# Measuring Cortisone Concentrations in Human Hair

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Introduction

Cortisol, a hormone produced by the adrenal glands is widely accepted as the primary stress hormone. Cortisol and its metabolite cortisone are important biomarkers of stress in the human body. Stress-related biomarkers can provide objective indicators of the impact of psychological stress on the body's stress response system, both the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system[1]. Cortisol and cortisone are downstream factors resulting from the activation of the HPA axis, with cortisol being converted to inactive cortisone[2].

Figure 1. Chemical structures of cortisol (A, on the left) and cortisone (B, on the right)
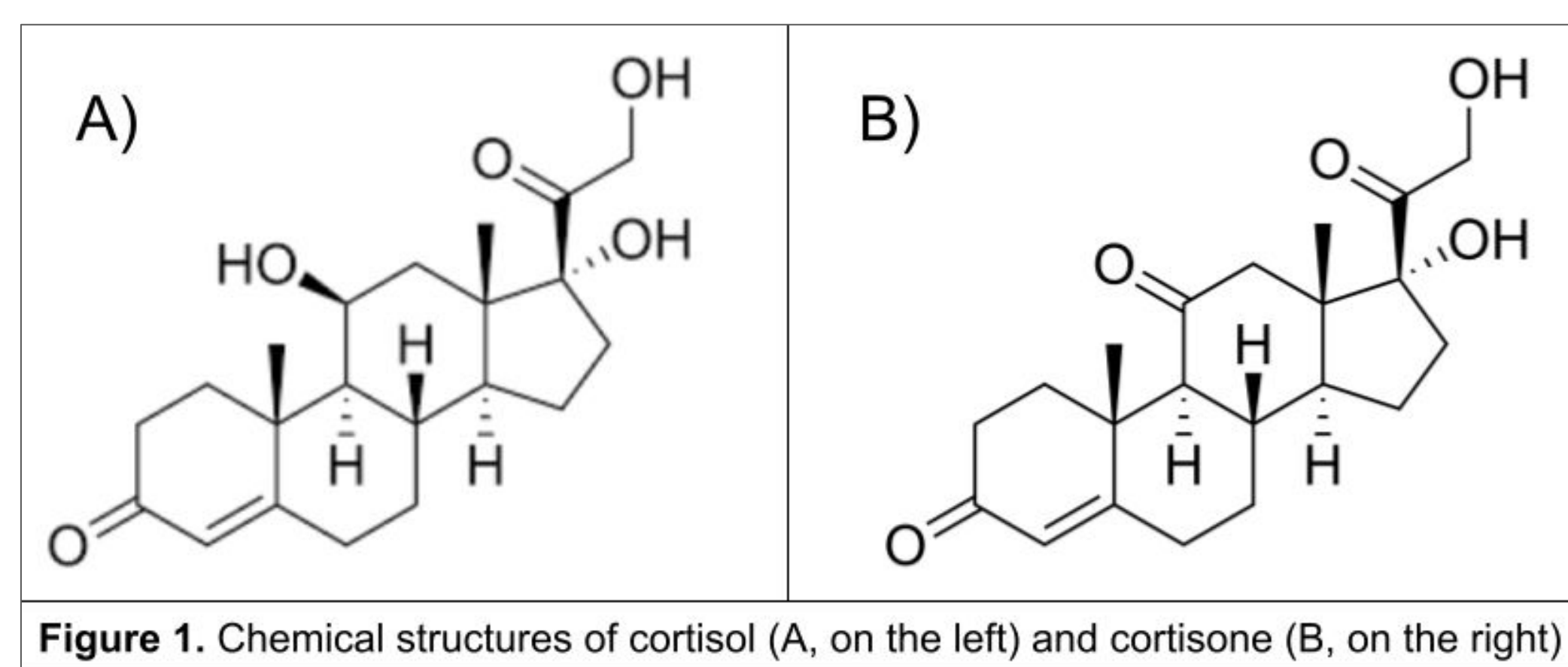
Cortisol is converted into inactive cortisone by the enzyme 11-beta-hydroxysteroid dehydrogenase (11β-HSD) type-2, with cortisone being converted back into active cortisol by the enzyme 11β-HSD type 1[3]. Cortisol and cortisone can be found in plasma, saliva, urine, hair, and nails. Cortisol and cortisone samples in plasma, saliva, and urine are affected by the "sleep-wakefulness" cycle (diurnal cycle) of the body, typically require special storage conditions, and are sensitive to transient fluctuations in HPA axis activity[4]. Hair samples for cortisol and cortisone, on the other hand, do not require the same storage concerns and are not affected by the diurnal rhythm[5]. Due to the more stable nature of hair and nails, they were chosen as the preferred sample medium for this research with the main focus to the time being on hair. This research is only a part of a much larger study. The goal of the summer was to refine the skills, methods, and procedures for measuring cortisone in hair.

