Application Note

Catecholamine Analysis in Plasma or Urine

AN08-0815

Here we outline our highly sensitive method for the detection of norepinephrine (NE) and epinephrine (E) from plasma, tissue or urine samples. This method utilizes a simple eight-step sample preparation protocol which also includes an internal standard (dihydroxylbenzylamine) to account for the recovery rate. The sample is added to a solution containing aluminum oxide, which under the basic conditions bonds the catecholamines to the aluminum. This complex is then washed repeatedly to remove any remaining contaminants from the sample. In the final step of the protocol, the catecholamines are recovered using acetic acid. This sample preparation allows concentration of NE, E and dopamine when the sample volume is more than a few hundred microliters.

During high stress conditions, such as blood sampling from an animal, NE and E levels are significantly elevated. For such cases, the extraction process may not be necessary because the concentrations of NE and E are much higher than the concentrations of the other analytes present in the sample. However, in the case of human blood sampling, stress levels are normally lower and therefore, the levels of NE and E are significantly lower than compared to the animals'. The extraction process is necessary to identify the NE and E peaks among the large number of unknown peaks present in the sample. Additionally, human blood contains more unknown compounds not found in animal blood. Thus, the extraction process is more important for the detection of NE and E from blood samples at lower concentrations. The extraction process is always required for urine samples.

This method reliably allows for a detection limit of 0.5 fmol for both NE and E in a 20 min analysis time. Typical NE and E levels in human plasma are approximately 200 pg/ml (1 pmol/ml) and 10 pg/ml (0.1 pmol/ml), respectively. Dopamine detection is also possible using this methodology.

To minimize the stress level on animals during blood sampling Eicom recommends using the DR-II. This automated blood sampling system samples via a catheterized implant which significantly reduces the stress level of the animal by removing the human and handling component.

Analytical Conditions

HPLC-ECD	Eicom HTEC-500
Separation column	Eicompak CA-5ODS (2.1 ID x 150 mm)
Precolumn	CA-ODS
Flow Rate	230 µl/min
Column Temperature	25˚C
Applied Potential	450 mV vs. Ag/AgCl
Working Electrode	Graphite Electrode (WE-3G) with Gasket (GS-25)

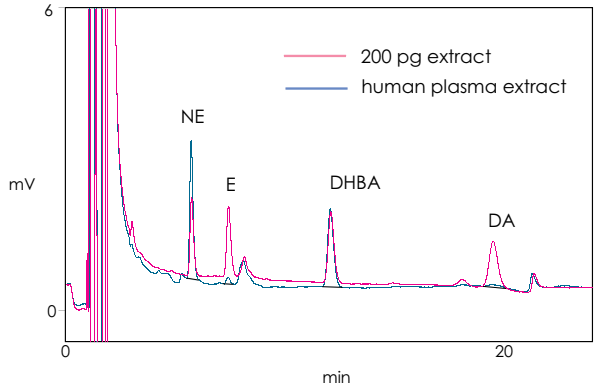

Fig. 1 Chromatogram for a 200 pg standard mixture of NE, E, DA and DHAB (internal standard) and a typical profile of human plasma sample at reduced stress levels.

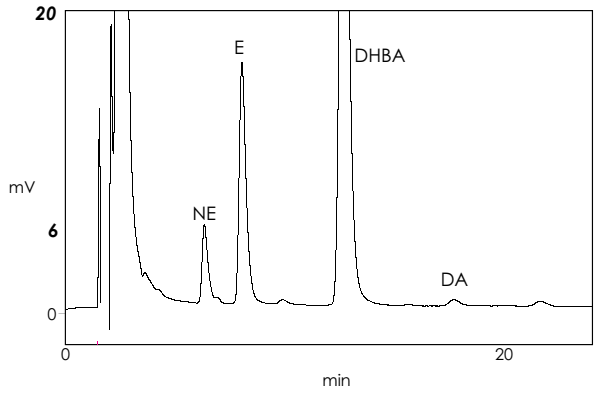

Fig. 2 Typical profile of rat plasma sample with trunk blood sampling.

Sample Preparation

Human Blood Sampling

Healthy volunteer. Blood samples are collected from the volunteer's arm and immediately mixed with EDTA (ethylenediaminetetraacetic acid). The samples are centrifuged within 2 hours of collection at 5,000 G for 20 min. The plasma supernatant can then be stored for one day at -20˚C.

Rat Blood Sampling

Trunk blood (750 µl) from a male Sprague Dawley rat (~300 g) was mixed in EDTA tubes. Samples were then centrifuged at 5000G for 15 min. The plasma supernatant was then used in the eight-step protocol (detailed in the Application Manual, **"Sample Preparation for Catecholamine Analysis in Plasma or Urine").**