

1. Determination Free Norepinephrine and Epinephrine in Urine by LC/MS/MS Analysis

Description: Norepinephrine (abbreviated NE), also called noradrenaline and Epinephrine (abbreviated E), known as adrenaline or adrenalin, are catecholamines playing important roles in the autonomic regulation of many homeostatic functions and a neurotransmitter in the central nervous system. Norepinephrine and Epinephrine are overlapped, but are separated in their receptor activation profile and consequent biological actions.

The measurement of urinary free Norepinephrine and Epinephrine is an important diagnostic test in biochemical screening for pheochromocytoma.

Free NE and E in human urine were complexed with 2-Aminoethyl Diphenylborinate at pH 7.5 - 9.5 before Solid Phase Extraction (SPE) for the sample pretreatment; NE and E are separated by High Performance Liquid Chromatography (HPLC), and determined 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 – d₆ and E-d₆ are utilized as internal standards for the calibration of NE and E respectively for the quantification analysis.

Collection considerations

Urine samples should be acidified to a pH of 2.0. For 24hour collections add 50uL of 6 N HCl in 10mL of urine

Performances

Lower limit of Quantization (LOQ):

Norepinephrine: 1.0 ng/mL

Epinephrine: 1.0 ng/mL

Linear range: (R ≥0.999)

Norepinephrine: 1 - 200 ng/mL

Epinephrine: 1 - 100 ng/mL

Precision (CV%)

Intra-assay CV: See table

Inter-assay CV: See table

Concentration (ng/mL)	Norepinephrine		Epinephrine	
	Intra assay, RSD(%)	Inter assay, RSD(%)	Intra assay, RSD(%)	Inter assay, RSD(%)
1	15.9		7.8	
5	8.22	6.37	4.58	6.12
10	5.47	5.77	3.65	5.34
50	4.90	6.64	4.13	4.21
100	4.41	6.93	2.90	3.41