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

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1. Introduction

Catecholamines and their metabolites in circulation are readily excreted to urine, both in free form and in conjugated forms (sulfate or glucoronate), and their levels are higher in urine than in plasma by orders of magnitude. Given this and also the non-invasive nature of sample collection, urinary catecholamines and their metabolites are growing research target in clinical context. Moreover, analysis for such research is increasingly performed by tandem mass spectrometry (LC/MS/MS) since it can selectively detect free and conjugated metabolites at high sensitivity. Our aim in this study is to accelerate clinical research by providing a fast and robust LC/MS/MS method to meet the requirement to quantitate both total (deconjugated by acid hydrolysis) and free catecholamine metabolites.

2. Methods

2-1. Sample Processing

Described herein are two methods: one method analyzed norepinephrine (NE), epinephrine (E), dopamine (DA), metanephrine (MN) and normetanephrine (NMN) after acid hydrolysis of urine samples followed by WCX-SPE cleanup; the other method was a dilute-and-shoot method to quantitate vanillylmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA).

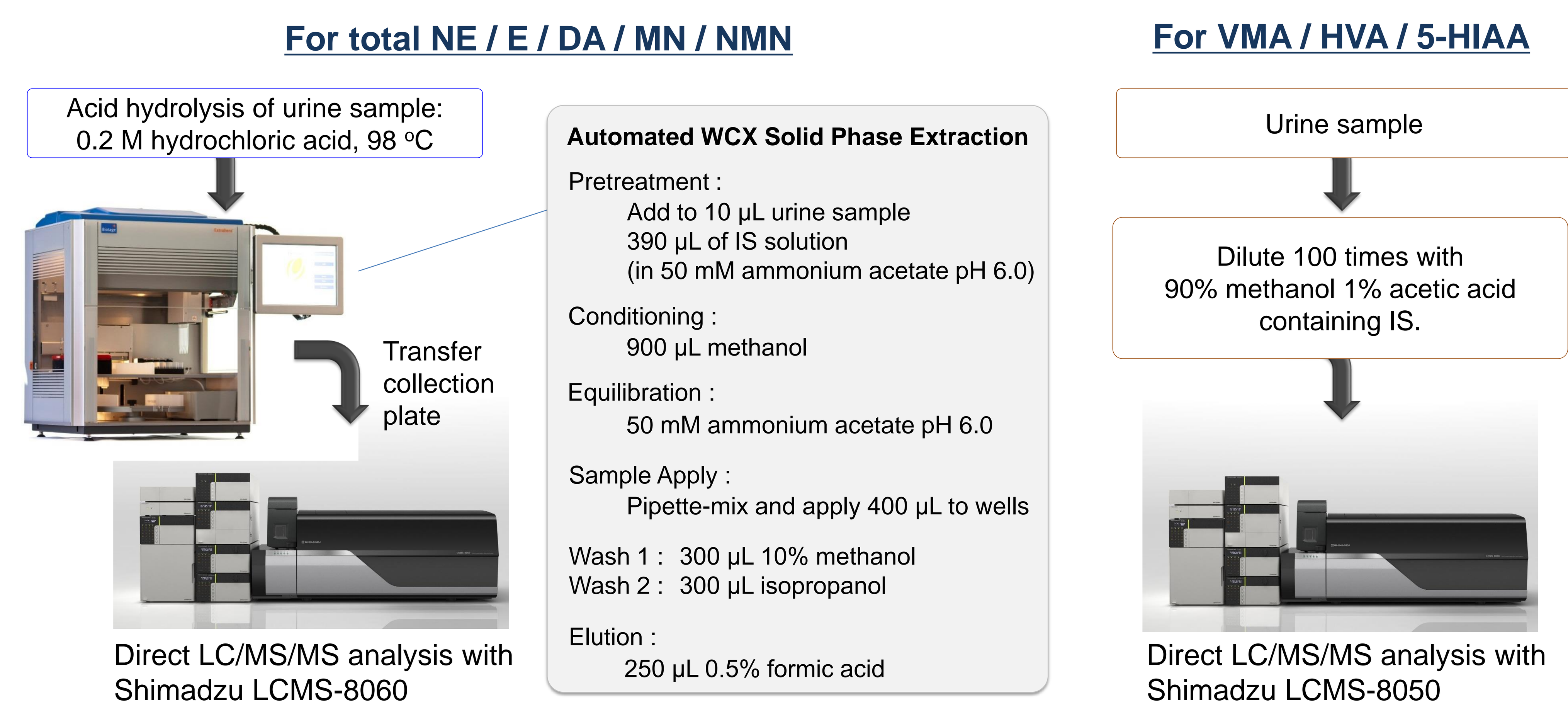

Fig. 1 Workflow for urinary catecholamine and catecholamine-related metabolite determination

2-2. Analytical Conditions

Determination of urinary catecholamines and catecholamine-related metabolites by LC/MS/MS was performed under the following conditions.

