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High-sensitivity, high-throughput quantitation of catecholamine metabolites in urine by LC/MS/MS for clinical research

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Introduction

Catecholamines and other neurotransmitters in circulation are readily metabolized to give vanillylmandelic acid (VMA), homovanillic acid (HVA) or 5-hydroxyindolacetic acid (5-HIAA), which are excreted to urine at much higher abundance than their precursor molecules. Given this and also the non-invasive nature of sample collection, urinary neurotransmitter metabolites are growing research target in clinical context. Our aim in this study is to accelerate clinical research by providing a fast and robust LC/MS/MS method for determining urinary neurotransmitter metabolites VMA, HVA and 5-HIAA.

Methods

Sample Processing

Described herein is a dilute-and-shoot LC/MS/MS method for determining the urinary concentration of VMA, HVA, 5-HIAA using high-sensitivity triple quadrupole mass spectrometer Shimadzu LCMS-8060. Briefly, 20 µL of urine sample was mixed with 980 µL of diluent (methanol that contained deuterium labeled internal standard for each compound) in a deep-well plate. After mixing by gentle vortexing, the plate was transferred directly to autosampler and 1 µL was subjected to LC/MS/MS analysis.
The pretreatment steps are robot-friendly and can easily be automated, although the study described here has been carried out by manual manipulation.

Analytical conditions

Table 1 MRM Transitions

Compound	Polarity	Precursor *m/z*	Product *m/z*	CE (V)
VMA	–	197.0	137.1* 138.1	22 13
VMA-d3	–	200.0	137.1* 138.1	22 13
HVA	–	181.0 137.0*	122.1	29 22
HVA-d5	–	186.0 142.0*	127.1	29 22
5-HIAA	–	190.0	146.15 144.0	11 22
5-HIAA-d5	–	195.0	151.15 148.0*	11 22

*ions used for quantitation

Table 2 HPLC Conditions

Column	: Shim-pack GISS C18 (100 mm x 2.1 mm, 3 µm)
Mobile phase A	: 0.05% acetic acid in water
Mobile phase B	: Methanol
Flow rate	: 0.6 mL/min
Time program	: 2% B (0 min) → 5% B (0.8 min) → 30% B (0.81 min) → 35% B (1.9 min) → 100% B (1.91 – 2.5 min) → 2% B
Column temp.	: 40 °C
Injection volume	: 1 µL

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Shimadzu LCMS-8060 was used for MRM mass spectrometry

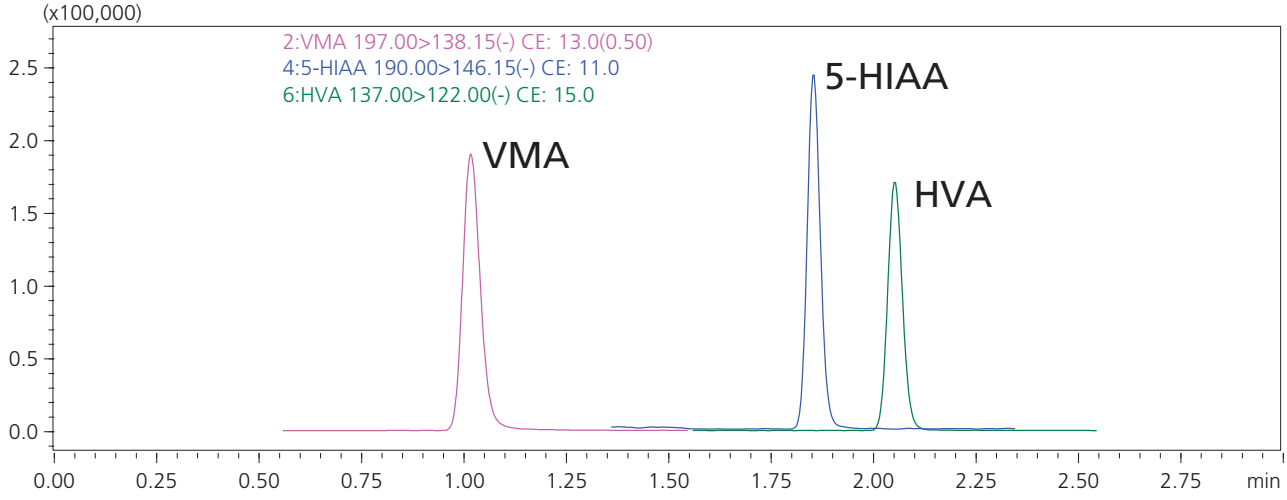

Fig. 1 Separation profile of VMA, HVA and 5-HIAA – 3 minutes in total

Results

Calibration range

We first evaluated sensitivity and quantitative range of HVA, VMA and 5-HIAA determination using freshly fortified neat standard solution. Table 3 summarizes the quantitative range; this calibration curve fulfilled the criteria of %RSD <15% and relative error <15% (<20% for LLOQ) for all calibration points in 5 repeat measurements.

