

**ASMS 2013**

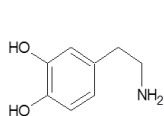
**TP-461**

Determination of Urinary  
Catecholamines and  
Metanephrines in a Single  
Run by LC-MS/MS for  
Clinical Research

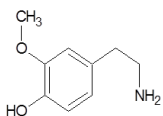
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## Introduction

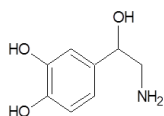
Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS method has been developed for the quantitation of catecholamines (dopamine, epinephrine and norepinephrine), metanephrine, normetanephrine and 3-methoxytyramine in urine. This method uses a single solid phase extraction procedure to simplify sample preparation.



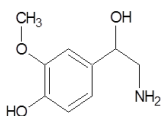
Dopamine (D)



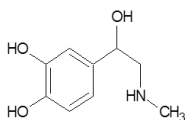
3-Methoxytyramine (3-MT)



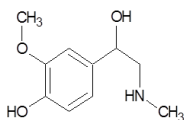
Norepinephrine (NE)



Normetanephrine (NMN)



Epinephrine (E)



Metanephrine (MN)

Figure 1. Structures for catecholamines and metanephrines.

A single, efficient solid phase extraction (SPE) sample preparation procedure was developed for the simultaneous extraction of dopamine, epinephrine, norepinephrine, metanephrine, normetanephrine and 3-methoxytyramine in urine. Calibrators were created by spiking clean urine with various concentrations of each analyte. The chromatographic system consists of a pentafluorophenyl column and a mobile phase comprised of methanol and water containing 0.2% formic acid. Quantifier and qualifier MRM transitions were monitored and deuterated internal standards were included for each analyte to ensure accurate and reproducible quantitation.

## Experimental

### Sample Preparation

Prepare complexed samples:

0.5 mL sample  
Add 40  $\mu$ L of internal standards mix  
Add 0.8 mL of complexing agent  
Verify pH is between 7.5-9.5

1. Condition SPE cartridge (Bond Elut Plexa, 30 mg, 3 mL) with 1 mL of MeOH and 1 mL of aqueous wash buffer (0.2 M NH<sub>4</sub>Cl-NH<sub>4</sub>OH).
2. Load complexed samples onto SPE cartridge.
3. Wash with 1 mL of 5% methanol wash buffer (0.2 M NH<sub>4</sub>Cl-NH<sub>4</sub>OH) and dry at full vacuum for 5 minutes.
4. Elute with 1 mL of 5% formic acid in water. Apply vacuum 5" Hg for 30 seconds. Transfer to autosampler vial.

### LC Method

Agilent 1290 HPLC binary pump, well plate sampler with thermostat, temperature-controlled column compartment

Parameter	Value
Analytical Column	Pursuit 3 PFP, 2x150mm, 3 $\mu$ m
Column Temp	40°C
Injection Volume	20 $\mu$ l
Autosampler Temp	4°C
Needle Wash	Flush port for 20 seconds
Mobile Phase A	0.2% Formic Acid in Water
Mobile Phase B	Methanol
Flow Rate	0.3 ml/min

