



Quantification of 2,5-Hexanedione in Urine Using High Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry

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Introduction

n-Hexane is a widely used industrial solvent; however, chronic exposure can lead to progressive polyneuropathy. Its primary metabolite, 2,5-hexanedione (2,5-HD), serves as a critical biological exposure index for monitoring worker exposure. Therefore, accurate and sensitive quantification of urinary 2,5-HD is essential for occupational health surveillance and risk assessment.

Aim

The objective of this study was to develop and validate a robust, highly sensitive, and specific analytical method utilizing high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the precise quantification of 2,5-HD in human urine samples.

Methods

The analysis was performed using an SCIEX ExionLC AE system coupled with a QTRAP 6500+ mass spectrometer. Sample preparation involved protein precipitation followed by filtration. Chromatographic separation was achieved on a biphenyl column via a 7-minute gradient elution. Analytes were detected in multiple reaction monitoring (MRM) mode. Method validation, including linearity, accuracy, precision, matrix effect, and recovery, was conducted. Data were processed using SCIEX OS software.

Figure 1. Workflow of sample preparation

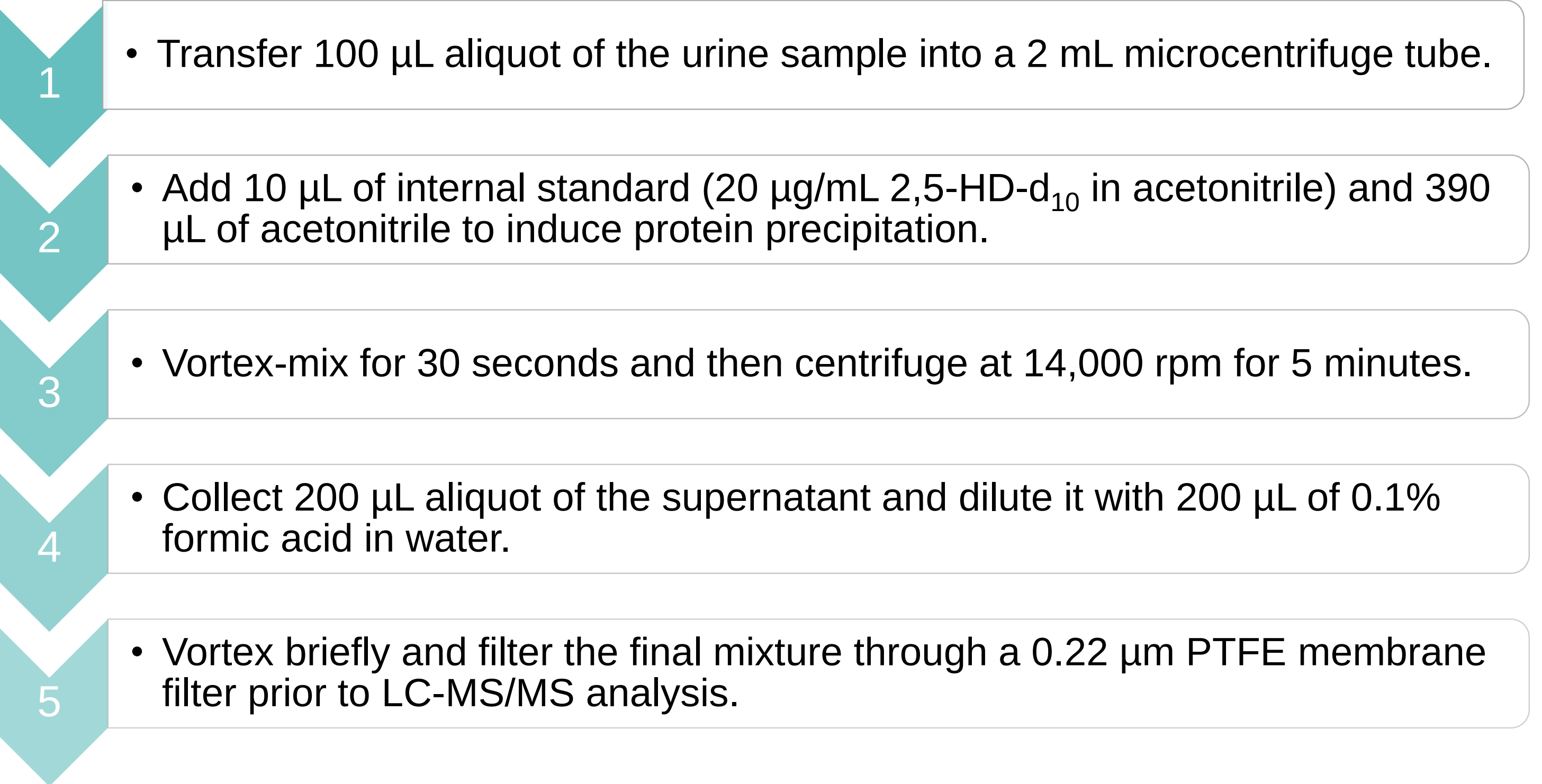
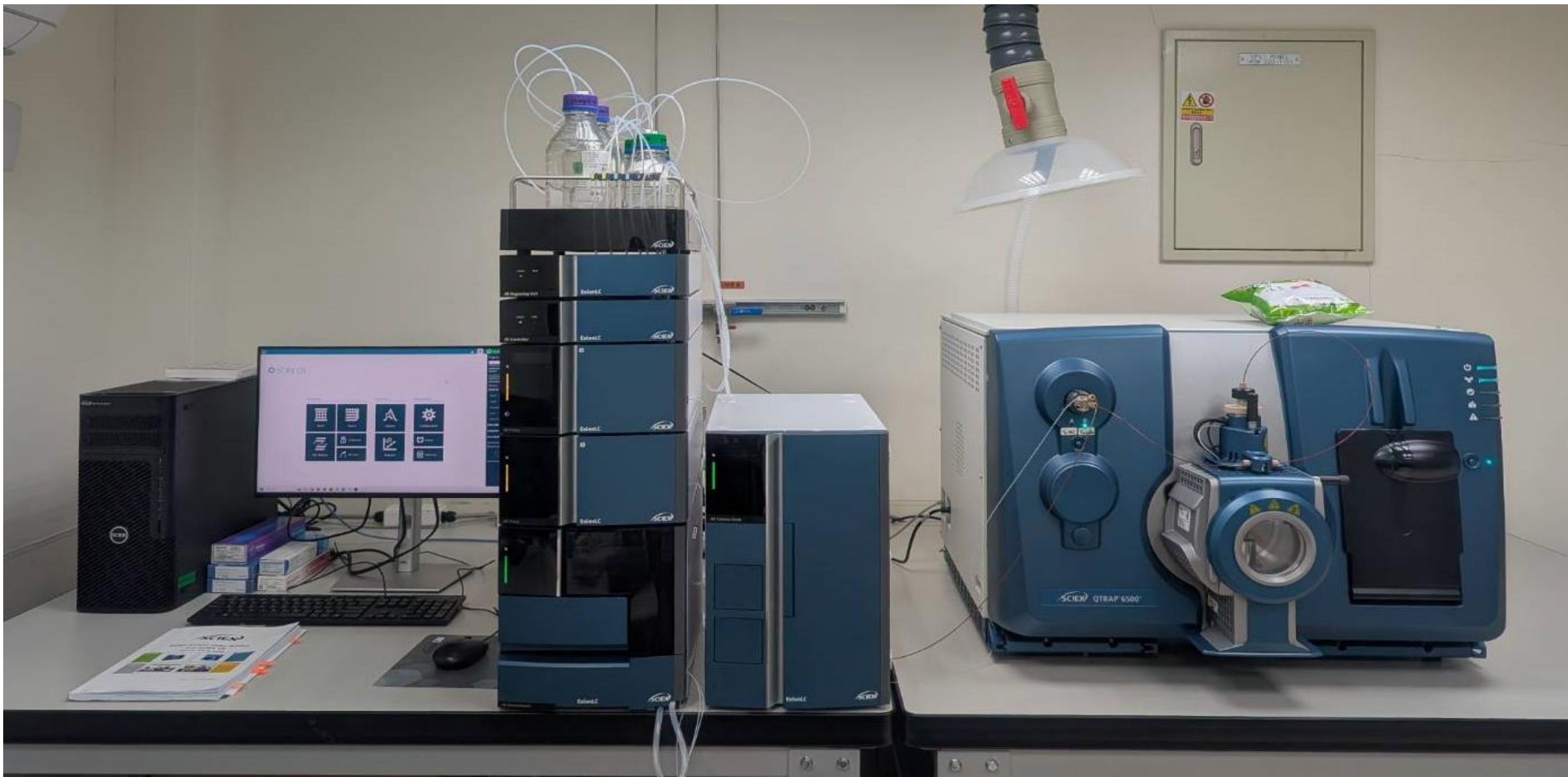


