Rapid, simultaneous analysis of plasma catecholamines and metanephrines by mixed-mode SPE and HILIC LC/MS/MS

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Background: In clinical research, elevated concentrations of plasma catecholamines can be used in conjunction with their O-methylated metabolites (metanephrines) to indicate the presence of conditions such as pheochromocytomas, neuroblastomas, ganglioblastomas and ganglioneuromas. However, these compounds (in particular, norepinephrine, epinephrine, and dopamine) can be a challenge to analyze via reversed-phase LC/MS/MS due to their high polarity. As a result, many research laboratories still analyze this panel using ion-pairing reagents and ECD detection. While reversed-phase LC/MS/MS has been used successfully, challenges still exist due to ion-suppression from matrix components, insufficient retention, and inadequate separation of normetanephrine and epinephrine. This work describes a single extraction and analysis method for monoamine neurotransmitters and metanephrines from human plasma. *Methods:* 250 µL plasma samples were pretreated with 50 mM NH_4CH_3COO, and loaded onto pretreated wells of mixed-mode µElution SPE plates. SPE wells were then washed with 20 mM NH_4CH_3COOH and 50:50 ACN:IPA and eluted with 2 x 25 µL aliquots of 85:15 ACN:H_2O with 2% formic acid. HILIC-based chromatographic separation was achieved using an UHPLC silica-hybrid amide column. MPA and MPB consisted of 30 mM NH_4COO dissolved in 95:5 H_2O: ACN and 15:85 H_2O: ACN, respectively. Compounds were detected by MRM in ESI positive ionization mode. *Results:* All compounds eluted within 2.1 minutes, with baseline separation between normetanephrine and epinephrine enabling their unambiguous identification and quantification. Recoveries ranged from 45-90% and averaged 76%. Matrix effects were less that 25% for dopamine and norepinephrine and under 10% for the remaining analytes. Calibration curves were linear from to 10-2000 pg/mL for dopamine, 3-MT, metanephrine and normetanephrine, and from 50-10,000 pg/mL for epinephrine and norepinephrine. Calibration curves for all compounds had R^2 values of 0.999 or greater. %CV and bias values for quality control samples were less than 10% for all analytes at even the lowest QC concentration (40 pg/mL). *Conclusion:* This combination of mixed-mode sample preparation and HILIC chromatography results in a

rapid, robust method with excellent linearity, accuracy, and precision that is suitable for measuring even the lowest endogenous concentrations of these compounds.

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