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Overview

PURPOSE

Compounds containing thiol moieties can undergo auto-oxidation in biological matrices. This research demonstrates the importance of evaluating multiple blood donors when establishing the stability characteristics of reactive functionalities.

METHOD

Captopril was fortified in commercial and freshly collected human blood (K₂EDTA) at concentrations of 30 ng/mL and 2250 ng/mL for various timepoints. Samples were gently mixed, then plasma was generated and captopril derivatized with N-Ethylmaleimide (NEM) to prevent further degradation. Samples were extracted by protein precipitation and analyzed by LC-MS/MS in positive ion mode.

RESULTS

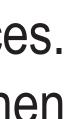
We investigated two blood sources, commercial and freshly collected, since enzymatic expression and activity may differ. The study demonstrates that when a drug is unstable in matrix, multiple donors of blood should be tested to determine optimal collection procedures.

INTRODUCTION

Captopril is an angiotensin-converting enzyme (ACE) inhibitor widely used in the treatment Commercial and freshly collected human blood (K2EDTA) was fortified at 30 ng/mL and of hypertension. Since captopril contains a sulfhydryl functional-group, it is susceptible to 2250 ng/mL with captopril, gently mixed and placed in an ice/water bath for the stability auto-oxidation and/or ion-catalyzed-oxidation in biological matrices. While quantitation of duration time (t_0 , t_5 , t_{10} , t_{15} and t_{30} minutes). such reactive compounds in blood presents a challenge, stability may be conferred by fortification with antioxidants, such as ascorbic acid.

Due to differing levels of enzymatic expression and activity, compound stabilities can differ precipitated using MeOH. between donors and as a function of blood source (i.e. commercial vs. freshly collected). Therefore, in the current investigation, captopril degradation is characterized in blood from multiple donors derived both commercially and freshly harvested to demonstrate the Captopril Disulfide conversion evaluation: necessity of mimicking actual sample composition when optimizing stabilization conditions Captopril disulfide conversion to captopril was evaluated at low (ratio 1:75) and high QC for collection in the clinic. (ratio 1:1) concentrations. Each sample was processed as captopril preparation.