Acknowledgements

Methods

- Cortisone extraction in 1 mL methanol, two separate overnight extractions followed by an acetone chase
- Arbor Assay cortisone ELISA kit procedure
- Modified conditions for cortisone testing:
  - Double the starting amount of hair used for extraction
  - Modify cortisone standard curve
  - Reconstitute with 0.20 mL instead of 0.25 mL Assay Buffer
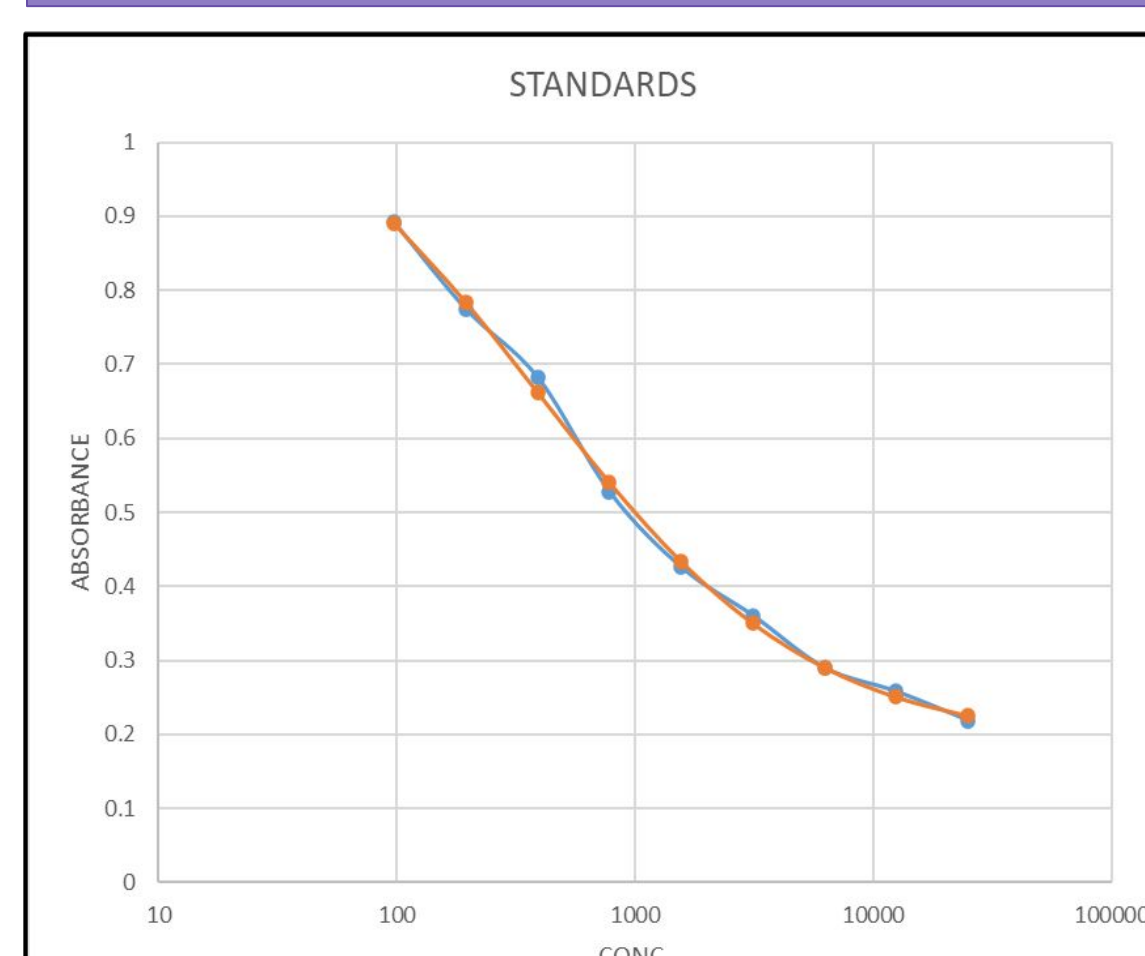
Results