Figure 2. SCIEX ExionLC AE system coupled with QTRAP 6500+ mass spectrometer.



Results

1. LC-MS/MS Method Development

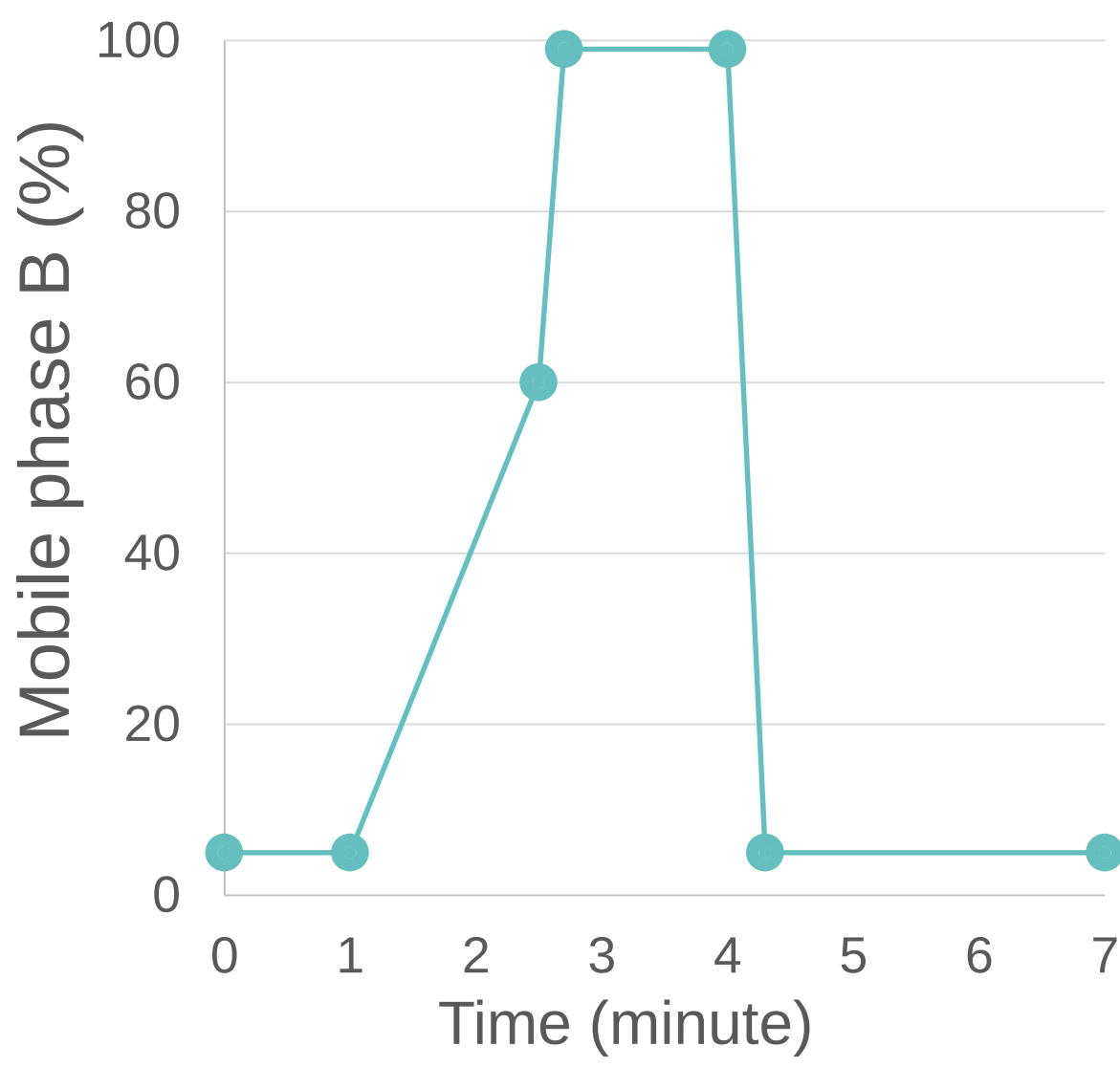
Table 1. Mass spectrometric parameters for 2,5-HD and internal standard 2,5-HD-d₁₀.

