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Rapid LC-MS/MS workflow for sensitive quantitation of Metanephrines and 3-Methoxytyramine in human plasma

Siji Joseph[1], Carrie Alder[2], Suet Ying Lee[3,4], Erhan Simsek[1], Robin Philp[1], Amaury Cazenave-Gassiot[3,4], Leroy S. Pakkiri [5,6], Chester L. Drum [5,6,7], David Bradley[8], Chee Sian Gan[1], Markus R. Wenk[3,4], and Anne K. Bendt[4]

1.Agilent Technologies, Singapore,
2.Agilent Technologies, UT, USA.,
3.Department of Biochemistry, National University of Singapore,
4.Singapore Lipidomics Incubator (SLING), National University of Singapore
5.Cardiovascular Research Institute, National University Health System, Singapore
6.Department of Medicine, National University of Singapore, Singapore
7.Department of Surgery, National University of Singapore
8.Agilent Technologies, Tasmania, Australia.

Introduction

This study presents a highly sensitive and specific LC-MS/MS method to quantitate MN, NMN, and 3-MT in plasma.

Metanephrine (MN), normetanephrine (NMN), and 3-methoxytyramine (3-MT) are the O-methylated metabolites of the catecholamines adrenaline, noradrenaline, and dopamine, respectively.

High-performance liquid chromatography combined with electrochemical detection (HPLC-ECD) or fluorescence detection (HPLC-FLD) are the typical analytical techniques to determine plasma metanephrines and 3-MT from the urine matrix.

However, quantitative measurement based on LC-MS/MS analysis has a huge potential to provide specificity and high sensitivity. Among the three targets, the chromatographic separation of MN and 3-MT is critical since these compounds share common fragments.

Experimental

Instrumentation

Agilent Ultivo LC/TQ equipped with an Agilent Jet Stream (AJS) Electrospray ion source was used for the simultaneous analysis of targets. MassHunter Data Acquisition (v: 1.2) and quantitative analysis (v: 10.0) software were used to acquire and process the data.

A 10-minute gradient method using an Agilent Pursuit 3 PFP column with water/ methanol/ formic acid mobile phase system was used to separate all three analytes chromatographically.

Sample preparation

Details of solid-phase extraction sample preparation procedure is given as Figure 1.

Experimental

MRM Transitions and sensitivity assessment

Specific MRM transitions of targets and deuterated internal standards were included to ensure a sensitive and reproducible MS/MS acquisition.

Table 1: MRM Transitions

Analyte	Precursor *(m/z)*	Fragmentor voltage (V)	Quant		Qual	
			m/z	CE (V)	*m/z*	CE (V)
MN	180	125	165	16	148	16
NMN	166	110	134	13	106	16
3-MT	151	130	91	20	119	10
MN-d3	183	125	168	16		
NMN-d3	169	110	137	13		
3-MT-d4	155	130	95	20		

Verification using certified standards

Method sensitivity was assessed using internal standard spiked plasma samples.

Method characteristics such as precision, accuracy, linearity, recovery, recovery repeatability, and method reproducibility were evaluated using ChromSystems certified reference standards (blank control, six-level calibrators, and three QC levels).

Recovery (%) and repeatability (%RSD) values were calculated from three intra-day technical preparations of each QC level. Inter-day QC recovery deviation (%RSD) was calculated to assess the workflow reproducibility.

Method efficiency for simultaneous separation of metanephrines and catecholamines were also evaluated.

