

## Application News

SSI-LCMS-086

Liquid Chromatography Mass Spectrometry

### Analysis of Catecholamines and Metanephrines in Urine



Liquid Chromatograph Mass Spectrometer

## LCMS-8060



#### ■ Summary

Catecholamines and Metanephrines were analyzed using a LCMS-8060 triple quadrupole mass spectrometer. Calibrators were prepared in synthetic urine and extracted using solid phase extraction.

#### ■ Background

Catecholamines act as neurotransmitters, and have a key role in regulating central nervous system functions such as motor control, cognition, and memory processing.

#### ■ Method

A C18-PFP column available from ACE-Excel available from MAC-MOD provided baseline separation for each analyte. 0.25 mM Ammonium Formate was used for mobile phase A, and 0.25 mM Ammonium Formate in MeOH was used for mobile phase B. Overall run time was 7.5 minutes using a flow rate of 0.4 mL/min.

Standards were purchased from Cerilliant, and synthetic urine was purchased from UTAK. Blank urine was spiked at varying concentrations to reflect clinical significance.

Isotopically labeled internal standards were used for all compounds except for 3-methoxytyramine.

#### ■ SPE Protocol

Extractions were performed using weak cation exchange on the EVOLUTE<sup>®</sup> EXPRESS WCX 10 mg 96-well plate from Biotage. Automated SPE plate processing was performed using the Biotage Extrahera<sup>®</sup> Table 1 below outlines the extraction procedure

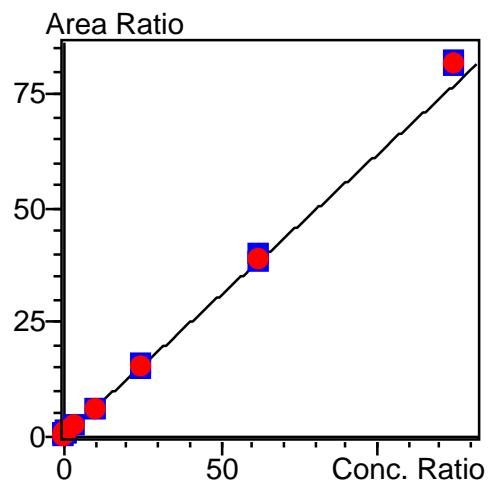
Pretreatment	Mix 75 uL urine with 10 uL internal standard solution and 150 uL of 250 mM ammonium acetate solution
Wash 1	Elute interferences with 10 mM ammonium acetate (500 uL)
Wash 2	Elute interferences with isopropyl alcohol (500 uL)
Elution	Elute analytes with 125 uL of water:isopropyl alcohol (85:15 v/v) containing 0.1% formic acid