Name	Precursor ion	Product ion	DP (V)	EP (V)	CE (V)	CXP (V)
2,5-HD	115	97	20	10	14	10
		69	20	10	17	10
2,5-HD-d ₁₀	125	105	20	10	18	10
		62	20	10	18	10

Table 2. LC gradient.

Time (minute)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	95	5
1.0	95	5
2.5	40	60
2.7	1	99
4.0	1	99
4.3	95	5
7.0	95	5

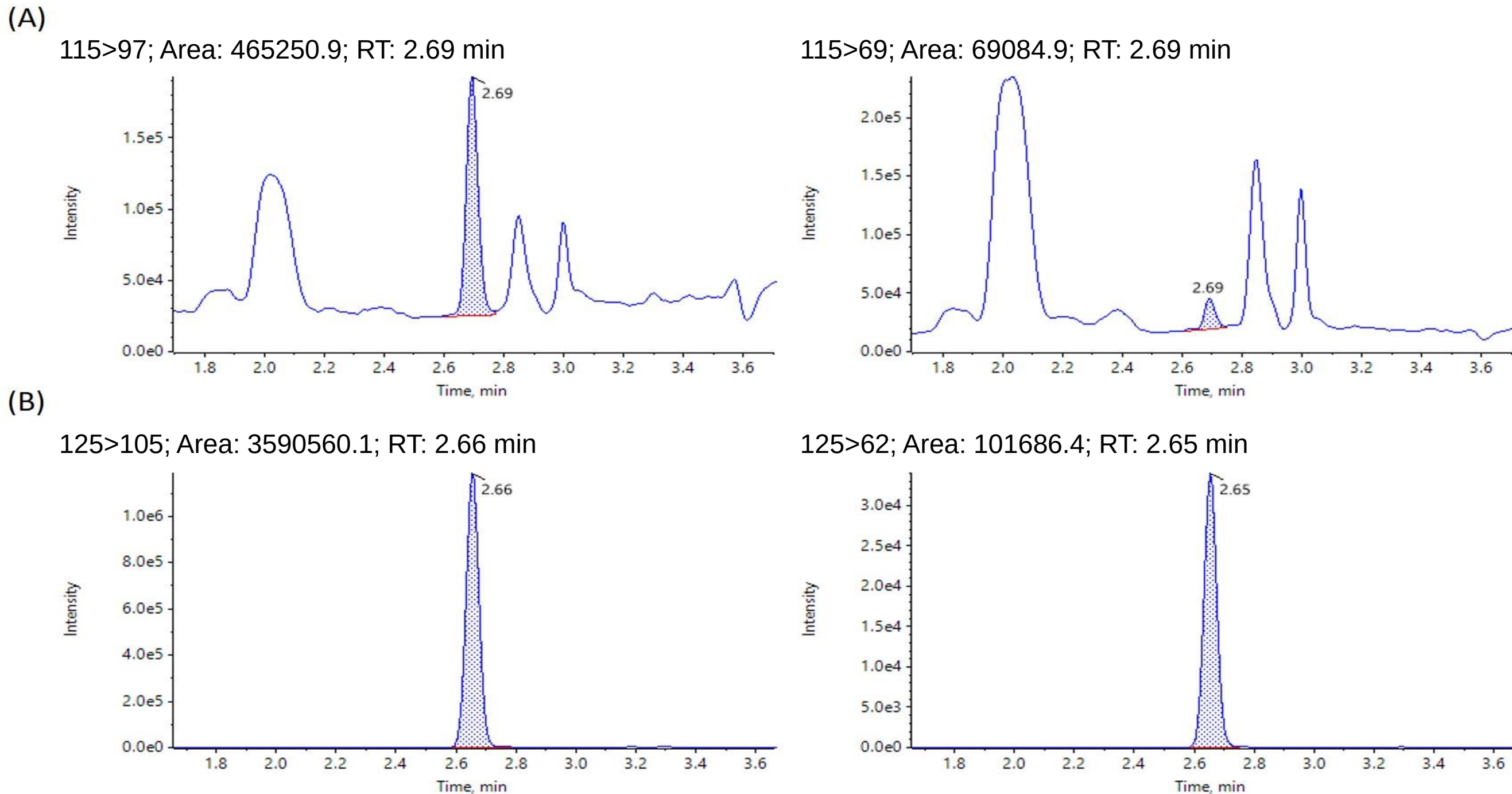
Figure 3. LC gradient curve.



