Long- And Short-Term Stability of Methylphenidate and Its Metabolites in Blood

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INTRODUCTION

Methylphenidate (MPH) is a common prescription medication that works to combat attention-deficit hyperactivity disorder (ADHD), but may also be abused recreationally. Though MPH has two chiral centers, *d*-threo-MPH is responsible for the pharmaceutical effects. Few studies have analyzed methylphenidate and its metabolites, ritalinic acid (RA) and ethylphenidate (EPH), in blood. Stability studies are crucial in a forensic setting to provide insight on ideal storage conditions and analysis time for forensic samples. This is the first method to our knowledge that analyzes the long-term stability of *d,l*-MPH, *d,l*-EPH and RA in blood.

MATERIALS & METHODS

Sample	• 250 µL whole blood • 25 µL internal standard solution
Preparation	• 1 mL 100 mM phosphate buffer (pH 6) • Centrifuge (2000 *rpm*); 10 min
Condition SPE	• 1 mL methanol • 1 mL 100 mM phosphate buffer (pH 6)
Load	• UCT Clean Screen DAU Columns (3 mL)
Wash	• 1 mL acetic acid (0.1 M) • 1 mL methanol
Elute	• 2 mL ammonium hydroxide (2%) in methanol (v/v)
Reconstitute	• Evaporate under nitrogen • Reconstitute in 100 µL of 2:98 mobile phase

Instrumentation
- Agilent 1290 Infinity II Liquid Chromatograph
- Agilent 6470 Triple Quadrupole MS

Column
- Agilent Poroshell Chiral-V (2.1 x 100 mm, 2.7 µm)

Mobile phase
- A: diH20
- B: 0.025% Ammonium acetate (w/v) + 0.0125% trifluoroacetic acid (v/v) in methanol

Flow rate
- 0.6 mL/min (isocratic, 2% A: 98% B)

RESULTS

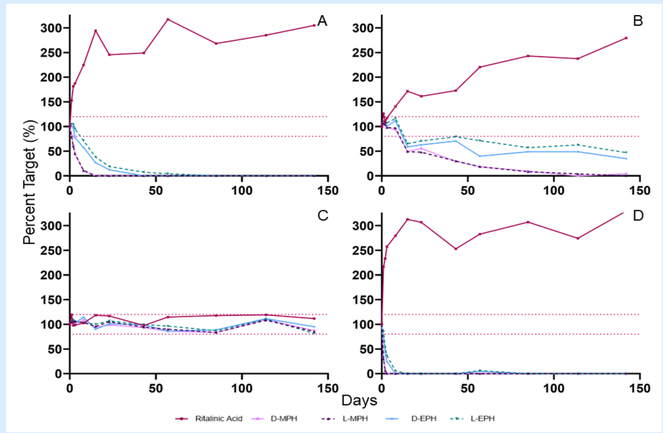

Figure 1. MPH, EPH and RA stability at the LQC (15 ng/mL) at room temperature (A), refrigerated temperature (B), frozen temperature (C) and elevated temperature (D).

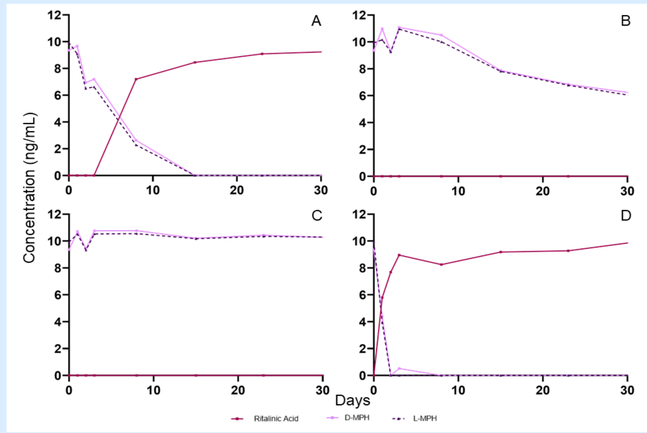

Figure 2. MPH and RA concentration vs time at the LQC (15 ng/mL MPH) at room temperature (A), refrigerated temperature (B), frozen temperature (C) and elevated temperature (D).

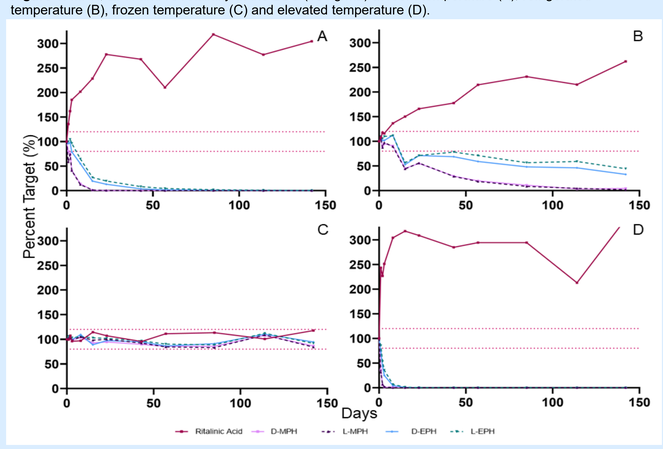

Figure 3. MPH, EPH and RA stability at the HQC (150 ng/mL) at room temperature (A), refrigerated temperature (B), frozen temperature (C) and elevated temperature (D).

