A convenient method for extraction and analysis with High-Pressure Liquid Chromatography of catecholamine neurotransmitters

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The extraction and analysis of catecholamine neurotransmitters in biological fluids is of great importance in assessing nervous system function and related diseases, but its precise measurement is still a challenge. Many protocols have been described for neurotransmitters measurement by a variety of instruments, including high-pressure liquid chromatography (HPLC). However, there are shortcomings, such as complicated operation or hard-to-detect multiple targets, that can no longer be avoided. Presently, the dominant analysis technique is still HPLC, due to its high sensitivity and good selectivity. Here, a detailed protocol is described for the pretreatment and detection of catecholamines with high pressure liquid chromatography coupled with ultraviolet detection (HPLC-UV) in plasma and brain samples of mice. The calibration curve of catecholamines was established in the concentration range of 0.01 – 5.00 ug/mL. 100 μl of plasma or brain samples were extracted by protein precipitation using 300 ul of freeze solution of formic acid 0.5%v/v in acetonitrile. Each sample was vortexed for at least 15 s and then stocked in freezer at -20°C for 15’ and later centrifuged at 4,000 rpm for 10 min. The 250ul of surnatant was  transferred to an injection vial. Chromatographic separation was performed at 35°C, using a column oven, on a RP column (Atlantis T3 4.6 x 50 mm, 5 μm, Waters, USA). A gradient chromatographic elution was executed by mixing three solutions: water; acetonitrile; 100mM Ammonium Formate, pH = 3.00 (with formic acid). The flow rate was set at 1 mL/min. Catecholamines plasma concentrations were reported as ug/mL, instead brain amount were converted in ng/mg of tissue weight. Previously, prior to start extraction procedure, weighted brain samples were frozen in liquid nitrogen, sonicated for 1 min, reconstituted in 1 mL of water and sonicated for another min. The established protocol was applied to assess the differences of plasma and brain levels of catecholamines between genetically different mice. Applications of our methods are very broad as wide is the field of neurodegerative diseases.