Table 1. LC Parameters

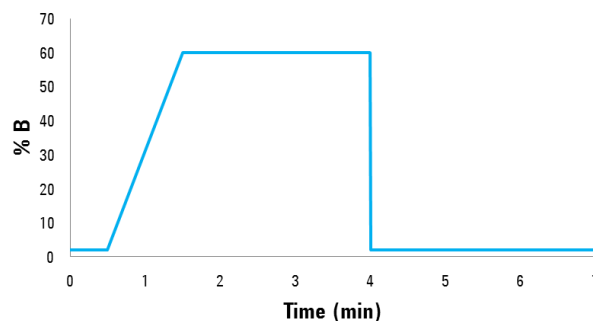


Figure 2. LC gradient

## Experimental

Compound	Prec	Prod	Frag	CE	CAV
Dopamine*	154.1	137.1	75	8	3
Dopamine	154.1	91.1	75	28	3
Dopamine-D4	158.1	141.1	75	8	3
Norepinephrine*	170.1	152.1	65	4	5
Norepinephrine	170.1	107	65	20	5
Norepinephrine-D6	176.1	158.1	65	4	5
Epinephrine*	184.1	166.1	70	8	5
Epinephrine	184.1	107.1	70	24	5
Epinephrine-D6	190.1	172.1	70	8	5
3-Methoxytyramine*	151.1	91.1	135	20	3
3-Methoxytyramine	151.1	119	135	12	3
3-Methoxytyramine-D4	155.1	95.1	135	24	3
Normetanephrine*	166.1	134	105	16	3
Normetanephrine	166.1	106.1	105	20	3
Normetanephrine-D3	169.1	137.1	105	16	3
Metanephrine*	180.1	165.1	120	16	5
Metanephrine	180.1	148.1	120	16	5
Metanephrine-D3	183.1	168.1	120	16	5

Table 2. MRM transitions (\*Quantifier)

### MS Method

Agilent 6460 QQQ with JetStream technology

Ion mode:	AJS ESI+
Gas temperature:	325 °C
Drying gas (nitrogen):	5 L/min
Nebulizer gas (nitrogen):	35 psi
Sheath gas (nitrogen):	375 °C
Sheath flow:	12 L/min
Capillary voltage:	3000V
Nozzle voltage:	0V
Q1/Q3 Resolution:	0.7 unit
Dwell time:	20 msec
Delta EMV:	200V

MRM transitions (table 2) were determined and optimized automatically using Agilent Optimizer software.

## Results and Discussion

Chromatographic separation of all analytes (figure 3) is achieved in less than four minutes through the use of a pentafluorophenyl column. The separation of epinephrine and normetanephrine, and the separation of metanephrine and 3-methoxytyramine are especially critical since these compounds share common fragments. Without proper separation by retention time, fragmentation of these compounds can cause interferences with one another and lead to inaccurate quantitation.

Commercially available quality control (QC) material (BioRad) was used to measure the accuracy and precision of this method. Results (table 3) show agreement with expected values and excellent precision at both levels.

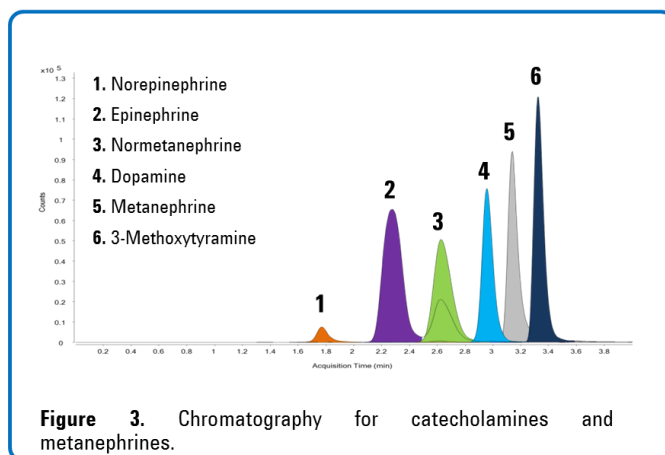


Figure 3. Chromatography for catecholamines and metanephrines.

Compound	Free/Total	Range	Level 1		Level 2		
			Measured	CV (%)	Range	Measured	CV (%)
Dopamine	Free	44.4 – 75.0	61.4	3.4	377 – 629	509	2.8
Norepinephrine	Free	31.3 – 51.6	38.4	5.8	156 – 239	192	4.8
Epinephrine	Free	9.62 – 19.1	14.3	5.3	67.8 – 104	86.7	2.0
3-Methoxytyramine	Total	28.6 – 48.7	44.7	3.8	381 – 572	557.7	2.2
Normetanephrine	Total	220 – 366	300.7	2.4	1084 – 1630	1379.2	2.8
Metanephrine	Total	69.0 – 116	91.2	2.0	434 - 655	612	2.5