Table 1 MRM Transitions

Compound	Polarity	Precursor ion *m/z*	Product ion *m/z*	CE (V)	Dwell Time
NE	+	152.1 170.1	77.1* 107.1	-32 -21	10 msec 10 msec
NE-d6	+	158.1 176.1	81.1* 111.1	-32 -21	5 msec 5 msec
E	+	184.1 184.1	107.1* 166.1	-21 -11	10 msec 10 msec
E-d6	+	190.1 190.1	112.1* 172.1	-21 -11	5 msec 5 msec
DA	+	154.1 154.1	91.1 137.1*	-24 -14	10 msec 10 msec
DA-d4	+	158.1 158.1	95.1 141.1*	-24 -14	5 msec 5 msec

*transition for quantitation

Compound	Polarity	Precursor ion *m/z*	Product ion *m/z*	CE (V)	Dwell Time
VMA	–	197.1 197.1	137.2* 138.2	22 13	5 msec 5 msec
VMA-d3	–	197.1 197.1	137.2* 138.2	22 13	5 msec 5 msec
HVA	–	181.1 181.1	122.1* 137.1	13 10	15 msec 15 msec
HVA-d5	–	186.1 186.1	127.1 142.1	13 10	5 msec 5 msec
5-HIAA	–	190.1 190.1	146.0 144.1*	11 22	15 msec 15 msec
5-HIAA-d5	–	195.1 195.1	151.0 148.1*	11 22	5 msec 5 msec

*transition for quantitation

Table 2 HPLC Conditions

Column:	Shimpack MAqC-ODS I (150 mm x 2.0 mm, 5 µm)
Mobile phase A:	0.05% formic acid in water
Mobile phase B:	methanol
Flow rate:	0.2 mL/min
Time program:	1% B (0-0.5 min) → 50% B (3 min) → 99% B (3.1-6 min) → 1% B (7.1-10 min)
Column temp.:	40 °C
Injection volume:	1 µL

Column:	Shim-pack GISS (100 x 2.0 mm, 3 µm)
Mobile phase A:	0.05% acetic acid in water
Mobile phase B:	methanol
Flow rate:	0.5 mL/min
Time program:	2% B (0-0.2 min) → 25% B (0.5 min) → 30% B (1.8 min) → 40% B (2.5 min) → 95% B (2.5-3.5 min) → 2% B
Column temp.:	40 °C
Injection volume:	1 µL

Disclaimer: Shimadzu LCMS-8050 CL, LCMS-8060 CL and certain Nexera UHPLC components are registered in the U.S. as a Class I device and is not specifically cleared for the analysis of catecholamines and metabolites in urine. Other Shimadzu UHPLC components, Shim-pack MAqC-ODS I, Shim-pack GISS and other instruments, reagents as well as devices are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

3. Results and discussion

3-1. Sensitivity and quantitative range

We first evaluated sensitivity and quantitative range of catecholamine determination using freshly fortified neat standard solution. Table 4 summarizes the quantitative range; these calibration curves fulfill the criteria of %RSD <15% and relative error <15% (<20% for LLOQ) for all calibration points in 5 repeat measurements.

Table 4 Quantitative range

Compound	Calibration curve (ng/mL on column)	Quantitative range in urine
NE	0.1 – 40	3.5 – 1,400 ng/mL
E	0.05 – 20	1.75 – 700 ng/mL
DA	1 – 200	35 – 7,000 ng/mL
MN	0.05 – 20	1.75 – 700 ng/mL
NMN	0.05 – 20	1.75 – 700 ng/mL
VMA	1 – 200	0.1 – 20 µg/mL
HVA	5 – 200	0.5 – 20 µg/mL
5-HIAA	1 – 200	0.1 – 20 µg/mL