Figure 2. Standard curve generated from the standards from Experiment O results.
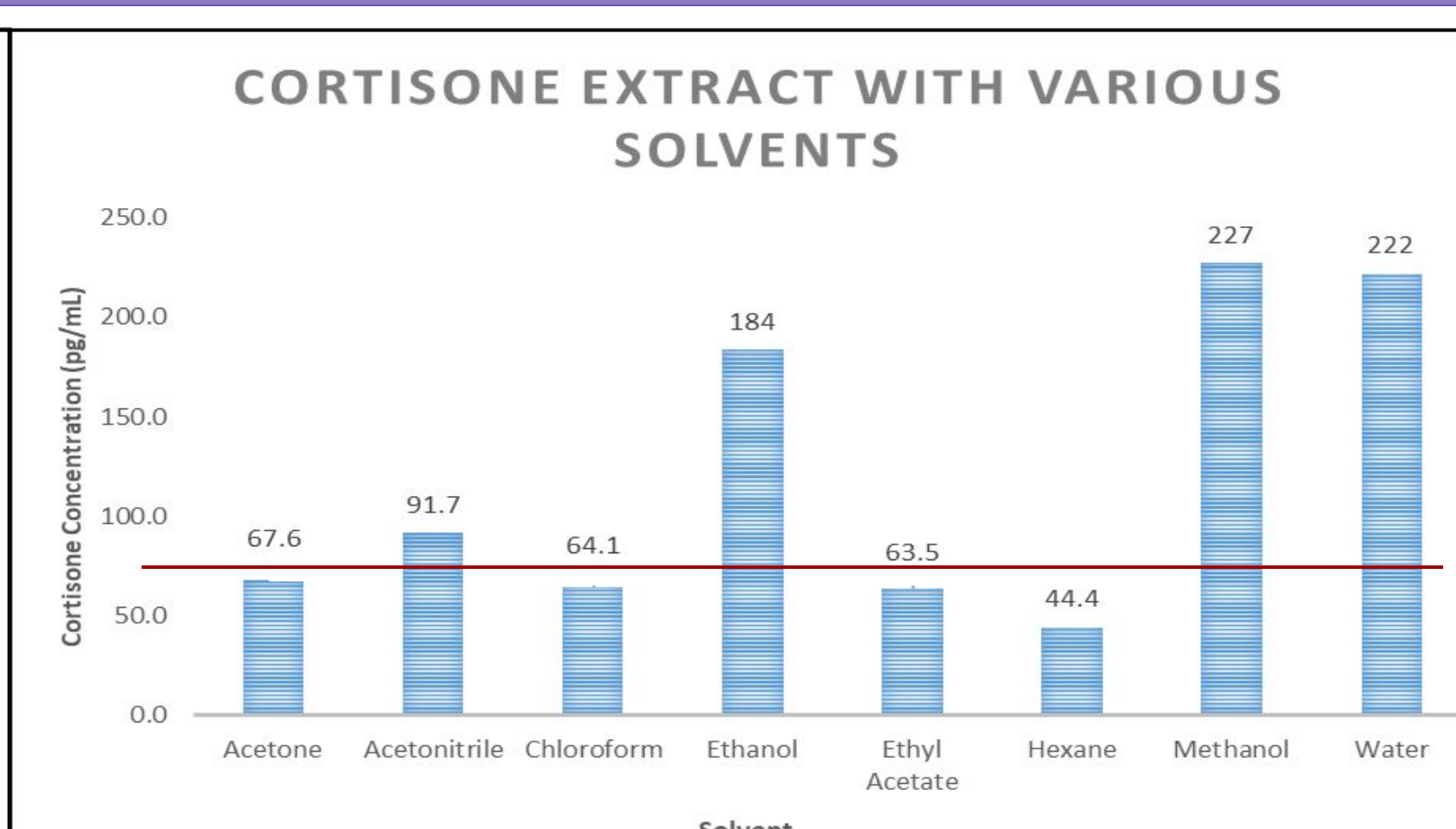