Results

1. LC-MS/MS Method Development

Figure 4. Extracted ion chromatograms of (A) 2,5-HD and (B) 2,5-HD-d₁₀ in the LLOQ urine sample.



2. Method Validation

Figure 5. Calibration curve of 2,5-HD in human urine.

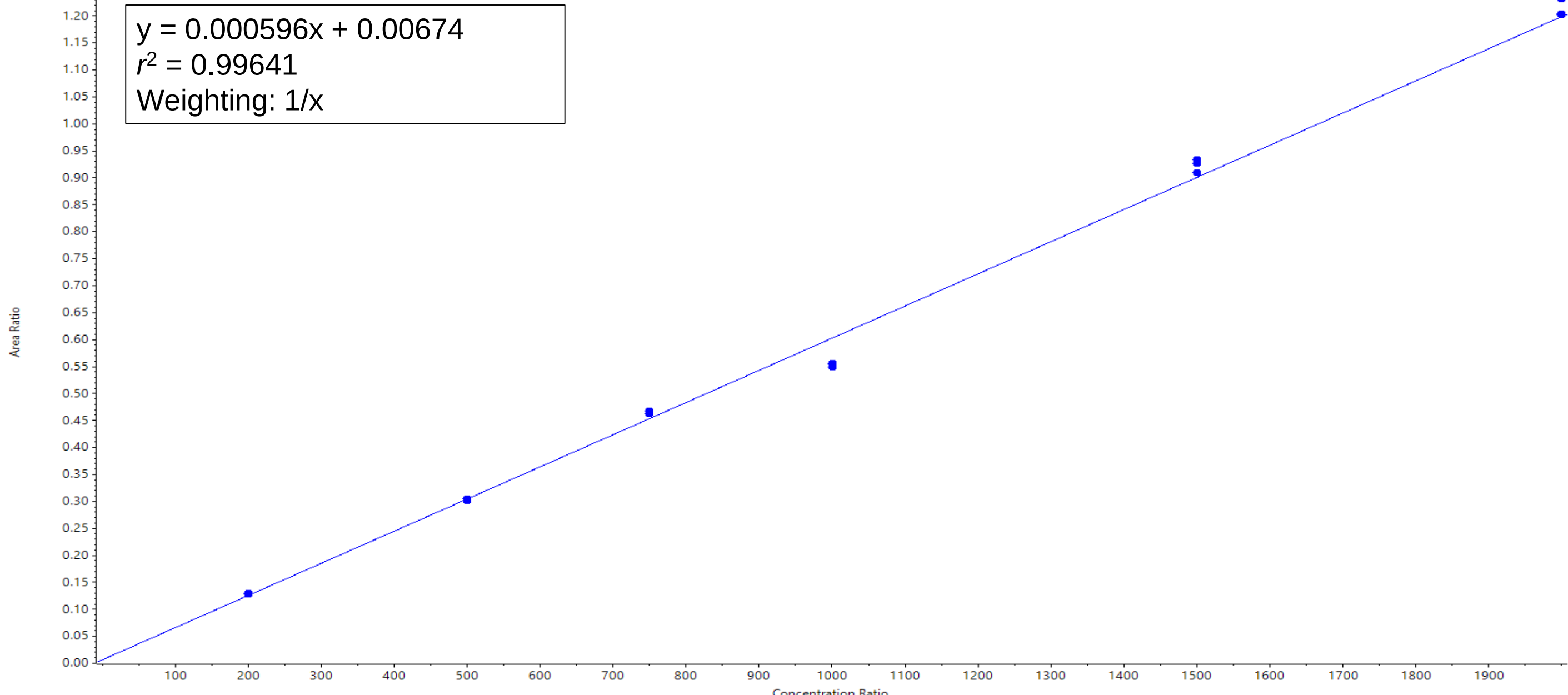


Table 3. Within-Run and between-Run accuracy and precision for 2,5-HD in urine.

Sample	Nominal Conc. (ng/mL)	Within-Run (n=5)			Between-Run (3 days, n=9)		
		Mean (ng/mL)	Accuracy (%)	CV (%)	Mean. (ng/mL)	Accuracy (%)	CV (%)
LLOQ	200	207.7	103.8	0.8	204.8	102.4	2.0
LQC	600	585.4	97.6	1.1	580.1	96.7	1.4
MQC	900	965.9	107.3	0.5	976.9	108.5	1.0
HQC	1600	1649.6	103.1	0.6	1625.9	101.6	1.6

LLOQ, lower limit of quantification; LQC, low quality control; MQC, medium quality control; HQC, high quality control; Conc., concentration.

Table 4. Accuracy and precision of 2,5-HD in six individual lots of urine.

Lot (n=3)	LQC				HQC			
	Nominal Conc. (ng/mL)	Mean (ng/mL)	Accuracy (%)	CV (%)	Nominal Conc. (ng/mL)	Mean (ng/mL)	Accuracy (%)	CV (%)
1	600	548.1	91.4	1.0	1600	1631.4	102.0	0.9
2		668.9	111.5	2.5		1665.4	104.1	1.3
3		614.5	102.4	2.9		1506.1	94.1	0.8
4		596.1	99.3	0.4		1445.0	90.3	0.2
5		643.4	107.2	0.1		1457.8	91.1	1.2