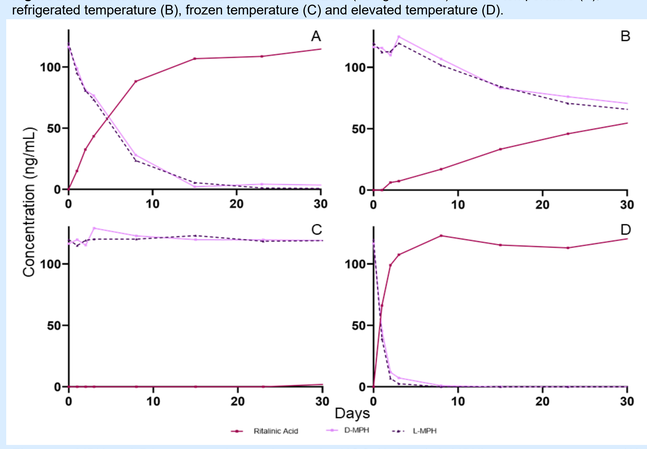

Figure 4. MPH and RA concentration vs time at the HQC (150 ng/mL MPH) at room temperature (A), refrigerated temperature (B), frozen temperature (C) and elevated temperature (D).

Table 1. Summary of data for MPH, EPH and RA stability at the LQC (15 ng/mL) and HQC (150 ng/mL).

	Analyte Change % Loss/Increase (Timepoint deemed unstable)[1]							
	Room temp. (~25°C)		Refrigerated (4°C)		Frozen (-20°C)		Elevated (35°C)	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
***d*-MPH**	-18.1 (24h)	-20.1 (24h)	-50.5 (2wk)	-54.8 (2wk)	-16.7 (5mo)	-14.3 (5mo)	-66.1 (24h)	-64.1 (24h)
***l*-MPH**	-20.6 (24h)	-41.5 (24h)	-50.9 (2wk)	-56.0 (2wk)	-16.4 (5mo)	-16.9 (5mo)	-70.7 (24h)	-68.7 (24h)
***d*-EPH**	-22.3 (48h)	-21.0 (48h)	-41.4 (2wk)	-47.1 (2wk)	-13.6 (5mo)	-13.4 (5mo)	-26.7 (24h)	-27.2 (24h)
***l*-EPH**	-28.8 (72h)	-35.4 (72h)	-34.8 (2wk)	-53.5 (2wk)	-18.5 (5mo)	-12.1 (5mo)	-47.5 (48h)	-46.8 (48h)
RA	+52.8 (24h)	+35.8 (24h)	+40.8 (1wk)	+36.6 (1wk)	+18.5 (5mo)	+17.7 (5mo)	+116.7 (24h)	+143.5 (24h)

[1]Data are displayed as %difference from 100% with the corresponding timepoint at which the analyte was deemed unstable.

Table 2. Summary of data for MPH only at the LQC (15 ng/mL) and HQC (150 ng/mL).

	Analyte Change % Loss/Increase (Timepoint deemed unstable)[2]							
	Room temp. (~25°C)		Refrigerated (4°C)		Frozen (-20°C)		Elevated (35°C)	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
***d*-MPH**	-25.9 (48h)	-30.4 (48h)	-26.9 (2wk)	-28.7 (1wk)	+6.5 (1mo)	+1.3 (1mo)	-53.1 (24h)	-61.1 (24h)
***l*-MPH**	-34.5 (48h)	-32.6 (48h)	-29.6 (1wk)	-29.6 (1wk)	+2.8 (6mo)	+0.8 (1mo)	-60.3 (24h)	-67.4 (24h)
RA	+7.2 (1wk)	+15 (24h)	-	+6.2 (48h)	-	-	+7.2 (1wk)	+15 (24h)

[2]For MPH, data are displayed as %difference from 100% with the corresponding timepoint at which the analyte was deemed unstable. For RA, data are displayed as a %difference from 0% due to no RA being present in the T_0 samples. The timepoint at which RA became quantifiable is indicated.

MATERIALS & METHODS

Blood with sodium fluoride and potassium oxalate preservatives was fortified with *d,l*-MPH, *d,l*-EPH and RA at 15ng/mL and 150ng/mL. Analytes quantified with validated method.

Storage Conditions:
- Room temperature (25°C)
- Refrigerated (4°C)
- Frozen (-20°C)
- Elevated (35°C)

Time points (n=3):
- 24h, 48h, 72h, 1wk, 2wk, 3wk, 4wk, 6wk, 2mo, 3mo, 4mo, 5mo, 6mo, 7mo, 8mo, 9mo

CONCLUSIONS

- Results indicate that -20°C is the optimal storage temperature for these analytes in blood as they remained stable for up to 9 months.
- Methylphenidate degrades to ritalinic acid under non-frozen conditions.
- This study displays the importance of stability studies, gives useful information on storage conditions, and proves that quantitative values may be inaccurate due to breakdown products.

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REFERENCE

Smith CR and Swortwood MJ. Short- and Long-Term Stability of Methylphenidate and Its metabolites in Blood. *Journal of Analytical Toxicology* (2021). https://doi.org/10.1093/jat/bkab063.