Figure 3. Cortisone extraction concentrations in pg/mL from various solvents (Experiment O). Red line indicates the limit of detection calculated from the standards in the experiment.
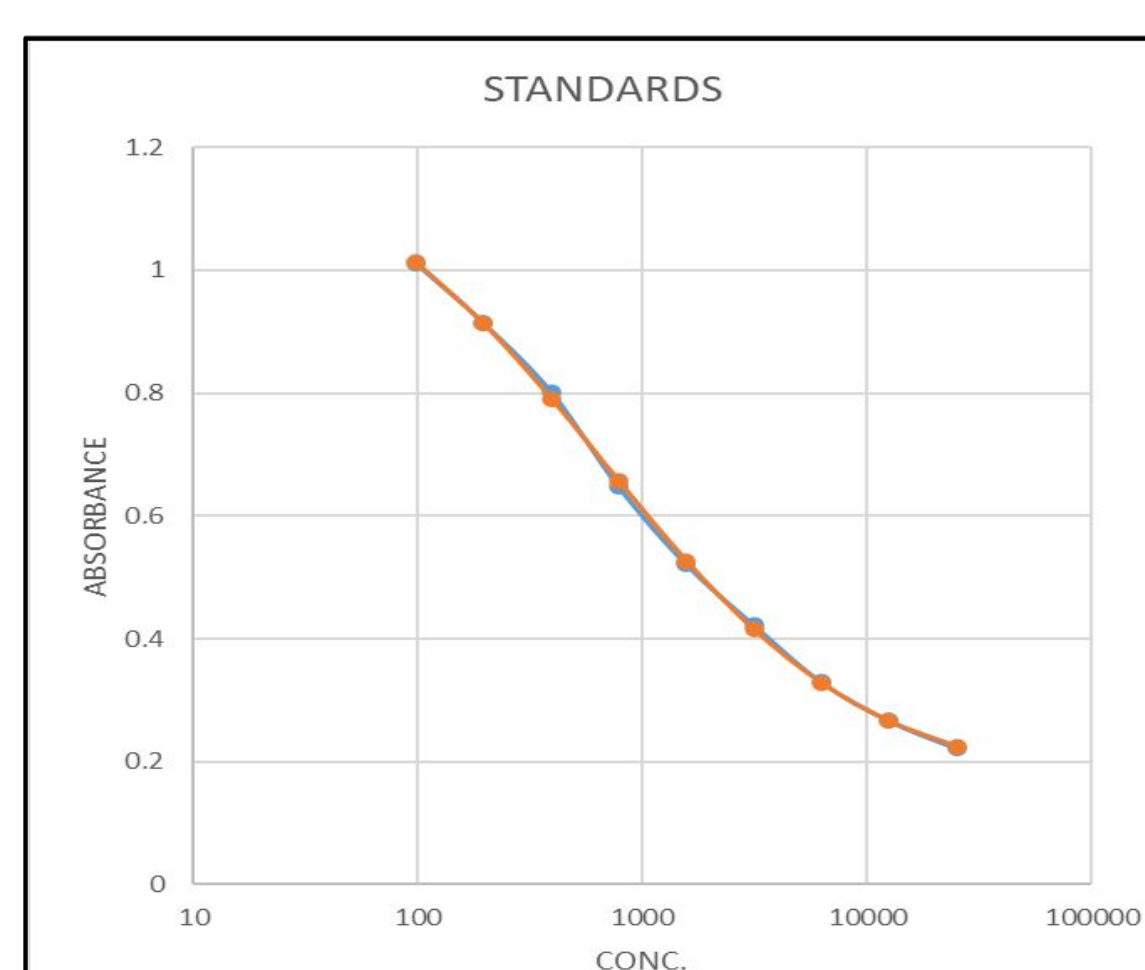