3-2. Detection of endogenous metabolites in urine sample

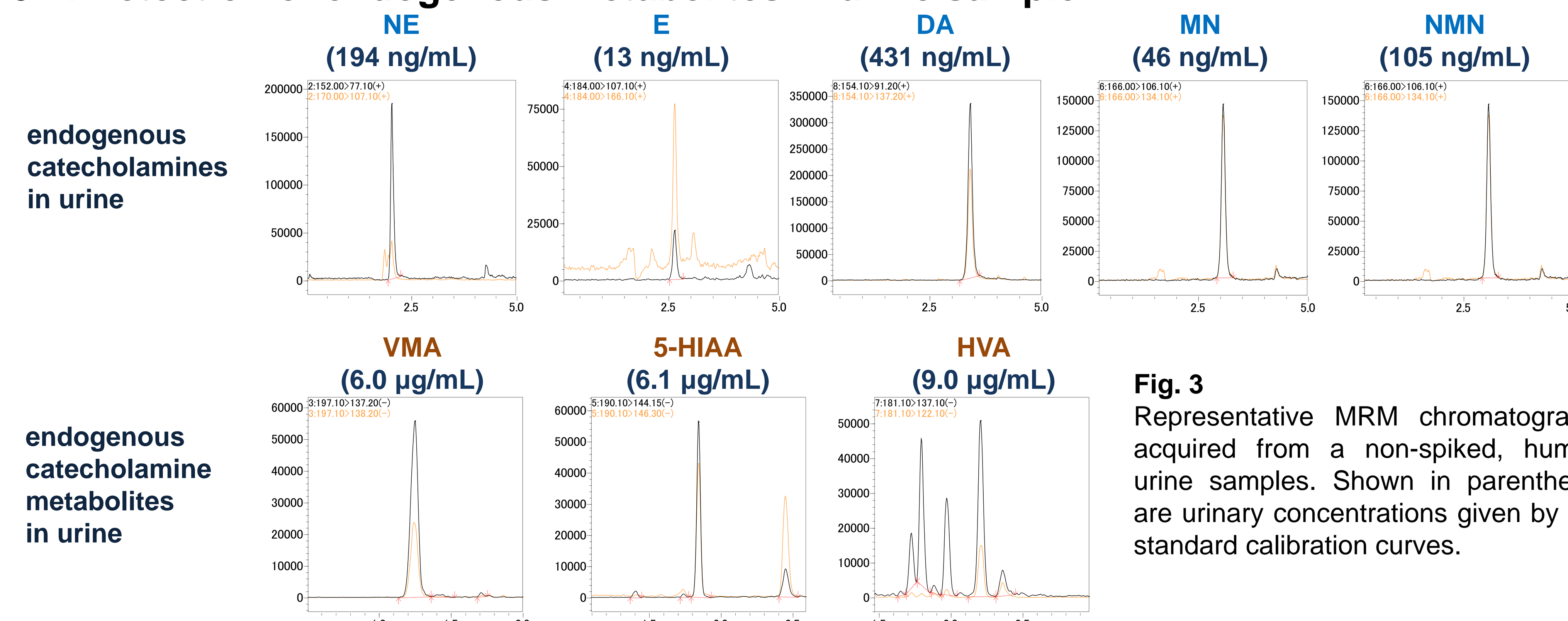

Fig. 3 Representative MRM chromatograms acquired from a non-spiked, human urine samples. Shown in parenthesis are urinary concentrations given by the standard calibration curves.

3-3. 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 urine sample that contained endogenous target compounds. As summarized in Table 5, urinary concentrations determination by the neat standard curve were demonstrated to reproduce the matrix calibration curve at 85-115% accuracy.

Table 5 Analytical performance of VMA/HVA/5-HIAA in urine

Compound	Spiked Conc.	True Conc. (matrix calib)	Determined Conc. by neat calib. curve	Accuracy	%RSD (n = 5)
VMA	0	5.90	6.41	108.6%	4.4
	0.5	6.40	7.01	109.6%	5.7
	1	6.90	7.40	107.3%	5.0
	5	10.90	11.79	108.2%	2.9
	10	15.90	17.02	107.1%	4.0
HVA	0	7.88	8.90	112.9%	2.7
	0.5	8.38	9.23	110.2%	4.7
	1	8.88	10.60	119.3%	7.2
	5	12.88	14.48	112.4%	10.2
	10	17.88	20.44	114.3%	6.2
5-HIAA	0	6.12	5.63	92.1%	11.8
	0.5	6.62	6.10	92.2%	9.5
	1	7.12	6.43	90.3%	3.0
	5	11.12	10.50	94.5%	12.2
	10	16.12	14.79	91.8%	3.8

3-4. Data correlation with HPLC-based methods

To evaluate whether or not the present LC/MS/MS platform gives consistent results relative to conventional methods, the same aggregate of samples were analyzed side-by-side using an established predicate devices available in Japan, which are based on (1) fluorescent HPLC detection for catecholamines, and (2) ECD detection for metanephrines. Results were presented as scatter plots shown in Fig. 4. For all compounds, both regression slope and correlation coefficient (r^2) were close to 1; the data acquired by the present LC/MS/MS method may be regarded equivalent to those given by conventional methods.