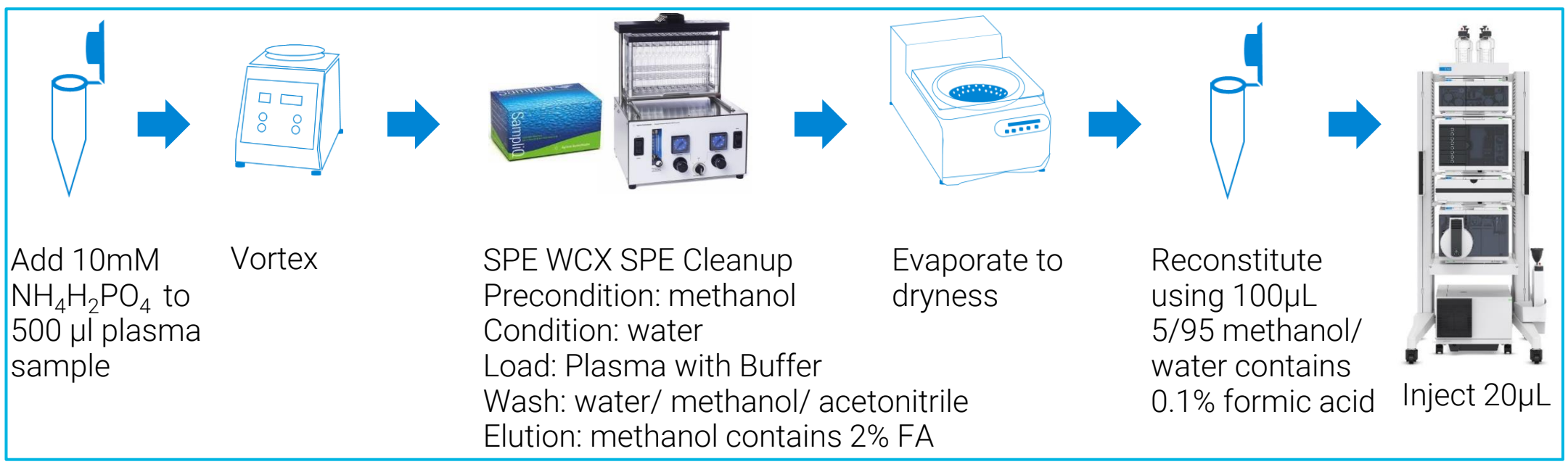

Figure 1: SPE protocol to remove biological interferences and to extract the analytes from 500 µL plasma samples

Target separation and workflow sensitivity

The Agilent Pursuit 3 PFP column offered baseline separation of all three analytes in under four minutes. The chromatographic baseline separation, together with MRM based triple quadruple detection, helped unambiguous identification of MN and 3-MT.

Method sensitivity estimation using target spiked plasma samples was impacted due to endogenous interferences. To eliminate this challenge, sensitivity was assessed using plasma samples spiked with deuterium-labeled targets at various concentrations. For MN d3 and 3-MT d4, the limit of detection (LOD) was 4 ng/L, whereas the Limit of Quantitation (LOQ) was 10 ng/L. For NMN d3, the LOD was 20 ng/L, and the LOQ was 40 ng/L.

Linearity, accuracy and precision

Method linearity, accuracy, and precision results using ChromSystems certified reference standards were satisfactory.
When compared with the control matrix, the analyte response was significantly higher for the lowest calibrator level ensuring method sensitivity.

All three targets displayed excellent linearity, with R^2 > 0.999.

Method accuracy was within 90 to 110% across the linearity range (%RSD < 4%).

Analyte absolute peak area response showed RSD < 11% (including LOQ), and retention time %RSD was < 0.3%.