Table 3. Results in ng/mL of BioRad QC run by LC/MS/MS (range determined by BioRad using HPLC)

## Results and Discussion

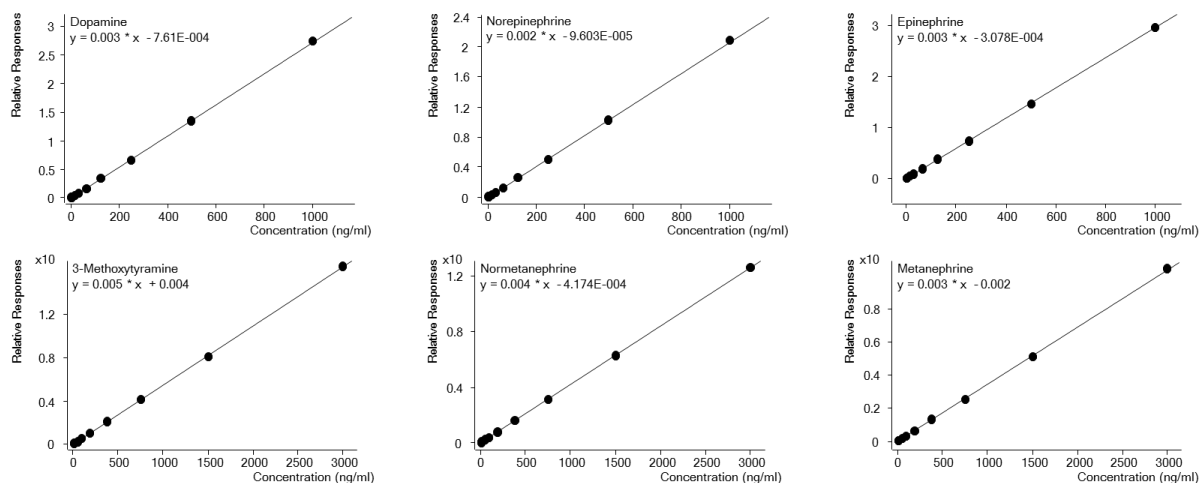


Figure 4. Calibration curves for catecholamines and metanephrines.

Compound	R <sup>2</sup>	Concentration (ng/mL)	Accuracy (%) n = 3	Intraday CV (%) n = 3	Interday CV (%) n = 5
Dopamine	0.9997	1.56	107.5	1	2.7
		62.5	99.1	1.7	2
		1000	101.3	0.1	0.3
Norepinephrine	0.9999	1.56	102.9	0.9	5.4
		62.5	101.1	3.5	4
		1000	101.1	0.6	0.6
Epinephrine	0.9998	1.56	101.6	4.3	2.7
		62.5	100.9	2.5	2
		1000	100.3	0.4	0.3
3-Methoxytyramine	0.9999	4.69	95.7	1.1	3.6
		187.5	102.9	0.9	2
		3000	100	0.2	0.3
Normetanephrine	0.9999	4.69	100.1	1.5	3.2
		187.5	102	1.1	2.5
		3000	100.7	0.2	0.2
Metanephrine	0.9999	4.69	100.5	0.3	2.8
		187.5	102	0.5	2.2
		3000	100.8	0.1	0.2

Table 4. Summary of analyte performance for catecholamines and metanephrines.

## Conclusions

A four minute method has been developed for quantifying catecholamines and their metabolites. Offline solid phase extraction (SPE) for simultaneous extraction of all six analytes from urine is shown with excellent recoveries. Chromatographic separation of all six analytes with conditions compatible with LC-MS/MS have been developed. Typical method performance results are well within acceptable criteria.

Reference: Whiting, M J. "Simultaneous measurement of urinary metanephrines and catecholamines by liquid chromatography with tandem mass spectrometric detection." *Ann Clin Biochem* 46 (2009): 129–136.

Agilent LC/MS products are for research use only and not to be used in diagnostic procedures