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High-sensitivity, high-throughput quantitation of catecholamines and metanephrine in plasma by automated WCX-SPE coupled to LC/MS/MS for clinical research

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Introduction

Catecholamine levels in plasma, namely norepinephrine (NE), epinephrine (E) and dopamine (DA), give indication of disease states and are routinely measured for clinical research. Recently, plasma catecholamine metabolites such as metanephrine (MN) and normetanephrine (NMN) are increasingly studied as an alternative of or to complement catecholamine levels. Therefore high-sensitivity and high-throughput analytical system that covers all of these compounds is warranted.

Methods

Sample Processing

In order to detect plasma catecholamines with high sensitivity and accuracy, and minimize instrument maintenance intervals, plasma samples need preparation to remove interfering molecules such as proteins. Such routine work is best multiplexed and automated to maximize efficiency. Fig. 1 describes the 96-plex sample preparation protocol using EVOLUTE WCX extraction plate and Extrahera™, a positive-pressure liquid handling device for automation of solid phase extraction.

WCX Solid Phase Extraction	
Pretreatment	: Add to 300 µL plasma sample 300 µL of 0.05% formic acid containing deuterated IS compounds
Conditioning	: 900 µL methanol
Equilibration	: 50 mM ammonium acetate pH 6.0
Sample Apply	: Pipette-mix and apply 500 µL to wells
Wash 1	: 300 µL 10% methanol
Wash 2	: 300 µL isopropanol
Elution	: 250 µL 30% isopropanol 0.5% formic acid

Direct LC/MS/MS analysis using the LCMS-8060

Fig. 1 Plasma sample preparation for high-throughput catecholamine and metanephrine determination

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Analytical conditions

Table 1 MRM Transitions

Compound	Polarity	Precursor *m/z*	Product *m/z*	CE (V)
NE	+	152.1	77.1	-32
NE-d6	+	158.1	81.1	-32
E	+	184.1	107.1	-21
E-d6	+	190.1	112.1	-21
DA	+	154.1	137.1	-14
DA-d4	+	158.1	141.1	-14
MN	+	180.0	148.1	-20
MN-d3	+	183.0	151.1	-20
NMN	+	166.0	106.1	-21
NMN-d3	+	169.0	109.1	-21

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	: 5 µL

Results

Calibration range

We first evaluated sensitivity and quantitative range of catecholamine 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

Compound	Calibration range (pg/mL)	R^2
NE	6.8 – 3380	0.9995
E	7.4 – 3680	0.9998
DA	6.1 – 3060	0.9994
MN	4.4 – 2190	0.9991
NMN	4.8 – 2400	0.9994

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Detection of endogenous catecholamines in plasma sample

Next, human plasma samples were processed with Extrahera as described above and the endogenous catecholamine and metabolite compounds were determined by LC/MS/MS for research purpose. The MRM chromatograms acquired (Fig. 2) showed no apparent interference from plasma components, demonstrating low pg/mL sensitivity in real sample.

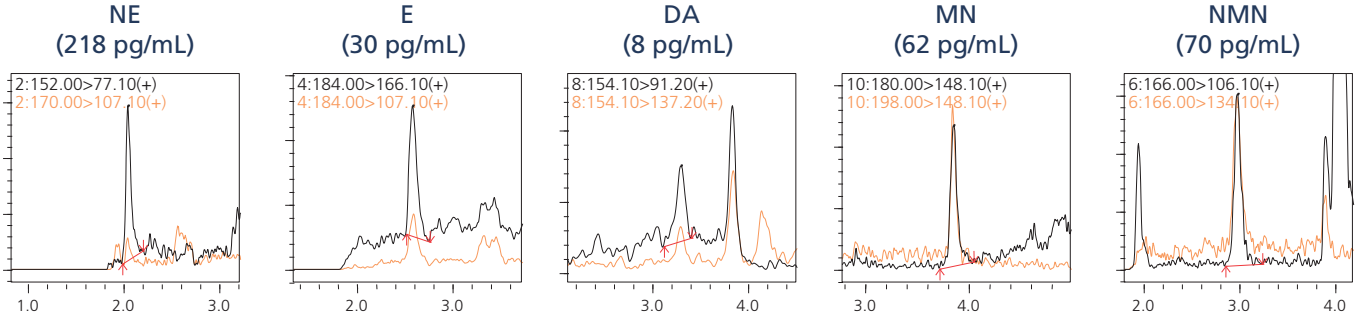

Fig. 2 Representative MRM chromatograms acquired from a non-spiked, human plasma. Concentrations in parenthesis were determined by the standard calibration curve.

Data correlation with a predicate device

To evaluate whether 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 device available in Japan, which is based on fluorescent HPLC detection. Results were summarized in Table 5 and represented as scatter plots shown in Fig. 3. Given on the scatter plots are the equation for linear regression (assumed to pass through the origin). For norepinephrine and epinephrine, 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.