Figure 4. Standard curve generated from the standards from Experiment P results.
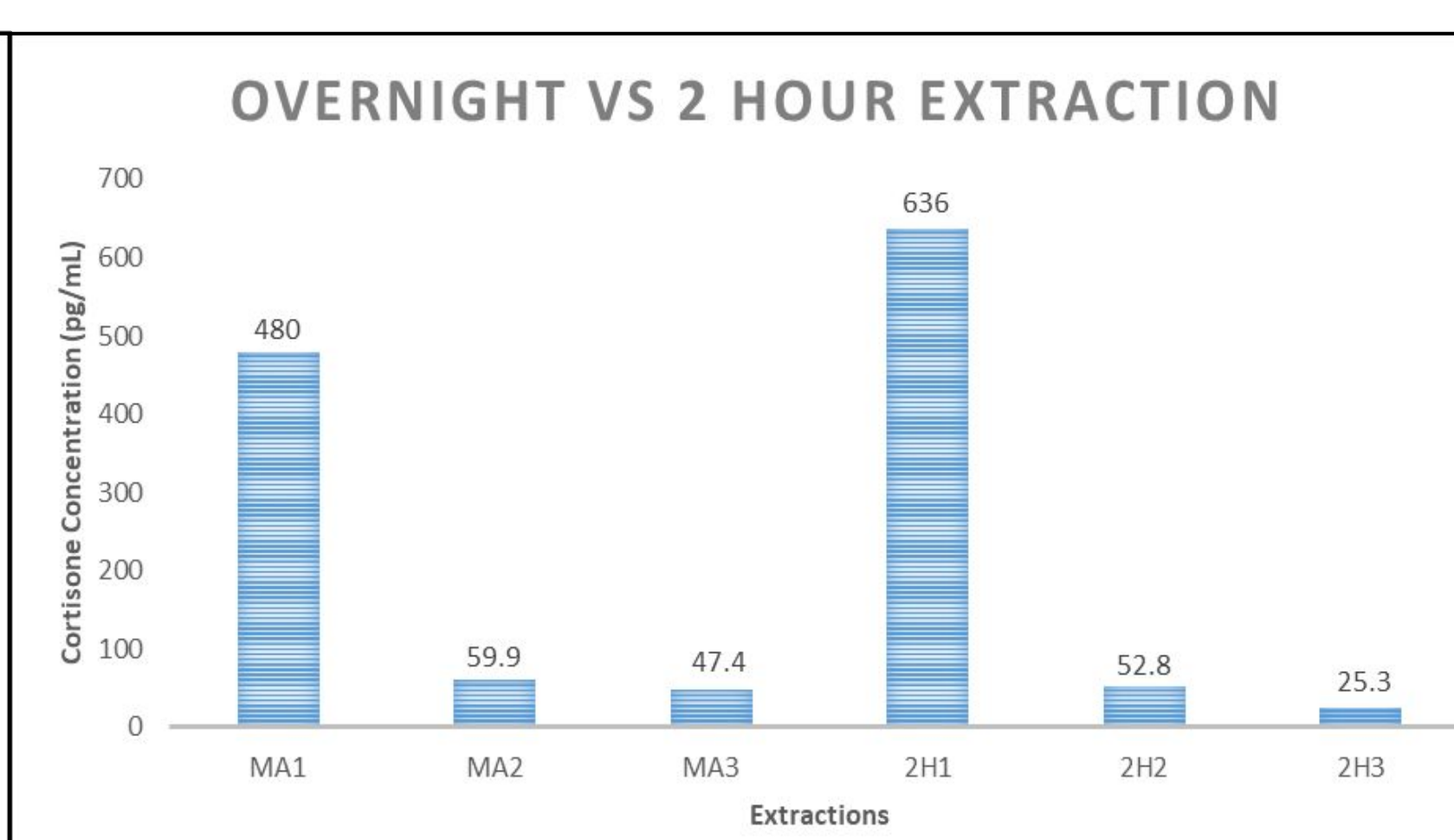

Figure 5. Cortisone extraction concentrations in pg/mL from comparing three consecutive overnight extractions (MA1, MA2, MA3) and three consecutive 2-hour extractions (2H1, 2H2, 2H3).

Future Work

Moving forward, validation experiments for linearity, parallelism, and reproducibility will be conducted to validate this procedure as an effective method and reliable method. Once validated it would be reasonable to start the process of collaborating with other researchers and universities that have samples they would like measured for cortisone in addition to cortisol that the lab currently measures. Additionally, more experiments should be done to further test the ability to replace overnight extractions with two-hour extractions. If two-hour extractions can be used the processing time for the sample would be decreased dramatically. Another future goal is to optimize and validate the same method for measuring cortisone in nail samples.

