Analysis of Free Norepinephrine and Epinephrine in Human Plasma by LC/MS/MS Analysis

Free NE and E in human plasma were pretreated by protein precipitation for removeing plasma protein; the supernatant is evaporated and reconstituted by recuctive ethylation labeling reagents (with d_4- acetaldehyde) to form ethyl derivatives of NE and E before LC/MS/MS analysis; the d_4-acetaldehyde labeled NE and E are separated by High Performance Liquid Chromatography (HPLC), and determinated by Mass Spectrometry (MS) in Electrospray Ionization (ESI) source at positive ionization mode. Multiple Reaction Monitoring (MRM) of transitions are used for the quantification of NE and E. Deuterated stable isotopes NE – d_6 and E-d_6 are utilized as internal standards for the calibration of NE and E respectively for the quantification analysis.

Performances

Lower limit of Quantization (LOQ): 2.5pg/mL
Linear range: 2.5 – 5000pg/mL ($R \geq 0.999$)

Precision (CV%)

Intra-assay CV: See table
Inter-assay CV: See table

Epinephrine	**Intra assay, RSD(%)**	**Inter assay, RSD(%)**
Low level (5pg/mL)	8.30	11.7
Middle level (50pg/mL)	4.30	7.05
High level (500pg/mL)	2.35	6.07
Up high level (5000pg/mL)	5.12	8.07
Norepinephrine	**Intra assay, RSD(%)**	**Inter assay, RSD(%)**
Low level (5pg/mL)	10.41	8.77
Middle level (50pg/mL)	4.36	7.53
High level (500pg/mL)	4.49	13.22
Up high level (5000pg/mL)	7.05	11.29