A Simple, Effective Method for Quantitative Analysis of the Biomarker Norepinephrine in Human Plasma by LC-MS/MS  
Bao Hoang, Casey Bonner, Alicia Petrasiewicz, and Steven Wilshire  
Agilux Laboratories – A Charles River Company, Worcester, MA

**PURPOSE**

Measuring endogenous catecholamines such as norepinephrine in plasma is important in the discovery, evaluation, and monitoring of drugs in many disease/disorder areas including diabetes, cancer (for example neuroblastoma), heart disease, pain, and anxiety. Quantification of catecholamine concentrations in plasma can provide a direct biomarker measurement of disease state and/or proper target engagement. Norepinephrine analysis in plasma has challenges including but not limited to; low concentrations, instability, and interferences. We were able to address the challenges and qualify a simple, fast, and effective method for quantitative analysis of norepinephrine in human plasma.

**METHOD**

Norepinephrine is an endogenous compound in plasma. Given a variety of considerations including norepinephrine instability, we chose a “depleted matrix” approach for quantitation. This approach consists of four key features: (1) calibrants and QCs are prepared from which endogenous norepinephrine is removed as described below, (2) true analyte is utilized for spiking calibrants and stable-label norepinephrine is utilized as internal standard, (3) derivatization with d4-acetaldehyde for improved chromatography, sensitivity, and stability, and (4) additional stability considerations including sodium metabisulfite addition, with on-ice and yellow-light extraction conditions.

To generate the “depleted matrix” for calibrants and QCs, K2 EDTA human plasma was exposed to precipitation coupled with derivatization with d4-acetaldehyde. Analysis was performed utilizing Agilent 1290 UHPLC pumps, a Leap CTC Pal autosampler, and Sciex API6500. Waters UPLC BEH C18 chromatography was employed for separation and total run-time was 3 minutes at a flow rate of 0.7 mL/min. Sciex Analyst 1.6 was utilized for integrations and calculations.

**RESULTS**

The method was successfully qualified using a combination of true analyte, depleted matrix, and stable-labeled internal standard. The qualified assay range is 0.0500 ng/mL to 100 ng/mL, and the assay met all parameters evaluated including intra-day accuracy/precision, inter-day accuracy/precision, carryover, dilution linearity, selectivity, and specificity. In addition, in-process stability was evaluated and addressed, including the time required for blood to plasma conversion as well as subsequent stability in plasma during handling and freeze/thaws. Representative qualification data (calibrants and batch acceptance QCs) are shown in the following two tables along with a representative norepinephrine calibration curve from Analyst®. The method has been utilized successfully for analysis of a variety of study samples.

**CONCLUSION**

A simple, effective method was developed with a 50 pg/mL lower limit of quantification utilizing conventional protein precipitation coupled with derivatization with d4-acetaldehyde. This method enables measurement of norepinephrine biomarker levels in normal, disease state, and treated-patients in support of a variety of clinical studies.

**REFERENCE**

"Simultaneous Determination of Plasma Epinephrine and Norepinephrine using an Integrated Strategy of a Fully Automated Protein Precipitation Technique, R educutive Labeling and UPLC-MS/MS". Chengjie Ji, Justin Walton, Yi Su, Max Tella, Analytica Chimica Acta, 670 (2010) 84-91