Stability Evaluation of Captopril in Human Blood by LC-MS/MS



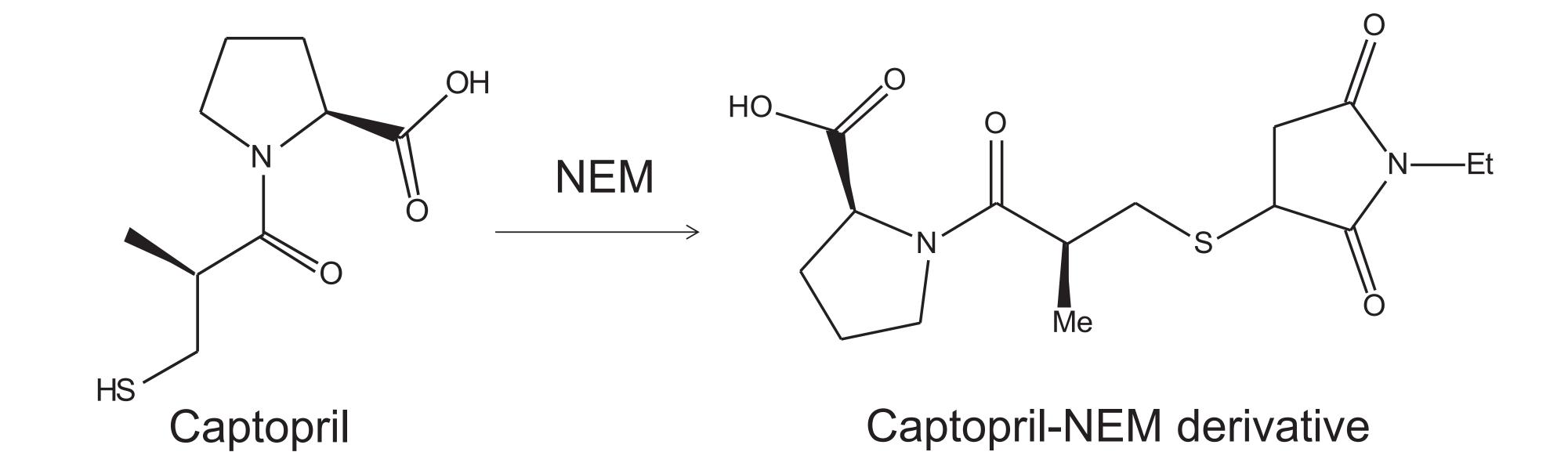


Figure 1. Structures of captopril and captopril-NEM-derivative.

Figure 2. Structure of captopril disulfide.

METHODS

SAMPLE PROCESSING

At each timepoint, plasma was generated and captopril was derivatized with 10% of 1M N-Ethylmaleimide (NEM). A 100 µL aliquot of sample was spiked with captopril-d3 NEM and

Methods CHROMATOGRAPHY

- Analytical column: Zorbax SB-C18, 50 X 2.1 mm, 5 µm
- Gradient elution using formic acid and MeOH

DETECTION

- SCIEX API 5000 using TurbolonSpray.
- MRM acquisition ESI(+):
- Captopril NEM: *m/z* 343.1 > *m/z* 228.1
- Captopril-d3 NEM: m/z 346.1 > m/z 228.1

RESULTS

Captopril stability was tested at two concentration levels up to t_{30} minutes, in two commercial and three freshly collected blood donors (Figure 3). Of the two commercial blood donors, captopril response was well within acceptance criteria ($\leq 15\%$ relative to t₀). However, incubation in freshly collected blood demonstrated a captopril response within acceptance criteria up to t₃₀ minutes for only one donor, while stability in the second donor was only obtained up to t_{15} . Stability in a third donor was achieved up to t_{10} with 25% and 18% degradation noted at t_{30} for low and high concentration levels, respectively (Figure 4).