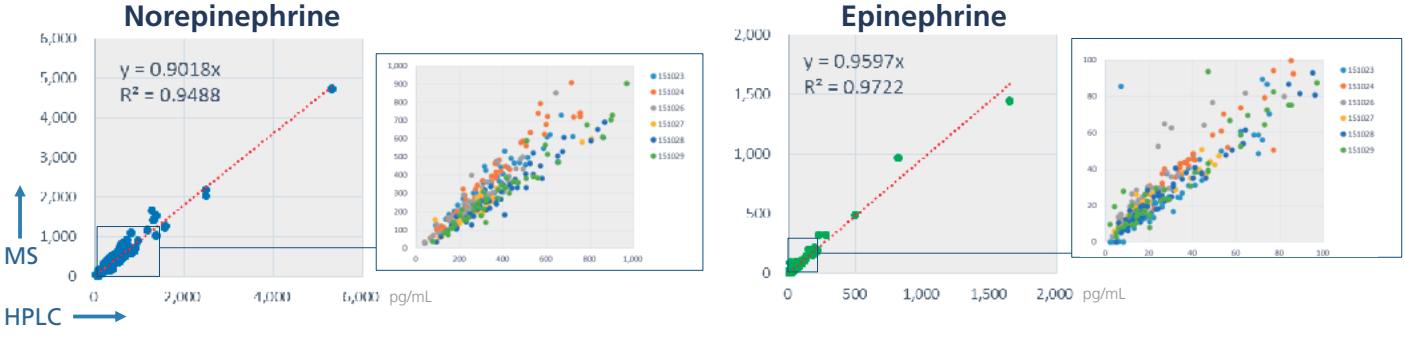

Fig. 3 Scatter plots showing the correlation between quantitation given by a predicate device (HPLC) and LC/MS/MS method presented herein (MS). Left panels show the full-scale plot for each compound and right panels are the same plots but enlarging about the origin. Within the enlarged image, color coding of the data points represent sample process batch.

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In contrast, we observed a strong negative bias in dopamine quantitation by LC/MS/MS (Fig. 4). This may be attributed to the difference in sample pretreatment. While WCX-SPE involves mild pH change, conventional methods employ protein precipitation by perchloric acid that causes acid-driven hydrolysis of dopamine conjugates, thus elevating the apparent level of plasma dopamine.

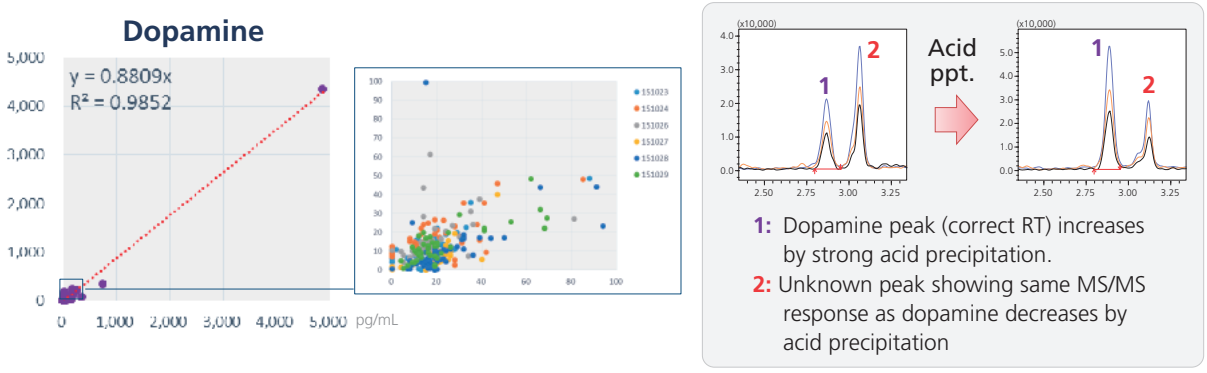

Fig. 4 Scatter plot displayed in the same format as Fig.3 that demonstrates limited correlation for dopamine. Right panel shows pilot result that suggests possible explanation to the observed discrepancy.

Table 4 Summary of catecholamine quantitation by HPLC and MS.

	Group †	Conc. Range (HPLC)	n	Mean (HPLC)	Mean (LCMS)	Bias by LCMS
NE	Ex. Low	< 100	16	76.6	78.1	+2.0%
	Low	101 - 300	116	214.3	188.8	-11.9%
	Mid	301 - 1000	148	468.0	431.6	-7.8%
	High	1001 - 5000	9	1622.7	1495.6	-7.8%
	Ex. High	> 5000	1	5313.0	4724.0	-11.1%
E	Ex. Low	< 10	42	7.1	10.6	+49.3%
	Low	11 - 30	136	18.8	21.1	+12.2%
	Mid	31 - 50	56	39.2	40.1	+2.3%
	High	51 - 200	45	80.9	81.1	+0.2%
	Ex. High	> 200	7	553.1	561.5	+1.5%
DA	Ex. Low	< 10	39	7.9	7.4	-6.8%
	Low	11 - 30	190	18.0	13.9	-23.2%
	Mid	31 - 50	26	37.7	21.8	-42.2%
	High	51 - 200	16	102.6	59.3	-42.2%
	Ex. High	> 200	5	1293.0	1044.5	-19.2%

† Samples were grouped according to concentrations determined by HPLC

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Conclusion

The described high-throughput method achieved sufficient sensitivity and linearity to cover biologically relevant concentration range in plasma and was demonstrated for robustness and consistency with conventional methodology.

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

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