Determination of plasma methanephrines and 3-methoxytyramine by LC-MS/MS for clinical research

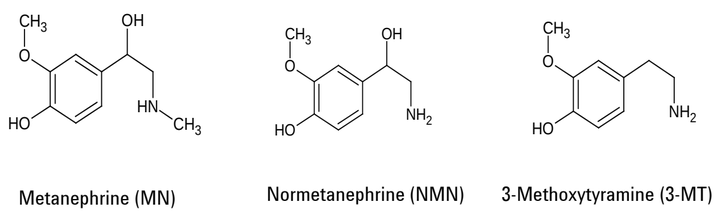
Linda Côté[1], Christophe Deckers[1], Kevin McCann[2], [1]Agilent Technologies, Inc., Montréal, QC, [2]Agilent Technologies, Inc.,Santa Clara, CA

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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 metanephrine, normetanephrine and 3-methoxytyramine in plasma. This method uses a solid phase extraction procedure for efficient sample preparation.



Metanephrine (MN) Normetanephrine (NMN) 3-Methoxytyramine (3-MT)

Figure 1. Structures of studied compounds.

An efficient solid phase extraction (SPE) sample preparation procedure was developed for the simultaneous extraction of metanephrine, normetanephrine and 3-methoxytyramine in plasma. Calibrators were created by spiking clean plasma with various concentrations of each analyte. The chromatographic system consists of a pentafluorophenyl (PFP) 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

Calibrators (Cerilliant, Medical Isotopes) are prepared with clean plasma matrix (Golden West Biologicals). Isotopically labelled Internal standards (Cerilliant, Cambridge Isotopes) and ChromSystems plasma controls were used.

Pretreatment of samples:

0.5 mL plasma, calibrators, QCs
Add 50 µL of internal standards mix
Add 0.5 mL of 10 mM NH4H2PO4 buffer pH 6.5

1: Condition SPE cartridge (SimpliQ WCX, 30 mg, 1 mL) with:
1 mL of MeOH
1 mL of 10 mM NH4H2PO4 buffer pH 6.5

2: Add samples

3: Wash with 1 mL H2O, 1 mL Methanol, 1 mL 0.2% formic acid in acetonitrile
Dry at full vacuum for 5 minutes

4: Elute with 2 x 250 µL of 2% formic acid in acetonitrile.
Apply vacuum 5" Hg for 60 seconds
Evaporate under nitrogen flow at 40 deg. C
Reconstitute with 100 µL of 0.2% formic acid in water
Note: use of silanized glassware is recommended for optimum recoveries

LC Method

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

Parameter	Value
Analytical Column	Agilent Pursuit 3 PFP, 2x150mm, 3µm PN: A3051150X020
Guard Column	Agilent Meta Guard column Pursuit 3 PFP, 2 mm PN: A3051MG2
Column Temp	40°C
Injection Volume	20 µl
Autosampler Temp	4°C
Needle Wash	Flush port for 20 seconds
Mobile Phase A	0.2% Formic Acid in Water
Mobile Phase B	0.2 % Formic Acid in Methanol
1290 Pump Gradient	Time (min.) %B Flow (mL/min.) 0.0 2 0.3 0.5 2 0.3 1.5 60 0.3 4.0 60 0.3 4.01 95 0.3 6.0 95 0.3 6.01 95 0.5
Stop Time	7.5 min.
Post Time	3 min.

Table 1. LC Parameters

Experimental

MS Method

Agilent 6460 QQQ with JetStream technology

Ion mode:	AJS ESI(+)
Gas temperature:	350 °C
Drying gas (nitrogen):	5 L/min
Nebulizer gas (nitrogen):	40 psi
Sheath gas (nitrogen):	400 °C
Sheath flow:	11 L/min
Capillary voltage:	3000V
Nozzle voltage:	0V
Q1/Q3 Resolution:	0.7 unit
Dwell time:	40 msec
Delta EMV:	200V

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

Compound	Prec Ion	Prod Ion	Frag (V)	CE (V)	CAV (V)
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	106.1	105	20	3
Normetanephrine	166.1	121	105	20	3
Normetanephrine-D3	169.1	109.1	105	20	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 table (*Quantifier)

Results and Discussion

Chromatographic separation of all analytes (figure 2) is achieved in less than four minutes through the use of a pentafluorophenyl (PFP) column. An extra wash can be performed at the end of every run or at the end of a batch for superior ruggedness. Although not measured with this method, 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, these compounds can cause interferences leading to inaccurate quantitation. The separation also achieved with these compounds is shown (figure 3).

Matrix effect were observed but were compensated for by the internal standards and gave acceptable recovery efficiency (table 3).

Commercially available quality controls (QC) material (ChromSystems) were used to measure the precision of this method. Results (table 4) show excellent precision at both levels.

