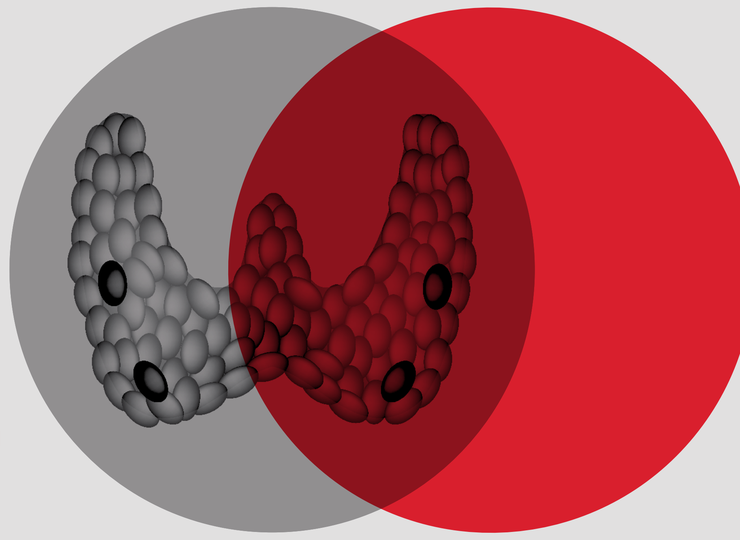
Stabilising PTH within RCPAQAP Materials: Exploring the Efficacy of Mg^{2+}, Dextran, and Triton X-100



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Introduction

The stability of external quality assurance (EQA) materials is a pillar for meaningful interpretation of both inter- & intra- laboratory performance. As stability declines, both the accuracy and precision of results becomes increasingly dependent on the time of measurement and, if not accounted for, can compromise data integrity. Whilst the 2019 RCPAQAP Endocrine material shows good stability for the majority of its analytes, the median CV for PTH measurements during the first half of the year was 54%. EDTA, protease inhibitors, and manufacturing techniques already contribute to maximizing the preservation of PTH, however it remains a challenge to mediate the chemical activity of all hormones and proteins within the material. Literature suggests that select divalent metal cations[1], polyanions[2], and non-ionic detergents[3] can aid in stabilizing the PTH molecule. Here, the effects of Mg^{2+}, Dextran, and TritonX-100 are explored.

Method

A series of $MgCl_2$ solutions were developed with concentrations of $4e^{-7}$mM, 30mM, and 120mM. In addition, two unique solutions of 120mM $MgCl_2$ were spiked to produce respective TritonX-100 (0.1%) and Dextran (0.169g/L) mixtures. To isolate the effect of Dextran, a discrete 0.169g/L solution was also prepared. Each of these 6 diluents were used to reconstitute both a high and low level vial of lyophilized RCPAQAP 2019 Endocrine material (i.e. high and low concentration PTH respectively). The samples were then left to stand for 20 minutes before PTH was measured (t_{20} = Initial Recovery Time) via. Immunochemiluminometric Assay on the Abbott Architect i2000 at Royal North Shore Hospital. Additional measurements were made at the 40 and 60 minute mark to chart stability.

Results

Initial recovery and stability data for all diluents are tabulated for both **Low Level PTH** (Table 1) and **High Level PTH** (Table 2). These result tables are in ascending order of loss rate (i.e. ranking diluents by their stabilizing effect). Comparing stability, Figures 1 & 2 depict Low & High Level PTH concentration as a percent over time. To regard the effect of PTH concentration on stability (e.g. kinetics), Figure 3 plots initial recovery against rate of loss for each diluent and level.

Table 1. Low Level PTH – Results.

Diluent	Initial Recovery (pg L^{-1})	Rate of Loss %* (min^{-1})
4	72	0.20%
5	43	0.42%
2	270	0.62%
3	258	0.65%
1	309	0.70%
6	144	1.55%

*Average

Figure 1. Low Level PTH – Stability.

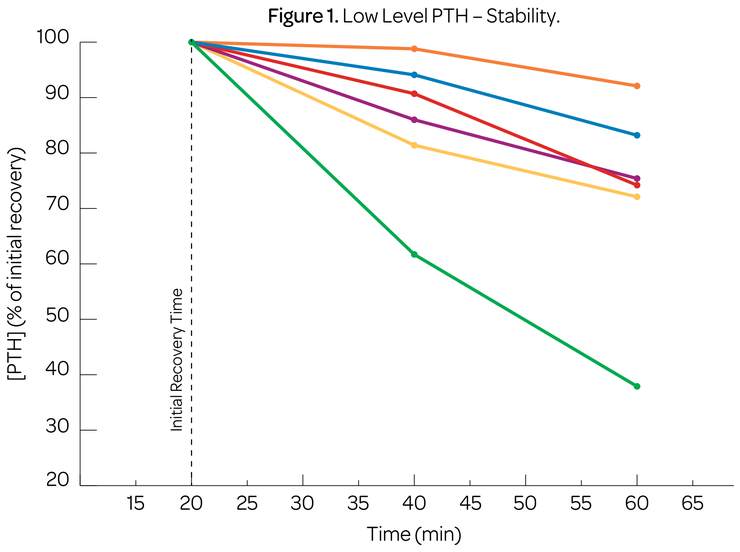


Table 2. High Level PTH – Results.