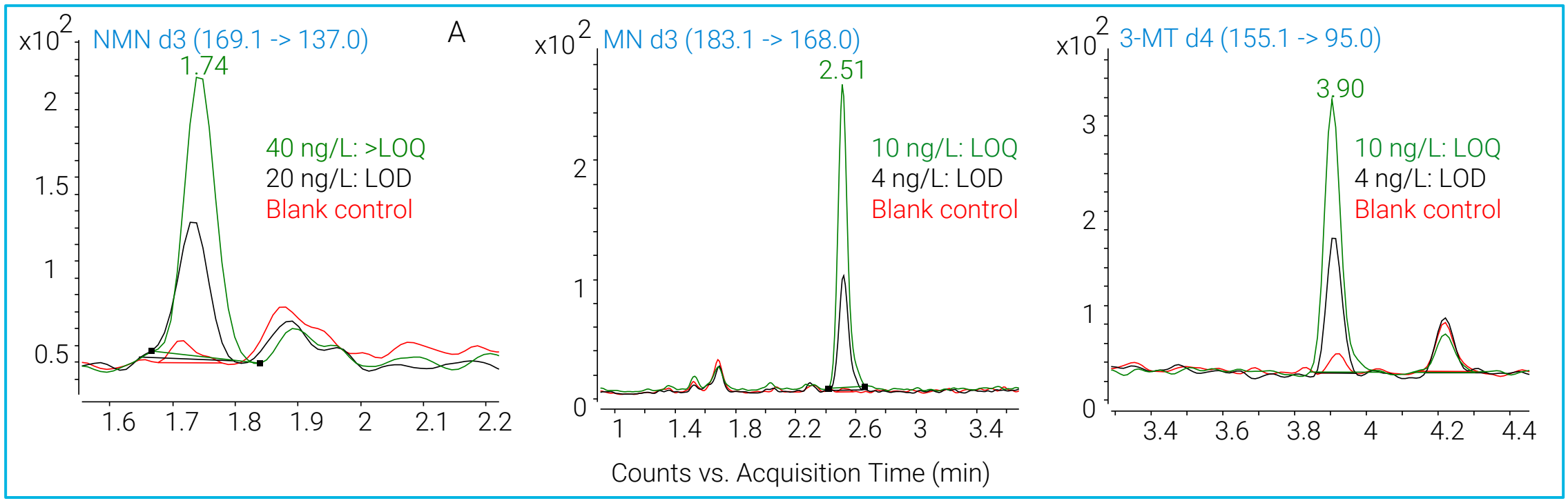

Figure 2: MRM trace overlay of control plasma (red trace), LOD (black trace), and LOQ (green trace) for NMN d3 (A), MN d3 (B), and 3-MT d4 (C).

Table 2: Intra-day recovery results using three technical preparations of three QC levels

QC	Technical Prep.	NMN			MN			3-MT		
		Expected amount (ng/L)	Calculated amount (ng/L)	Recovery (%)	Expected amount (ng/L)	Calculated amount (ng/L)	Recovery (%)	Expected amount (ng/L)	Calculated amount (ng/L)	Recovery (%)
LQC	1	127.0	124	98	58.4	58	99	22.7	23	101
	2		136	107		63	109		23	102
	3		128	101		61	105		24	105
MQC	1	279.0	281	101	182.0	175	91	92.8	87	94
	2		287	103		177	92		90	97
	3		283	101		183	95		92	99
HQC	1	1499.0	1477	99	950.0	921	97	878.0	822	94
	2		1553	104		937	99		836	95
	3		1527	102		916	96		807	92

Results and Discussion

Recovery and workflow repeatability

The impact of sample preparation on target recovery was assessed using three preparations of three levels of QC samples (LQC, MQC, and HQC).

Each preparation was injected into the LC/TQ in four replicates. Recovery (%) was calculated using respective calibration curve equations (Table 2).

The intra-day recovery repeatability was measured as %RSD of average recovery values, calculated using technical preparations. Recoveries for overall analytes were within 90 to 110%, with intra-day %RSD ≤ 5%.

Workflow day-to-day reproducibility

Average recovery results from three consecutive days were compared, and %RSD was calculated to assess inter-day workflow method reproducibility. The observed average recovery values for all three targets across three days were within 93 to 106%, and inter-day reproducibility was within 4 %RSD (Figure 3).

These results confirm the consistency of SPE extraction and LC/TQ methodology for routine quantitative analysis of MN, NMN, and 3-MT.

