## 1. Determination Melatonin in Human Plasma by LC/MS/MS Analysis

## Description:

Melatonin is a sleep hormone, which is primarily metabolized to a highly stable 6-hydroxymelatonin sulfate (aMT6) in urine. In humans, melatonin is produced by the pineal gland. 90% of melatonin is cleared in a single passage through the liver; a small amount is excreted in urine and found in saliva. Human melatonin production decreases as a person ages. Also, as children become teenagers, the nightly schedule of melatonin release is delayed, leading to later sleeping and waking times.

Melatonin in human plasma is a useful biomarkers to the physiological function of sleeping to humans and there is a demand for its measurement for research into sleeping disorders.

Melatonin in Human plasma was extracted by Solid Phase Extraction (SPE), separated by High Performance Liquid Chromatography (HPLC), and analyzed by Mass Spectrometry (MS) in Electrospray Ionization (ESI) source at positive ionization mode. Multiple Reaction Monitoring (MRM) of transition was used for the quantification of melatonin. The deuterated stable isotope melatonin-d<sub>7</sub> was utilized as internal standards for the quantification analysis.

## **Performances**

Lower limit of Quantization (LOQ): 5.0 pg/mL

Linear range:  $5 - 500 \text{ pg/mL} (R \ge 0.999)$ 

## **Precision (CV%)**

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

Concentration	Intra assay, RSD(%)	Inter assay, RSD(%)
Sub-Low Level (5pg/mL)	2.57	7.30
Low Level (42.4 pg/mL)	4.33	12.51
Medium Level (100.0 pg/mL)	5.33	14.31
High Level (242 pg/mL)	5.61	12.99