Calculations

	1	2	3	4	5	6
A	25000	6250	1562.5	390.63	97.66	Blank
B	25000	6250	1562.5	390.63	97.66	Blank
C	25000	6250	1562.5	390.63	97.66	Blank
D	25000	6250	1562.5	390.63	97.66	Blank
E	12500	3125	781.25	195.31	Blank	Blank
F	12500	3125	781.25	195.31	Blank	Blank
G	12500	3125	781.25	195.31	Blank	Blank
H	12500	3125	781.25	195.31	Blank	Blank

	1	2	3	4	5	6
A	0.205	0.29	0.421	0.681	0.902	1.015
B	0.221	0.277	0.419	0.701	0.873	0.99
C	0.223	0.309	0.424	0.673	0.902	1.062
D	0.225	0.283	0.439	0.674	0.89	0.982
E	0.285	0.356	0.583	0.79	1.084	0.999
F	0.236	0.348	0.499	0.758	1.057	0.978
G	0.24	0.355	0.525	0.783	1.053	1.051
H	0.27	0.382	0.553	0.767	1.069	0.972

	Std Dev of Standards	Std Dev-2
Average of Blanks: 1.026	0.009	0.000084
	0.024	0.000560
	0.014	0.000193
Std Dev of Blanks: 0.041	0.015	0.000223
	0.009	0.000082
	0.022	0.000500
Sum of Std Dev-2: 0.00221	0.013	0.000169
	0.015	0.000214
	0.014	0.000188

Limit of Blank: $L_B = \mu_{blank} - t(a, n-1)\sigma_{blank}$

$\mu_{blank} = 1.026$
$\sigma_{blank} = 0.041$
$t(0.05, 11) = 1.796$
$a = 0.05$
$n = 12$

Limit of Detection: $L_D = L_B + t[\beta, m(n-1)]\sigma_{test}$

$L_B = 0.953$
$\sigma_{test} = 0.016$
$t(0.05, 27) =$
$B = 0.05$
$m = 9$
$n = 4$

Limit of Blank, Absorbance: 0.953
Limit of Blank, Concentration: 59.306 pg/mL

Limit of Detection, Absorbance: 0.926
Limit of Detection, Concentration: 74.308 pg/mL

Conclusions

The standard curves are generated from the raw data and optimized for the specific ELISA experiment. The orange line is the standard curve generated from the expected numerical values of the standards while the blue line is generated from the measured absorbance that is then used to calculate the actual concentration of the standard. The more these two lines overlap the better the data is generally accepted to be, thus the results more meaningful to the researcher. Since Fig. 2 and Fig. 4 have good overlap the data can be used and accepted. The graph in Fig. 3 shows how effectively different solvents extract cortisone from hair. The red line on the graph shows where the limit of detection is for this data. The limit of detection was calculated as seen in the calculation section using a method[6] which is statistically valid. Water and methanol have the highest concentrations, while ethanol and acetonitrile can produce concentrations over the limit of detection. The graph in Fig. 5 compares how efficiently different lengths of extraction are for extracting cortisone from hair. Only one sample for each extraction was conducted so the specific values measured are not significant but the same general trend can be seen in the results of both extraction methods.

References

1) Papadimitriou, A.; Priftis, K. N. Regulation of the Hypothalamic-Pituitary-Adrenal Axis. Neuroimmunomodulation 2009, 16 (5), 265–271. DOI:10.1159/000216184.
2) Staufenbiel, S. M.; Penninx, B. W. J. H.; de Rijke, Y. B.; van den Akker, E. L. T.; van Rossum, E. F. C. Determinants of Hair Cortisol and Hair Cortisone Concentrations in Adults. Psychoneuroendocrinology 2015, 60, 182–194. DOI:10.1016/j.psyneuen.2015.06.011.
3) Davison, B.; Singh, G. R.; McFarlane, J. Hair Cortisol and Cortisone as Markers of Stress in Indigenous and Non-Indigenous Young Adults. Stress 2019, 22 (2), 210–220. DOI:10.1080/10253890.2018.1543395.
4) Spiga, F.; Walker, J. J.; Terry, J. R.; Lightman, S. L. Hpa Axis-Rhythms. Comprehensive Physiology 2014, 1273–1298. DOI:10.1002/cphy.c140003.
5) Musana, J. W.; Cohen, C. R.; Kuppermann, M.; Gerona, R.; Wanyoro, A.; Aguilar, D.; Santos, N.; Temmerman, M.; Weiss, S. J. Association of Differential Symptoms of Stress to Hair Cortisol and Cortisone Concentrations among Pregnant Women in Kenya. Stress 2019, 23 (5), 556–566. DOI:10.1080/10253890.2019.1696305.
6) Holstein, C. A.; Griffin, M.; Hong, J.; Sampson, P. D. Statistical Method for Determining and Comparing Limits of Detection of Bioassays. Analytical Chemistry 2015, 87 (19), 9795–9801. DOI:10.1021/acs.analchem.5b02082.