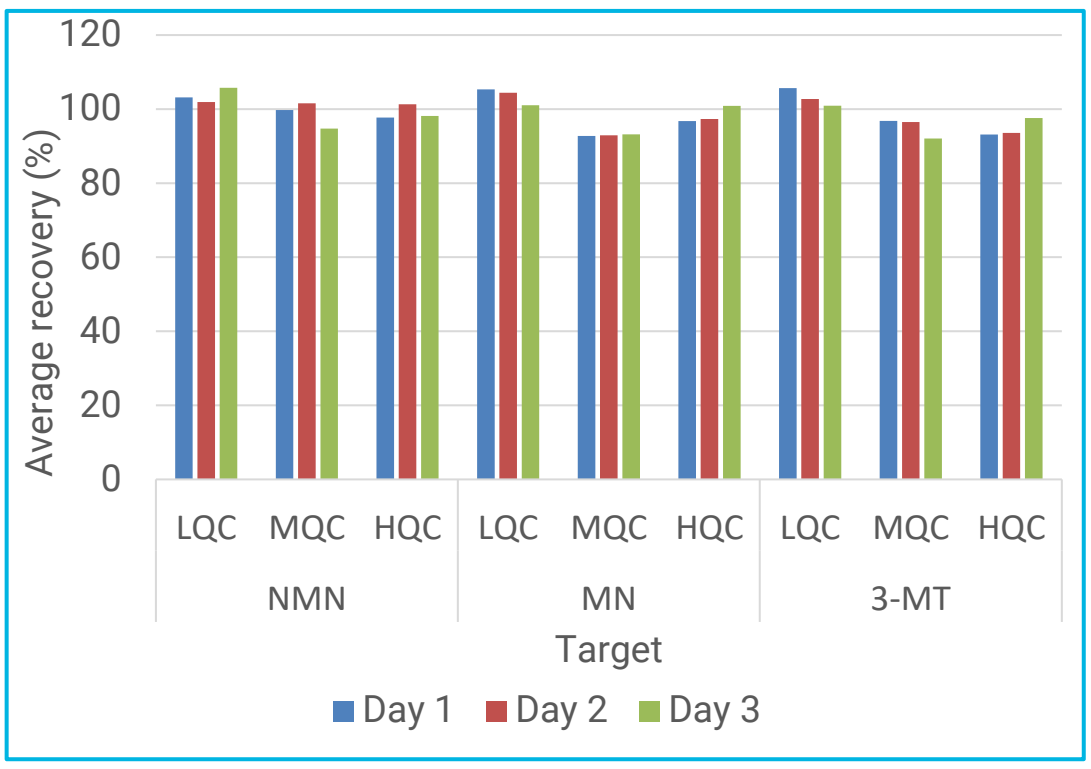

Figure 3: Recovery reproducibility of MN, NMN, and 3-MT across 3 days for all three targets

Simultaneous separation of metanephrines and catecholamines

The newly developed LC-MS/MS workflow can also be deployed for simultaneous analysis of metanephrines and catecholamines. The method also offered good chromatographic separation of norepinephrine (NEN), epinephrine (EN), and dopamine (Dopa) (Figure 4).

