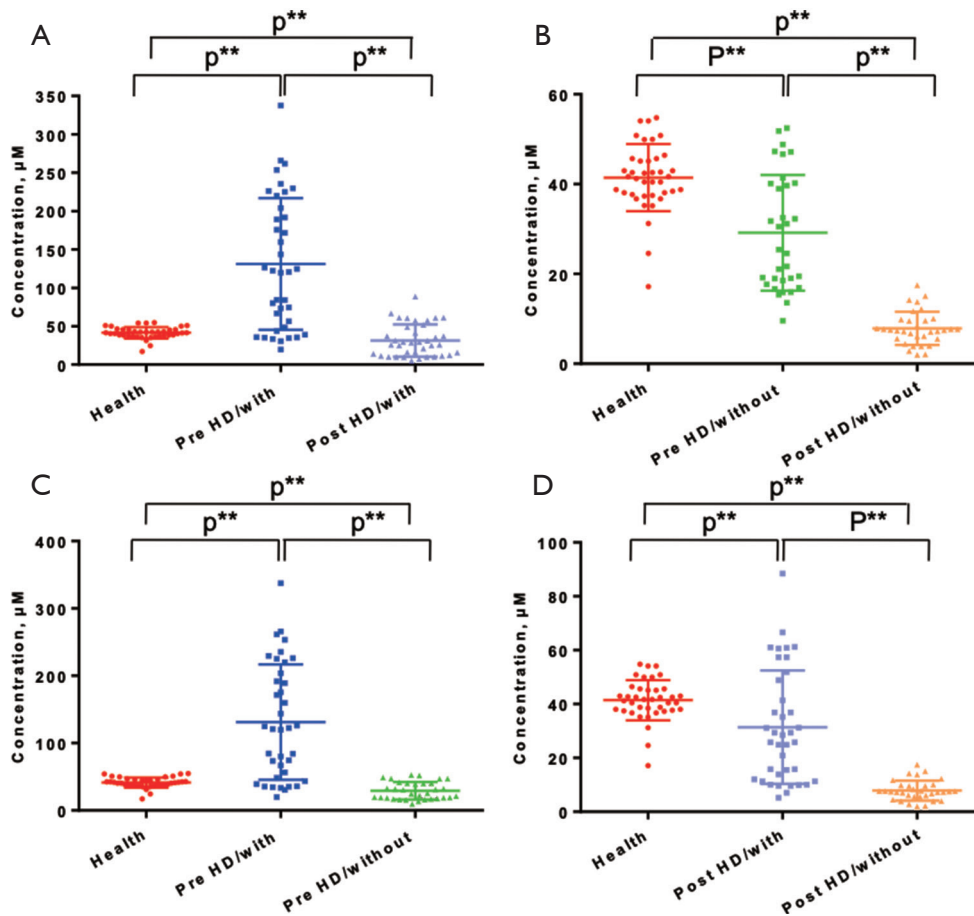


Figure S4 Statistical differences between healthy subjects and different HD groups. Patients with HD were divided into 4 groups according to pre/post HD and whether they were treated with L-carnitine or not. **, $P < 0.01$ is considered to indicate a statistically significant difference. HD, hemodialysis.

Table S1 Intra-day and inter-day precision and accuracy for QC samples

Nominal concentration		Intra-day			Inter-day		
		Mean (μM)	Accuracy (RE%)	Precision (RSD%)	Mean (μM)	Accuracy (RE%)	Precision (RSD%)
LLOQ (6.169 μM)	Day 1	7.424	20.000	7.600	6.688	8.400	12.500
	Day 2	6.909	12.000	6.000			
	Day 3	5.584	-9.500	5.300			
QL (18.506 μM)	Day 1	18.528	0.100	7.800	18.342	-0.900	6.200
	Day 2	17.810	-3.800	5.900			
	Day 3	18.724	1.200	5.100			
QM (49.349 μM)	Day 1	47.617	-3.500	5.900	47.457	-3.800	5.800
	Day 2	45.044	-8.700	5.100			
	Day 3	49.709	0.700	0.900			
QH (154.216 μM)	Day 1	140.867	-8.700	-3.600	143.121	-7.200	4.900
	Day 2	137.223	-11.000	2.200			
	Day 3	151.273	-1.900	1.500			

QC samples, quality control samples; LLOQ, lower limit of quantification; QL, low concentration QC; QM, middle concentration QC; QH, high concentration QC; RE, relative error; RSD, relative standard deviation.

Table S2 The matrix factor and recovery of the method developed

QC samples	Matrix factor (%)		Recovery (%)	
	Mean	RSD	Mean	RSD
QL	91.803	9.867	154.416	3.593
QM	96.229	3.510	123.053	5.745
QH	99.862	0.715	102.945	6.057

QC samples, quality control samples; LLOQ, lower limit of quantification; QL, low concentration QC; QM, middle concentration QC; QH, high concentration QC; RSD, relative standard deviation.

Table S3 The area ratio of different sample processing methods

Sample ID	Response intensity		Area ratio (AR)
	L-carnitine	MILD	
QM-1 ^a	31437052.889	3666389.647	8.574
QM-2 ^b	34716499.506	1662983.625	20.876
QM-3 ^c	58391600.575	3357444.474	17.392
QM-4 ^d	55451867.434	3221367.498	17.214

^aQM-1 is defined as the middle concentration quality control (QM) sample processed according to method A; ^bQM-2 is defined as the QM sample processed according to method B; ^cQM-3 is defined as the QM sample processed according to method C; ^dQM-4 is defined as the QM sample processed according to method D; MILD, mildronate.

Table S4 The recovery comparison of different sample processing methods

Recovery of QMs (H/V)	AR _{QM-3}	AR _{QM-4}
AR _{QM-1}	49.302%	49.811%
AR _{QM-2}	<i>120.035%</i>	121.275%

*H/V is defined as the ratio of the horizontal values and the vertical values and the italic fonts represents the recovery of the method developed here; AR, area ratio; QM-1 is defined as the QM middle concentration quality control (QM) sample processed according to method A; QM-2 is defined as the QM sample processed according to method B; QM-3 is defined as the QM sample processed according to method C; QM-4 is defined as the QM sample processed according to method D.

Table S5 The fortified validation of calculated concentrations of the endogenous L-carnitine with 6 different calibration curves

Calculated concentration (μM)	Calibration curves with corresponding matrix						Mean	RSD (%)
	A	B	C	D	E	F		
Blank-plasma A	40.587	39.055	38.165	44.166	44.955	45.960	42.148	7.822
Blank-plasma B	37.258	35.773	34.800	37.677	39.142	39.848	37.417	5.145
Blank-plasma C	36.947	35.467	34.461	37.542	39.805	40.577	37.466	6.347
Blank-plasma D	36.184	34.714	33.716	39.520	40.702	41.564	37.733	8.737
Blank-plasma E	35.734	34.270	33.278	37.375	39.517	40.260	36.739	7.660
Blank-plasma F	34.415	32.970	31.945	36.716	36.436	36.872	34.892	6.044

RSD, relative standard deviation.