

# Catecholamine Analyzer

The Catecholamine is a group of naturally occurring compounds in the sympathetic nervous system that function as hormones or neurotransmitters. This group includes such compounds as epinephrine, adrenaline, norepinephrine, and dopamine, which resemble each other chemically by having an aromatic portion (a catechol or alcohol and phenol based chemical structure) to which is attached an amine or nitrogen-containing group.

The amine as the functional group of Catecholamine tends to oxidize in either a weak basic or neutral solution. Because of this characteristic, Catecholamine has traditionally been detected by Electro Chemical Detector (ECD), utilizing the electron generated by the oxidation of the amine. The analysis for detection of Catecholamine has been problematic for researchers, often due to protein contamination of samples and degradation of samples due to improper care and storage. Additionally, errors often occur during sample preparation when reagents are mixed, which induces inaccuracy of the results by researchers. Lastly, the ECD requires a long time for a stabilization step. Improper optimization of the ECD procedure is known to decrease the sensitivity of the analysis for the detection, while increasing the range of errors.

In order to overcome these disadvantages and to analyze Catecholamine effectively and efficiently, YL provides a special analyzer for Catecholamine analysis that includes all the necessary sample preparation reagents, solvents, standard reagents, and an analytical column. Furthermore, YL Catecholamine Analyzer is the best solution in the market today to obtain accurate results for Catecholamine in an easy, convenient, and affordable process.

## • Methods of Sample Preparation of Catecholamines

### Sample preparation

#### (1) Catecholamine in Urine

**Solution 1:** Aliquot 3.0 ml of a urine sample and add 6.0 ml of the dilution buffer and 100  $\mu$ l of the internal standard, vortex to mix, and adjust the pH to fall within a range of 3 to 7 using 1N NaOH.

- Pass the solution 1 through the provided SPE Cartridge. Discard the effluent.
- Wash the SPE Cartridge with purified water (10 ml) and discard the effluent.
- Rinse the SPE Cartridge with 6.0 ml of the elution buffer and save the elution for HPLC analysis.

#### (2) Catecholamine in Plasma

**Solution 1:** Put 0.5 ml (500  $\mu$ l) of the extraction buffer to the sample preparation column and mix well by inverting.

- Add 50.0  $\mu$ l of the Internal Standard and 1.0 ml of plasma to solution 1 and mix well by inverting.
- Pass solution 1 through the provided SPE Cartridge, and discard the effluent.
- Wash the SPE Cartridge by adding 1.0 ml of the wash buffer and centrifuge. Then discard the effluent. Repeat this step three times.
- Add 120.0  $\mu$ l of the elution buffer to the SPE Cartridge, mix well by inverting the cartridge, and incubate the cartridge at room temperature for 2 minutes.
  - After incubating the cartridge for 2 minutes at room temperature, invert the cartridge several times to ensure sufficient mixing of the elution buffer.
  - Centrifuge the cartridge at 2000 rpm for 1 minute, and save the elution for HPLC analysis.

#### (3) Serotonin in Serum

- To a 200  $\mu$ l plasma serum sample, add 100  $\mu$ l of an Internal Standard and 50  $\mu$ l of the precipitation reagent. Vortex the solution for 30 seconds to mix thoroughly.
- Centrifuge the solution at 9000 rpm for 5 minutes. Remove the upper layer and save it for HPLC analysis.

#### (4) VMA, HVA, 5-HIAA in Urine

- To 50  $\mu$ l of a urine sample, add 1 ml of the Internal Standard. Mix well by vortexing. Pass the solution through a SPE Cartridge and centrifuge. Discard the effluent.
- Wash the SPE Cartridge by adding 3 ml of wash buffer I, and discard the effluent. Wash it by adding 3 ml of wash buffer II, and discard the effluent. Repeat this procedure one more time.
- To the SPE Cartridge, add 2 ml of the elution buffer. Save the elution for HPLC analysis.

### • Classes of Catecholamine Standards

	Reagents	M.W	Free M.W
NE	Norepinephrine	169.18	
Epi	Epinephrine	183.2	
MHPG	Hydroxymethoxyphenylglycol piperazinesalt	454.52	368.38
NM	Normethanephrine-HCl	219.67	183.2
DA	Dopamine-HCl	189.64	153.18
DOPAC	Dihydroxyphenylaceticacid	168.15	
HVA	Homovanillicacid	182.18	
3-MT	3-Methoxytyramine-HCl	203.6	167.24
5-HT	Serotonine-creatininesulfate	405.43	176.22
5-HIAA	5-Hydroxyindoleaceticacid	191.19	
ISO	Isoproterenol-HCl	247.72	211.26

### • Application

- Catecholamine analysis in Plasma
- Catecholamine analysis in Urine

### ■ Catecholamines in Plasma

- Mobile phase & Column : by Chromsystem kit
- Flow rate : 1.3 ml/min
- Column oven : 35 °C
- Detector : ECD (+500mV, 2nA)  
WE (Glassy Carbon)
- Injection volumn : 20 µl sample loop