Diluent	Initial Recovery (pg L^{-1})	Rate of Loss %* (min^{-1})
2	463	1.26%
1	474	1.32%
3	451	1.35%
4	331	1.79%
6	258	1.83%
5	230	1.96%

*Average

Figure 2. High Level PTH – Stability.

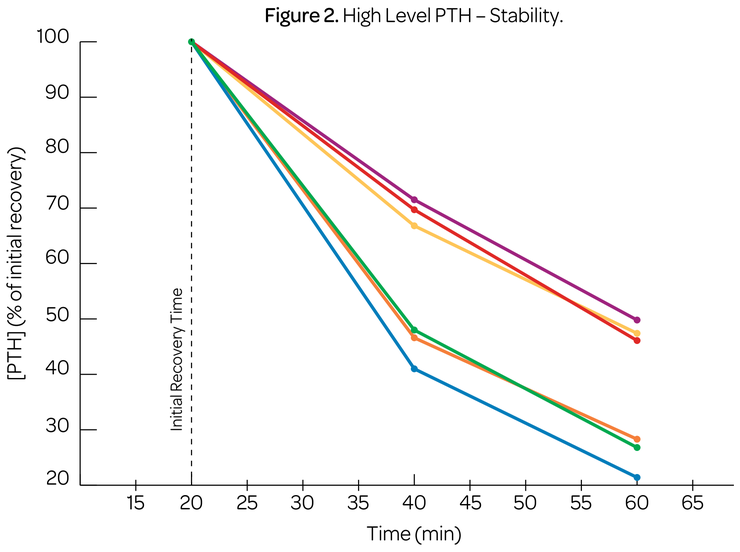
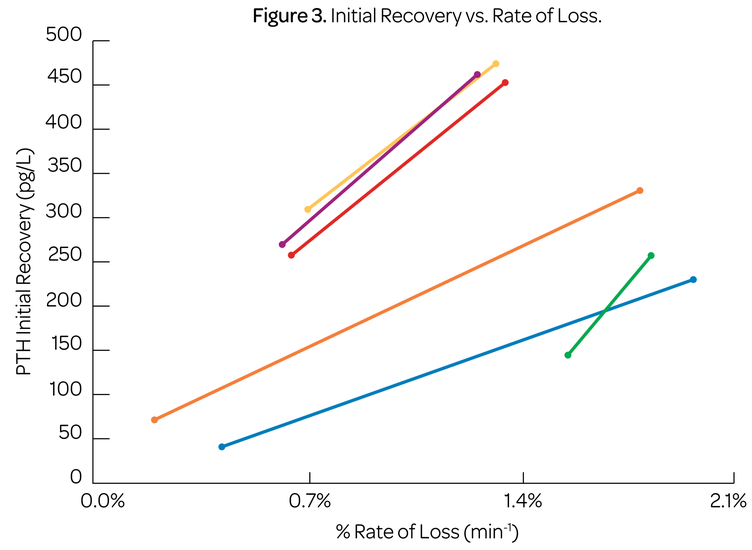


Figure 3. Initial Recovery vs. Rate of Loss.



Discussion and Conclusion

There appears to be a proportional relationship between PTH concentration and its rate of loss, demonstrated by the increasing gradient from Figure 1 to 2, as well as correlation data in Figure 3. Comparing Diluents 4 & 5 (two lowest $MgCl_2$ concentrations of 30mM and $4e^{-7}$ mM respectively), it does seem that the higher $MgCl_2$ diluent tempers these kinetics (both higher recovery and lower rate of loss across both levels). However, this effect isn't observed for the highest $MgCl_2$ diluent (120mM for Diluent 3), suggesting a threshold between the stabilising effect of $MgCl_2$ and the kinetics of PTH loss. Diluents 4, 5, and 6 are not satisfactory due to their substantial rate of loss for the high level. Of the remaining mixtures (Diluents 1-3; all containing a base of 120mM $MgCl_2$), spiking with 0.169g/L Dextran (Diluent 2) appears to provide the greatest benefit to stabilising PTH across low and high levels. Further investigation with Dextran and Triton spikes for lower $MgCl_2$ concentrations (i.e. 30mM) could provide clarity, as the similar recoveries across Diluents 1-3 may either suggest yields representative of the true PTH concentration, or that recovery is primarily influenced by $MgCl_2$.

References

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