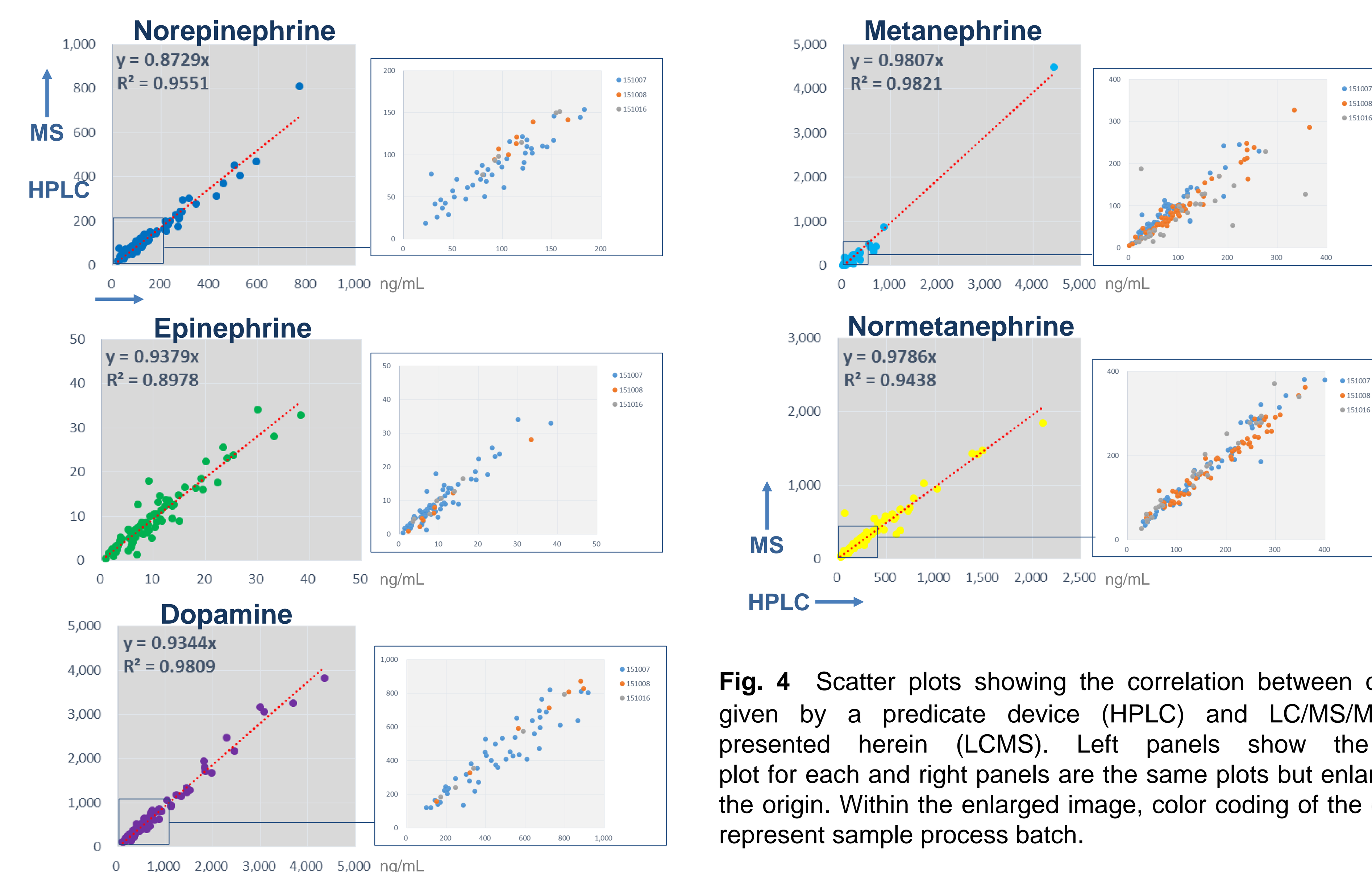

Fig. 4 Scatter plots showing the correlation between quantitation given by a predicate device (HPLC) and LC/MS/MS method presented herein (LCMS). Left panels show the full-scale plot for each and right panels are the same plots but enlarging about the origin. Within the enlarged image, color coding of the data points represent sample process batch.

4. Conclusion

In conclusion, the described high-throughput methods achieved sufficient sensitivity and linearity to cover biologically relevant concentration range in urine and was demonstrated for accuracy, robustness and consistency with conventional methodology.