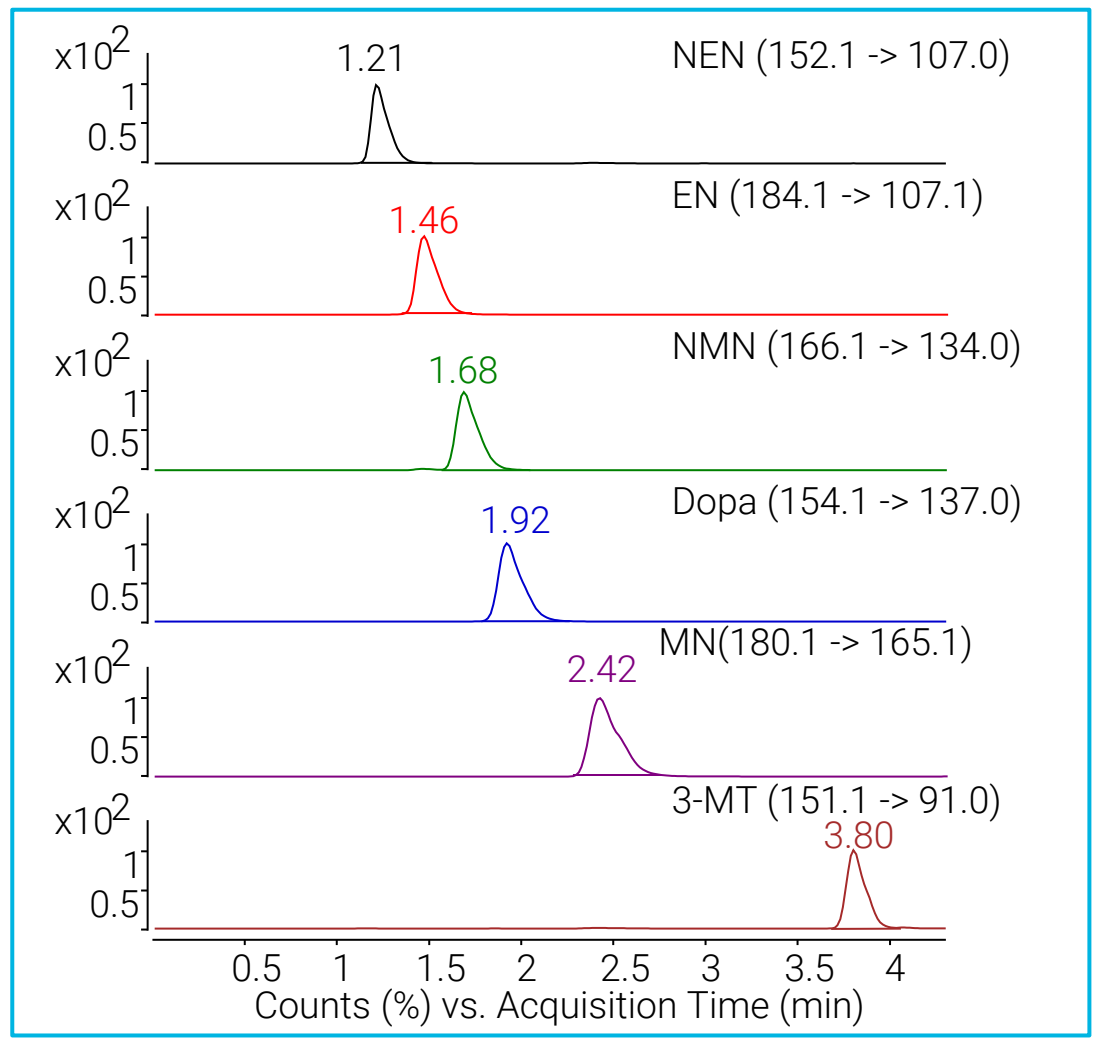

Figure 4: MRM trace of simultaneous separation of metanephrines (MN, NMN, 3-MT) and catecholamines (NEN, EN, Dopa). Concentration: 20 ng/mL in neat.

Conclusions

- A sensitive workflow was developed for the quantitation of MN, NMN and 3-MT in human plasma.
- The SPE sample cleanup protocol followed by a short gradient using an Agilent Pursuit 3 PFP column helped to achieve required sensitivity and selectivity for unambiguous measurement.
- The inter-day recovery reproducibility assessment using multiple QC levels illustrated the workflow reliability for confident day-to-day operation.
- The newly developed LC/MS/MS workflow for simultaneous measurement of metanephrines and catecholamines from plasma offers a convenient alternative and fast method to the standard practice of analyzing these targets from urine samples.

References

For more details, refer Agilent application note “Rapid and sensitive quantitation of metanephrines and 3-methoxy-tyramine in human plasma using an Agilent Ultivo Triple Quadrupole LC/MS (5994-4729EN).

https://explore.agilent.com/asms

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