Determination of Catecholamines and Metanephrines in Urine by SPE and LC-MS/MS

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Introduction

Catecholamines are hormones made by the adrenal glands and metanephrines are the metabolites of catecholamines. They are biomarkers of several kinds of tumors, such as pheochromocytoma tumors, paraganglioma tumors and neuroblastoma tumors. These tumors produce excess catecholamines which metabolize to metanephrines. Urinary catecholamines and metanephrines are tested for diagnosis of these tumors.

The challenge to analyze these compounds is that some of them are very hydrophilic compounds. They are hard to retain on a regular C18 HPLC column. Many HPLC methods use 100% aqueous mobile phase or use ion-pairing reagents. These are not ideal conditions for mass spectrometry. We have tested several phases of HPLC columns and chose one with good retention of these compounds under certain percentage of organic solvent.

Weak cation-exchange solid phase extraction (SPE) methods remove all neutral and acidic interference in the urine samples. LC-MS/MS is highly sensitive and selective. We developed SPE and LC-MS/MS methods to quantify urinary catecholamines and metanephrines.

Instruments and Materials

- **Instruments:** SPE extractions were carried out using Orochem's EZPRESS 48 positive pressure processor. All LC-MS/MS methods used AB Sciex Excion LC system coupled to API 4500 mass spectrometer with a turbo ion spray ESI source operated in positive ion mode.
- **Materials:** Agility Deluxe Polymeric WCX SPE, 30 mg/cc cartridges (Orochem Technologies Inc) were used for all extractions. Norepinephrine, epinephrine, dopamine, metanephrine and 3-methoxytyramine (3-MTY) standards and ammonium acetate were purchased from Sigma-aldrich. HPLC grade methanol, acetonitrile, IPA, water and formic acid (FA) were purchased from Pharmco-Aaper. Human urine was fortified with standards.

HPLC and Mass Spec Conditions

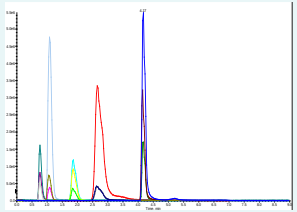
HPLC-MS/MS Conditions:
Column: Orochem Orosil C18-Polar HPLC column 3x50 mm, 3 µm.
Mobile phases: A: 0.1% FA/water; B: 0.1% FA /methanol. Hold 1.5 min of 0% B, then increase to 50% B in 6 min.
Flow rate: 0.4 mL/min
Injection volume: 10 µL
Mass spectrometer: API4500, ESI+, IS : 5000, Temp 500, MRM (see below table)

Procedures

Sample preparation : Analytes were spiked into blank urine. Agility Deluxe WCX cartridge was pre-conditioned with 1 ml of acetonitrile, then 1 ml of 50 mM NH4AC. Urine solution (0.2 mL) was loaded to SPE, then washed with 1 ml each of 10% methanol and IPA. Analytes were eluted with 1 ml of 5% formic acid in acetonitrile, and then evaporated and reconstituted in water.

Results

1) Mass chromatogram of catecholamines and metanephrines:



Compound	Retention Time (min)	Q1/Q3 (Quantifier ion)	DP	CE
Norepinephrine	0.65	170.1/152.1	20	15
Epinephrine	0.81	184.1/166.1	20	15
Dopamine	1.12	153.9/137.0	30	18
Metanephrine	1.49	197.9/180.0	15	15
3-Methoxytyramine	3.19	168.0/91.0	30	18

Without FA, the analytes retained on the column better, however, the peak shapes were poor. With 0.1% FA, the peak shapes and sensitivity increased significantly.

2) Linearity:

Calibration range is with 5-250 ng/mL for all analytes except norepinephrine. For norepinephrine, the range is 10-250 ng/mL. R^2 is above 0.99 for all analytes.

3) Recovery Test Results:

Recovery for all analytes are within 94-106%.

Conclusions

A weak cation exchange SPE method has been developed to extract catecholamines and metanephrines in urine, and extracts can be analyzed with a sensitive LC-MS/MS method.
Future study is needed to increase the retention of norepinephrine on the LC column, so there will be less matrix effect.