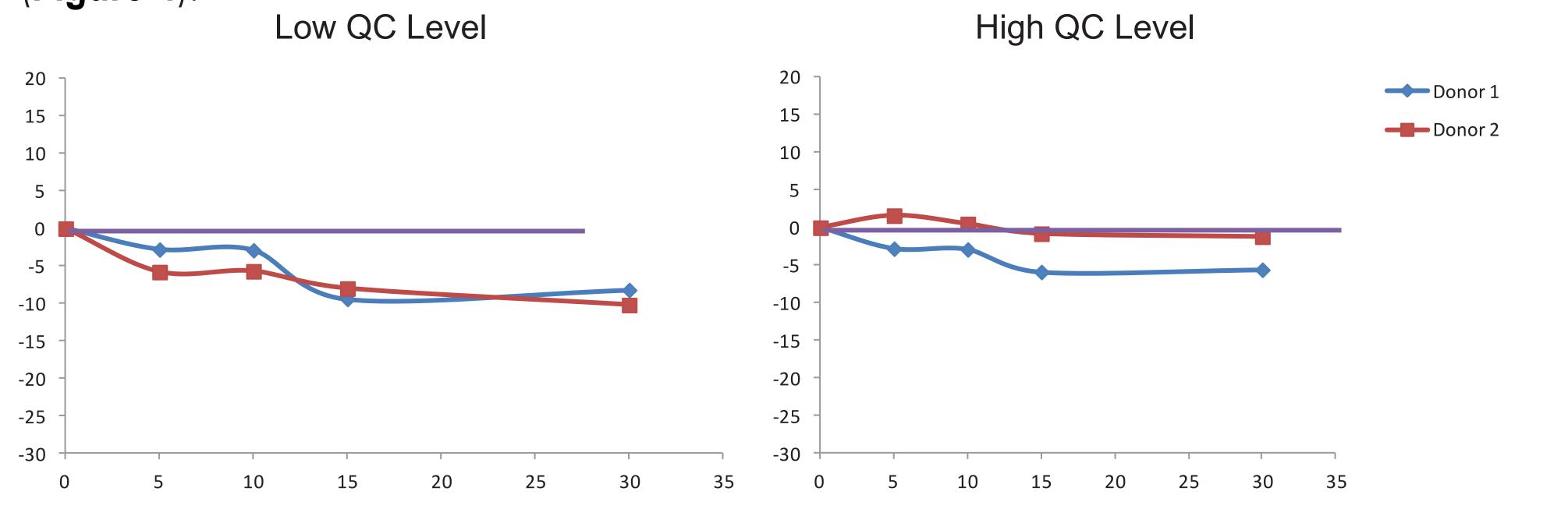


Figure 3. Captopril stability evaluation over time in two sources of commercially available blood at low and high concentration.

Captopril stability was also evaluated in the presence of potentially interfering metabolite captopril disulfide. As demonstrated in Figure 5, Donor 1 concentration increased over the acceptance limit of 15% for the high concentration sample within 15 minutes. At low concentration, the rate of degradation in Donor 3 was similar to that observed in the absence of disulfide metabolite. Accordingly, a 10 minutes time limit for the harvesting of plasma from freshly collected blood sample was defined for the captopril clinical study.

Results from the stability assessment of captopril were used in defining a 10 minutes time limit for the harvesting of plasma from freshly collected blood samples. Further, the study demonstrates that when drug or metabolite instability is expected in matrix, multiple donors from freshly collected blood should be assessed to determine the optimal collection strategy in order to minimize adulteration of *ex-vivo* drug concentration.