Table 3 Quantitative range of neat standard detection. The concentrations are of original level in urine.

Compound	Calibration range (mg/L)	R^2
VMA	0.1 – 77.5	0.9994
HVA	0.1 – 77.5	0.9996
5-HIAA	0.1 – 77.5	0.9996

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Detection of endogenous VMA, HVA, 5-HIAA in plasma sample

Next, human urine samples were pretreated and endogenous catecholamine metabolite compounds were determined by LC/MS/MS. The MRM chromatograms acquired (Fig. 2) showed no apparent interference from urinary components, demonstrating low ng/mL sensitivity in real sample.

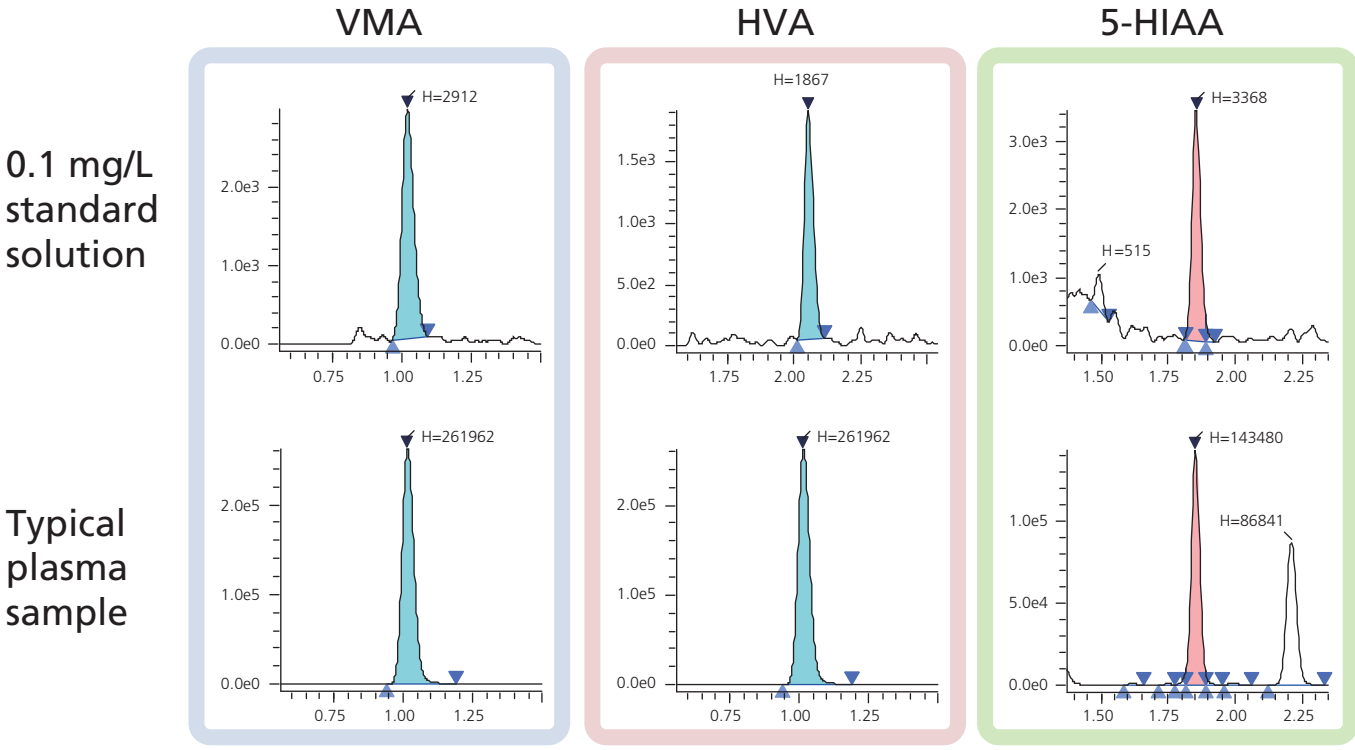

Fig. 2 Representative MRM chromatograms of VMA, HVA and 5-HIAA acquired from standard solution at LLOQ and non-spiked, human urine containing endogenous VMA, HVA and 5-HIAA at 6.2, 7.7 and 5.1 mg/L, respectively.