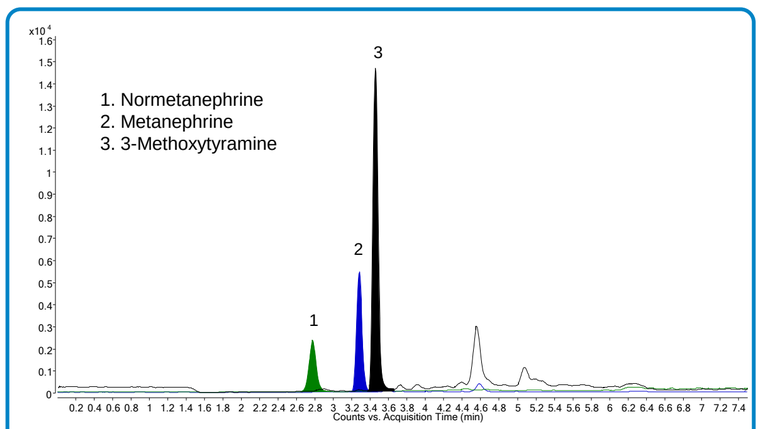


Figure 2. Chromatography for normetanephrine, metanephrine and 3-methoxytyramine

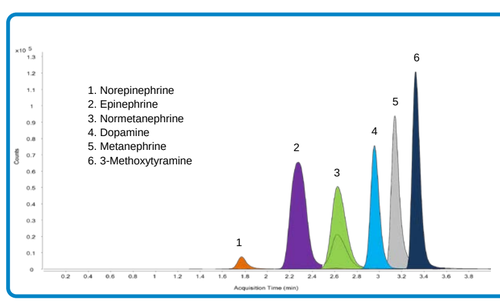


Figure 3. Chromatography for catecholamines and metanephrines

Results and Discussion

Compound	Matrix effects % (n = 9)		Recovery efficiency % (n = 9)	
	Average	SD	Average	SD
3-Methoxytyramine	78.3	7.2	99.1	16.9
Normetanephrine	36.0	13.7	87.7	27.0
Metanephrine	73.0	9.0	104.4	17.3

Matrix effect % = B/A *100
Recovery efficiency % = C/B *100
A: neat standard solutions
B: plasma extracted then spiked (post-ext)
C: spiked plasma then extracted (pre-ext)

Table 3: Matrix effects and recovery efficiency

	Level 1 (n=3)		Level 2 (n=3)	
Compound	Measured (pg/mL)	CV (%)	Measured (pg/mL)	CV (%)
3-Methoxytyramine	--	--	1768.9	0.8
Normetanephrine	95.9	5.1	7369.9	3.0
Metanephrine	85.0	2.2	1620.8	3.2

Table 4. Results of ChromSystems controls by LC-MS/MS

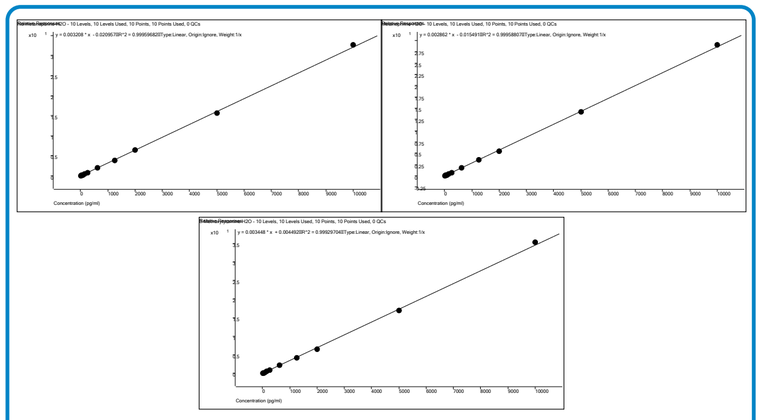


Figure 4. Calibration curves for metanephrine, normetanephrine and 3-methoxytyramine

Compound	R^2	Concentration (pg/ml)	Concentration (nmol/L)	Accuracy (%) n = 3	Intraday CV(%) n = 3	Interday CV (%) n = 5
3-Methoxytyramine	0.9997	15.63	0.09	115.4	2.4	1.9
		78.13	0.47	97.6	1.8	1.6
		1250	7.5	100.4	1.1	1.8
		10000	59.8	100.0	0.4	1.0
Normetanephrine	0.9996	15.63	0.09	117.3	2.7	4.9
		78.13	0.43	100.0	2.4	2.5
		1250	6.8	97.1	2.5	1.8
		10000	54.6	100.4	0.4	0.8
Metanephrine	0.9998	15.63	0.08	116.6	1.7	1.9
		78.13	0.40	96.5	2.5	2.2
		1250	6.3	100.2	1.1	0.9
		10000	50.7	100.3	0.6	0.6

Table 5: Summary of analyte performance.

Conclusions

A robust method for quantifying metanephrine, normetanephrine and 3-methoxytyramine in plasma with excellent reproducibility and accuracy has been developed.

References:
- 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.
- Gabler, J. "A sensitive and interference-free liquid chromatography tandem mass spectrometry method for measuring metanephrines in plasma." J Chromatograph Separat Techniq 2012, S2

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