Table 1: SPE protocol performed on Biotage Extrahera<sup>®</sup>

## ■ Calibration Curves

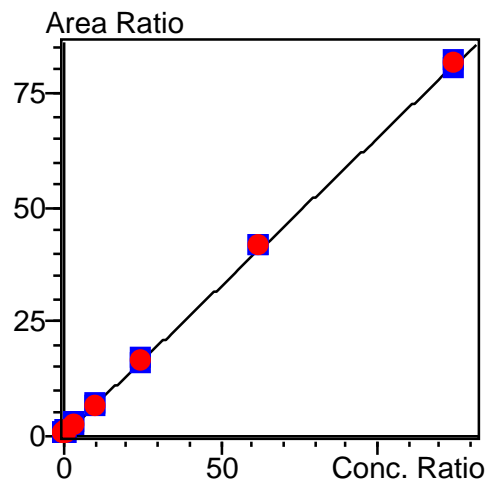
Metanephrine

$R^2=0.997$



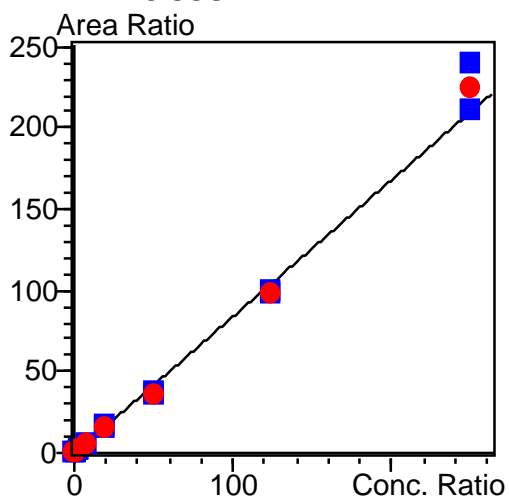
Normetanephrine

$R^2=0.999$



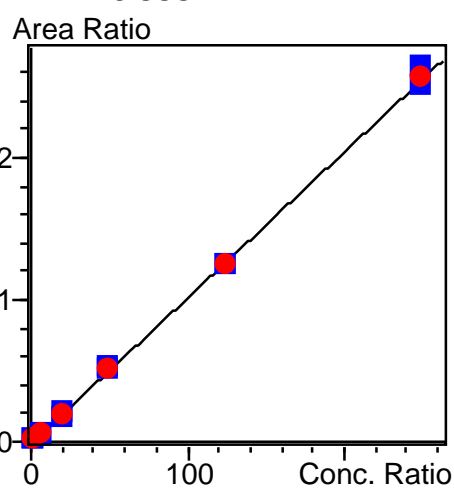
Epinephrine

$R^2=0.993$



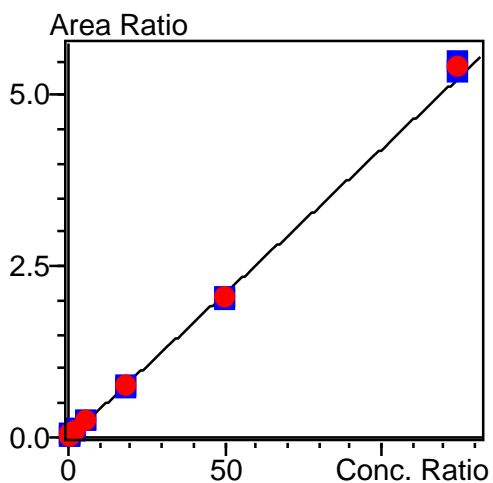
Norepinephrine

$R^2=0.999$



Dopamine

$R^2=0.997$



3-Methoxytyramine

$R^2=0.997$

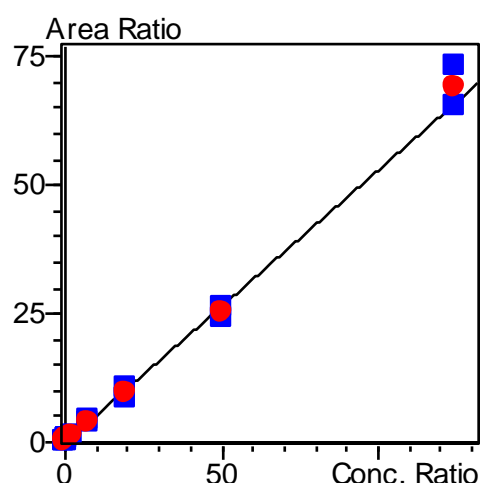


Figure 1: Calibration curves for reported compounds

Metanephrine	Area	ISTD Area	Area Ratio	Conc (ng/mL)	Std Conc (ng/mL)	Accuracy
Level 1	101,758	363,491	0.280	0.104	0.125	83.2
	95,412	316,562	0.301	0.139	0.125	111.1
Level 2	165,449	417,955	0.396	0.293	0.25	117
	147,426	412,432	0.357	0.23	0.25	92
Level 3	251,521	452,053	0.556	0.554	0.5	110.8
	228,309	450,277	0.507	0.474	0.5	94.7
Level 4	451,582	446,043	1.012	1.296	1.25	103.7
	347,155	351,719	0.987	1.255	1.25	100.4
Level 5	984,582	425,248	2.315	3.417	3.75	91.1
	1,144,924	477,275	2.399	3.553	3.75	94.7
Level 6	2,335,094	381,752	6.117	9.604	10	96
	2,321,947	380,067	6.109	9.592	10	95.9
Level 7	5,934,137	381,083	15.572	24.994	25	100
	5,876,835	394,679	14.890	23.885	25	95.5
Level 8	13,524,100	340,133	39.761	64.366	62.5	103
	13,368,910	349,633	38.237	61.885	62.5	99
Level 9	23,058,407	284,275	81.113	131.673	125	105.3
	24,885,067	303,741	81.929	133.001	125	106.4