6		547.1	91.2	2.8		1596.9	99.8	0.8

Table 5. Matrix effect and recovery of 2,5-HD in urine

Sample	Nominal Conc. (ng/mL)	Matrix Effect (% , n=3)	Recovery (% , n=3)
LQC	600	107.2	97.9
HQC	1600	98.8	105.0

Conclusions

The developed LC-MS/MS method provides a sensitive, accurate, and high-throughput approach for the quantification of 2,5-HD in urine, with the limit of detection (LOD) and limit of quantitation (LOQ) at 100 ng/mL and 200 ng/mL, respectively. The calibration curve exhibited strong linearity over a range of 200–2000 ng/mL ($r^2 > 0.990$). All within-run and between-run accuracy and precision were well within the acceptable limits of the ICH M10 guideline. Consequently, this method is highly suitable for monitoring *n*-hexane exposure and holds significant potential for broader clinical applications.

References

- Torres, M. Edite, et al. "Alternative biomarkers of n-hexane exposure: characterization of aminoderived pyrroles and thiol-pyrrole conjugates in urine of rats exposed to 2, 5-hexanedione." *Toxicology Letters* 224.1 (2014): 54-63.
- Guideline, I. H. "Bioanalytical method validation M10." *European Medicines Agency: Amsterdam, The Netherlands* (2019).