Primary Hyperoxaluria Screening and Monitoring: Quantitative Measurement of Plasma Oxalate by Gas Chromatography-Mass Spectrometry With High Sensitivity

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Supplemental Data Table S1. GC-MS instrument control parameters

GC summary		**Front stainless steel He inlet**	
Run time	20.25 mins	Mode	Pulsed splitless
Oven		Pressure	8.2317 psi
Maximum temperature	325℃	Total flow	104 mL/min
Initial temperature	60℃	Septum purge flow	3 mL/min
Rate	25℃/min	Injection pulse pressure	25 psi until 0.5 min
Value	90℃	Purge flow to split vent	100 mL/min at l min
Automatic liquid sampler		**Column**	
Injection volume	1 µL	P-pressure	8.2317 psi
Solvent A volume	8 µL		
Solvent B volume	8 µL		
Dwell time (pre-injection)	0 min		
Dwell time (post-injection)	0.05 min		

Abbreviations: GC-MS, gas chromatography-mass spectrometry; psi, pounds per square inch.

Supplemental Data Table S2. Between-day precision of oxalate measurements in plasma specimens containing low, medium, or high concentrations of oxalate*

Sample concentration	Sample size, N	Mean, µmol/L	SD, µmol/L	CV, %
Low	25	3.2	0.52	16.3
Medium	25	36.4	0.81	2.2
High	25	65.8	0.66	1.0

*We determined the between-day precision of the assay using pooled plasma samples with three different oxalate concentrations. We measured the oxalate concentrations five times per day for five days (N=25 replicates).

Supplemental Data Table S3. Recovery of oxalate from spiked samples

Sample	Spiked oxalate concentration, µmol/L		Recovery, %*
	Targeted	Measured	
1[†]	2.0	1.8	90.0
2	10.0	10.5	105.0
3	20.0	19.2	96.0
4[‡]	5.0	5.5	110.0
5	20.0	21.4	107.0
6	40.0	38.6	96.5

*We assessed recovery using spiked samples. We prepared spiked samples by adding oxalate powder to normal QC material. The final oxalate concentrations in the spiked samples were expected to be 2, 5, 10, 20, and 40 µmol/L. We analyzed each spiked sample four times. The average oxalate concentration in the spiked samples represents the total concentration of endogenous oxalate in the normal QC material plus the spiked oxalate. We calculated the measured concentration of spiked oxalate by subtracting the endogenous oxalate concentration from the total concentration. We calculated the percent recovery using the measured and targeted concentrations of spiked oxalate.

[†]We prepared samples 1–3 by spiking oxalate into normal QC material with an endogenous oxalate concentration of 1.13 µmol/L.

[‡]We prepared samples 4–6 by spiking oxalate into normal QC material with an endogenous oxalate concentration of 1.9 µmol/L.

Supplemental Data Table S4. Interference of Hb, intralipids, and bilirubin with oxalate plasma measurements*

Sample	Concentration, μmol/L	Difference, %
Plasma (neat)	1.2	–
Plasma spiked with Hb (5.0 g/L)	1.3	108
Plasma spiked with intralipids (17.0 mmol/L)	3.0	250
Plasma spiked with bilirubin (500.0 μmol/L)	1.9	158

*We assessed interference due to hemolysis, lipemia, and icterus by analyzing plasma samples spiked with human Hb (5.0 g/L), intralipids (17.0 mmol/L), and total bilirubin (500.0 mol/L), respectively. We measured oxalate in neat and spiked samples in quadruplicate.

Supplemental Data Table S5. Effects of pH and anticoagulants on oxalate plasma measurements*

Sample	Oxalate concentration, µmol/L		
	Acidified heparin plasma	**Non-acidified heparin plasma**	**Non-acidified EDTA plasma**
1	2.1	8.4	9.2
2	2.0	9.1	9.3
3	1.6	8.2	9.0
4	0.8	6.8	5.9
5	1.1	6.4	5.2

*We collected five blood specimens and aliquoted each specimen into two tubes, one with EDTA preservative and the other with lithium heparin, immediately after collection. We stored all tubes in an ice-water bath at 4°C until the plasma was separated. We further divided the heparinized plasma aliquot of each specimen into two aliquots: one acidified with concentrated HCl to pH <2.0 and the other was left untreated. We did not acidify the EDTA plasma aliquots. We measured the oxalate concentrations of 15 samples.

Supplemental Data Table S6. Comparison of methods used to measure plasma oxalate concentrations

Sample	Oxalate concentration, µmol/L		Absolute difference, µmol/L	Relative difference, %
	GC-MS	Enzymatic assay		
1	5.3	0.0	5.3	200
2	5.5	1.7	3.8	106
3	6.9	0.0	6.9	200
4	20.0	23.1	–3.1	–14
5	22.2	21.4	0.8	4
6	24.8	26.6	–1.8	–7
7	37.6	46.7	–9.1	–22
8	42.3	46.1	–3.8	–9
9	47.3	59.4	–12.1	–23
10	54.0	67.4	–13.4	–22
11	59.8	77.6	–17.8	–26
12	65.4	80.3	–14.9	–20

We analyzed specimens from 12 patients with oxalate concentrations ranging between 0 and 80 mol/L using GC-MS. We sent aliquots of these specimens to another laboratory for testing using a traditional spectrophotometric assay based on the oxalate oxidase reaction, as reported previously [16].
Abbreviation: GC-MS, gas chromatography-mass spectrometry.