**Table 2:** Metanephrine calibration data obtained from two separate extractions

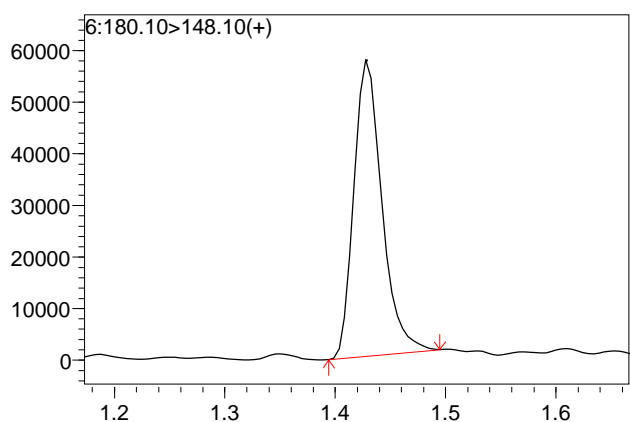
Normetanephrine	Area	ISTD Area	Area Ratio	Conc (ng/mL)	Std Conc (ng/mL)	Accuracy
Level 1	254,304	649,743	0.391	0.115	0.125	91.9
	251,858	643,279	0.392	0.115	0.125	92.1
Level 2	313,341	619,810	0.506	0.291	0.25	116.4
	302,923	602,861	0.502	0.286	0.25	114.5
Level 3	427,036	655,977	0.651	0.515	0.5	103
	418,150	656,980	0.636	0.493	0.5	98.5
Level 4	766,925	685,589	1.119	1.236	1.25	98.9
	624,768	564,523	1.107	1.218	1.25	97.4
Level 5	1,630,501	628,470	2.594	3.511	3.75	93.6
	1,858,675	696,834	2.667	3.623	3.75	96.6
Level 6	3,741,827	561,744	6.661	9.779	10	97.8
	3,971,640	583,349	6.808	10.006	10	100.1
Level 7	9,193,374	546,319	16.828	25.451	25	101.8
	8,619,207	551,477	15.629	23.604	25	94.4
Level 8	19,596,742	473,640	41.375	63.29	62.5	101.3
	20,415,069	488,718	41.773	63.903	62.5	102.2
Level 9	34,127,079	423,738	80.538	123.659	125	98.9
	35,380,645	432,351	81.833	125.655	125	100.5

**Table 3:** Normetanephrine calibration data obtained from two separate extractions

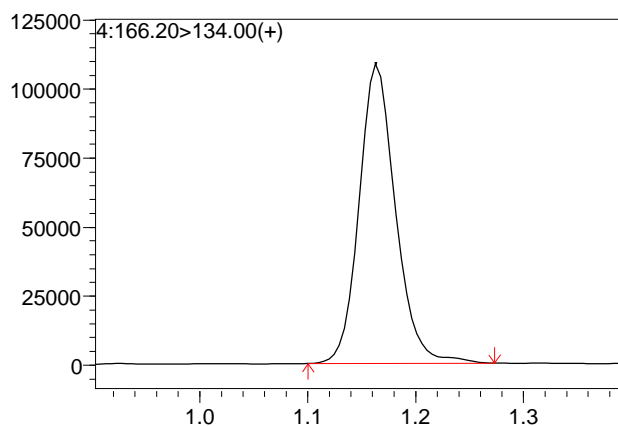
## ■ Repeatability

Compound	Transition	Trial 1	Trial 2	Trial 3	Trial 4	%RSD
Normetanephrine (0.125 ng/mL)	166.20>134.00	242,522	267,499	250,938	204,870	10.98
Metanephrine (0.125 ng/mL)	180.10>148.10	100,327	120,670	94,593	123,162	13.08
3-methoxytyramine (0.25 ng/mL)	151.10>91.10	19,941	22,598	24,000	21,575	7.76
Epinephrine (0.5 ng/mL)	184.10>166.10	26,683	20,518	23,297	26,497	12.09
Dopamine (0.625 ng/mL)	154.2>91.10	28,433	22,325	33,261	32,405	17.13
Norepinephrine (2.5 ng/mL)	152.2>107.05	6,248	7,034	4,797	6,622	15.76

**Table 4:** %RSD calculations performed on the LOQ for each compound



**Figure 2:** Chromatogram for Metanephrine at LOQ (0.125 ng/mL)



**Figure 2:** Chromatogram for Metanephrine at LOQ (0.125 ng/mL)

### ■ Results and Discussion

Figure 1 shows the calibration curves for epinephrine, norepinephrine, metanephrine, normetanephrine, and 3-methoxytyramine. All compounds were linear with a  $r^2$  value of at least 0.99. The linear range for metanephrine and normetanephrine was 0.125 ng/mL to 125 ng/mL. 3-methoxytyramine had a linear range of 0.25 ng/mL to 125 ng/mL. The linear range for dopamine was 0.625 ng/mL to 125 ng/mL. The linear range for epinephrine was 0.5 ng/mL to 250 ng/mL. The linear range for norepinephrine was 2.5 ng/mL to 250 ng/mL.

Tables 2 and 3 show the data used to generate the calibration curve for metanephrine and normetanephrine.

Table 4 shows the transitions monitored for each compound, as well as the %RSD for four separate extractions calculated at the LOQ for each compound. All transitions monitored used the dehydrated ion as the precursor, with the exception of epinephrine. This was done to reduce potential interferences from the matrix.

### ■ Conclusion

The LCMS-8060 showed to be successful at quantitating catecholamines and metanephrines in urine at ultra-low levels with good accuracy and repeatability.

# UPLC-MS

ULTRA FAST MASS SPECTROMETRY



LCMS-8040



LCMS-8045



LCMS-8050



LCMS-8060



LCMS-2020



LCMS-IT-TOF

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