Pre-validation study

As a pre-validation study, we evaluated the accuracy of the neat standard curve with respect to matrix calibration curve, prepared using fresh, pooled urine sample that contained endogenous target compounds. As a result, while the true endogenous concentrations of VMA, HVA and 5-HIAA as determined by matrix calibration curve were 4.26, 4.91 and 3.43 mg/L, quantitation based on neat standard curve were 4.16 (97.6%), 5.07 (103.2%) and 3.00 mg/L (87.6%), respectively. Similarly for urine samples with 2.5, 5, 10, 20 and 25 ng/mL spiked concentrations, the accuracy of developed quantitation were within 87-103% range and inter-assay precision was <3.3% (n=5), as summarized in Table 4.

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Data correlation with HPLC method

To evaluate whether the present LC/MS/MS platform gives consistent results relative to conventional methods, a sample aggregate (n=180) were analyzed side-by-side by LC/MS/MS and HPLC in the same laboratory. Results are represented as scatter plots in Fig. 3. All compounds demonstrated satisfactory correlation and slope.

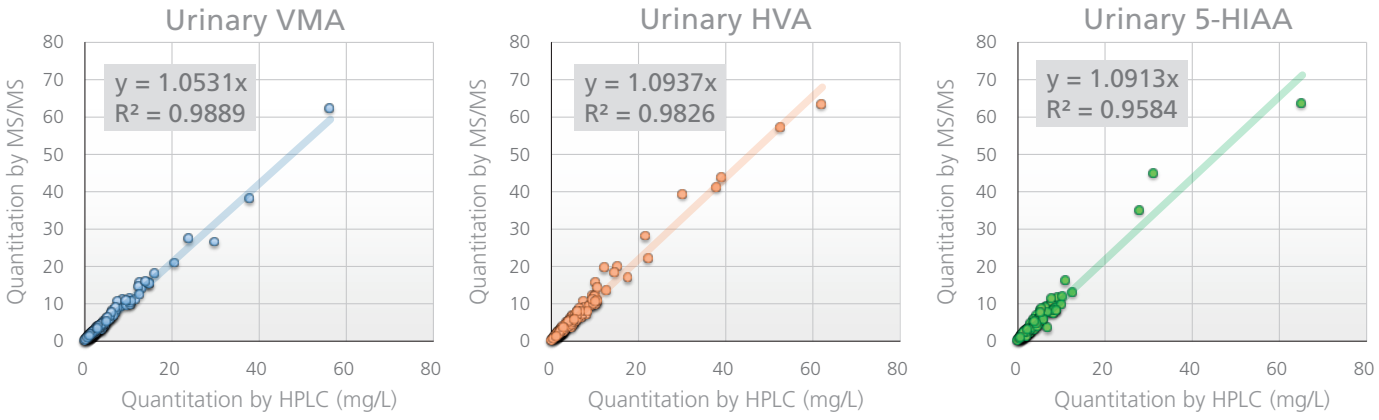

Fig. 3 Scatter plots showing the correlation between quantitation given by HPLC (X-axis) and LC/MS/MS method presented herein (Y-axis). Note that two samples that contained >100 mg/L of VMA and HVA were omitted from calculation as they dictated the correlation.

Table 4 Summary of pre-validation study

Compound	Added conc.	Target / IS area ratio	%RSD (n=5)	Determination based on		Accuracy (%)
				Matrix Calib.	Neat Calib.	
VMA	0.00	0.400	1.58%	4.256	4.160	**97.8%**
	2.50	0.631	0.57%	6.707	6.540	**97.5%**
	5.00	0.871	0.64%	9.260	9.022	**97.4%**
	10.00	1.341	0.59%	14.253	13.860	**97.2%**
	20.00	2.272	1.06%	24.146	23.480	**97.2%**
	25.00	2.759	0.53%	29.316	28.500	**97.2%**
HVA	0.00	0.497	0.43%	4.912	5.070	**103.2%**
	2.50	0.753	1.58%	7.447	7.684	**103.2%**
	5.00	1.035	1.03%	10.237	10.560	**103.2%**
	10.00	1.517	1.14%	15.007	15.480	**103.2%**
	20.00	2.518	2.88%	24.902	25.700	**103.2%**
	25.00	3.037	1.28%	30.044	30.960	**103.0%**
5-HIAA	0.00	0.283	2.05%	3.429	3.002	**87.6%**
	2.50	0.489	0.50%	5.922	5.188	**87.6%**
	5.00	0.697	3.34%	8.439	7.392	**87.6%**
	10.00	1.132	2.17%	13.706	12.020	**87.7%**
	20.00	1.948	1.27%	23.583	20.640	**87.5%**
	25.00	2.341	1.68%	28.340	24.820	**87.6%**

Concentration unit: mg/L

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Conclusion

We developed an extremely fast LC/MS/MS workflow that achieved sufficient sensitivity and linearity to cover biologically relevant concentration range of VMA, HVA and 5-HIAA in urine and was demonstrated for accuracy, precision and robustness.

Disclaimer: LCMS-8060 is intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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