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

Jonathan Danaceau, Erin Chambers, and Kenneth Fountain

Waters Corporation, 5 Technology Dr,, Milford, MA 01757

Background: In clinical research, elevated concentrations of urinary 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 urinary monoamine neurotransmitters and metanephrines.

Methods: 400 µL urine samples were pretreated with 1 mL of 500 mM NH_4CH_3COO, and loaded onto pretreated wells of mixed-mode weak cation exchange SPE plates. SPE wells were then washed with 1 mL of 20 mM NH_4CH_3COOH and 1 mL of MeOH and eluted with 2 x 250 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 consisted of 50 mM NH_4COO (pH 3.0) dissolved in 95:5 H_2O: ACN, and MPB was 30 mM NH_4COO (pH 3.0) 15:85 H_2O: ACN. 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. The chromatography can be seen in the accompanying figure. Recoveries ranged from 36-98.5% and averaged 72%. Matrix effects were - 35% for dopamine, -22% for epinephrine and under 10% for the remaining analytes. This represented a significant improvement over reversed-phase chromatography in which matrix effects were greater than

-50% for norepinephrine and epinephrine. Calibration curves were linear from to 0.5-500 ng/mL for all analytes with R^2 values of 0.99 or greater. Bias values for quality control samples were less than 10% for all analytes at even the lowest QC concentration (1.6 ng/mL). Precision was also excellent with all %CV values under 15% and most under 10%.

Conclusion: This combination of mixed-mode sample preparation and HILIC LC/MS/MS analysis 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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