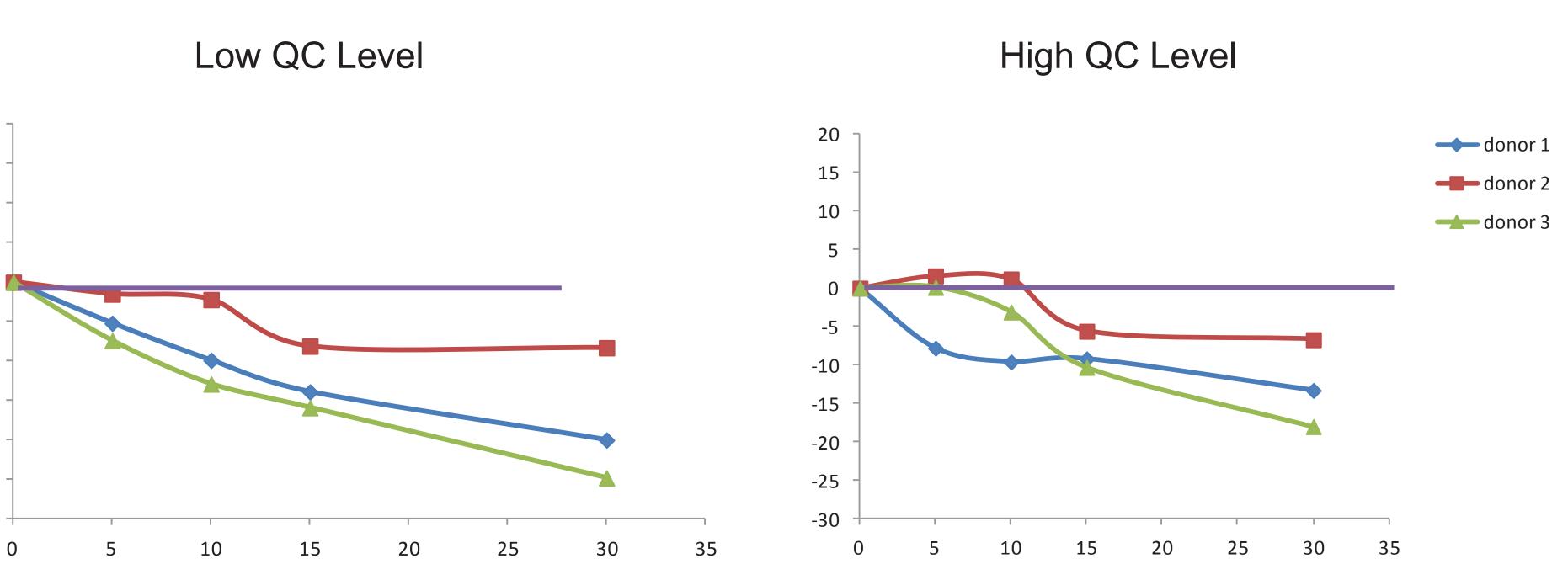
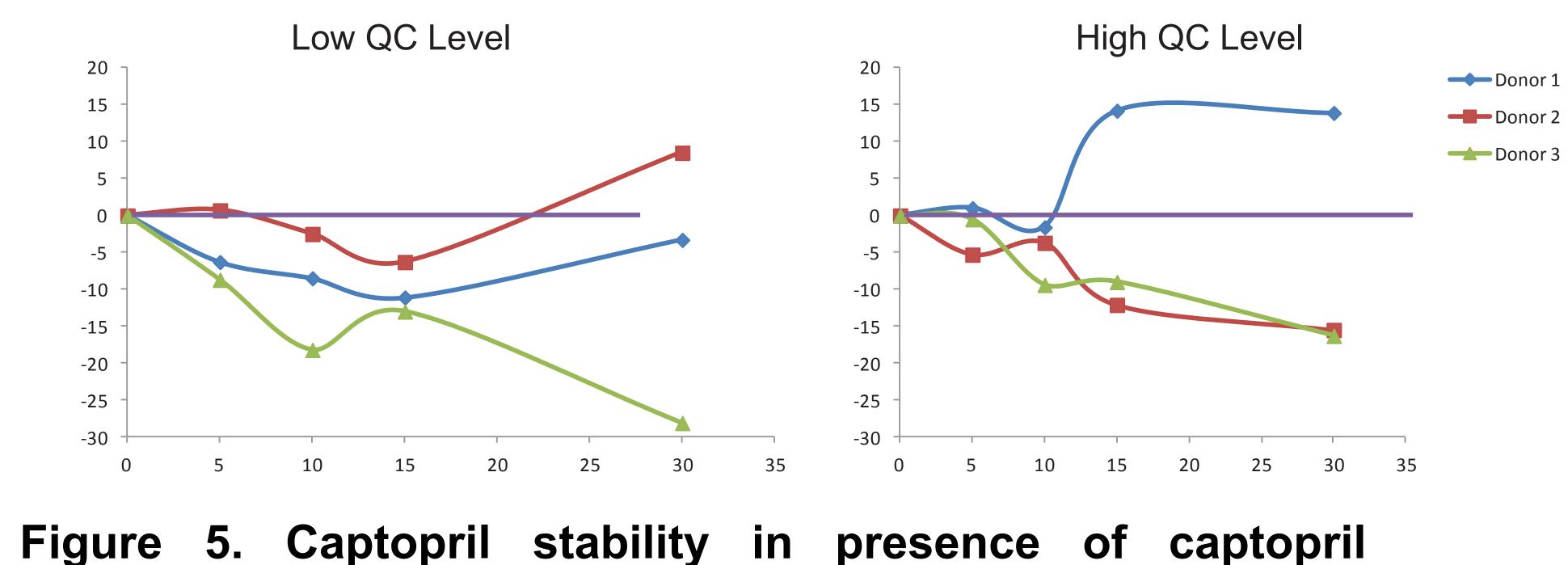


Figure 4. Captopril stability evaluation over time in three freshly collected blood donors, at low and high concentration.



disulfide metabolite at low and high concentration in freshly collected blood.

Conclusion

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