An Automated Sensitive Measurement of Estrone and 17β-Estradiol from Human Plasma on LC-MS using Solid-Phase Extraction and MassBoost Derivatization.

Emmanuel Chanco[1], Qi Huang[2], Phil Dimson[1]

[1]SPEware Corp., 14180 Live Oak Ave, Suite I, Baldwin Park CA. 91706
[2]Quantalytical Labs, 651 Via Alondra, #711, Camarillo, CA 93012

Estrogens are a set of hormones which have a large influence in reproduction, sexual maturation and metabolism. Disregulation and unusual levels of these compounds are associated with a variety of fertility issues, developmental disorders, and several types of cancers. Levels of the major estrogens (estrone, estradiol and estriol) vary depending on age, gender and reproductive status. Therefore, accurate quantification of these compounds, taken in context with a patient's current personal status, is important in determining possible health issues.

The importance of estrogens has been well-established, and methods for their measurement have been in use for several years. Radioimmunoassay (RIA) has been the choice method of analysis for many years, but has been replaced by mass spectrometry-based methods to avoid issues of cross-reactivity and low throughput. However, measurements of estrogen levels in plasma are difficult due to their low abundance and lack of ionizable groups. Additionally, the relevant concentration of estrogens vary significantly, ranging from less than 1 pg/mL for patients undergoing cancer treatment, to greater than 1000 pg/mL levels in patients undergoing reproductive therapy. Thus, methods of highly sensitive detection of estrogens are of great clinical relevance, but also need to span a large physiological range.

In this presentation, we describe an automated method for extracting estrone (E1) and 17β-estradiol (E2) from 100 uL of plasma. The method gives good linear response to both compounds from 5-500 pg/mL , with an LOD of 5 pg/mL for estradiol and 10 pg/mL for estrone using an AB Sciex Qtrap 4000 for detection coupled to an Agilent 1200 HPLC. Samples are extracted on SPEware Maestro A columns for cleanup and phospholipid removal, and derivatized with MassBoost for a significant increase in sensitivity. After derivatization, samples were directly injected onto LCMS and quantified. Good agreement with physiological reference ranges were seen for all samples tested. The stability of the derivatives was also evaluated. The workflow was then optimized for automation and high-throughput production. This developed method has the advantages of high sensitivity and specificity, automatable workflow and cleaner extracts.

References

1. Rosner W, et al. Challenges to the measurement of estradiol: an endocrine society position statement. **J Clin Endocrinol Metab**. 2013 Apr;98(4):1376-87.

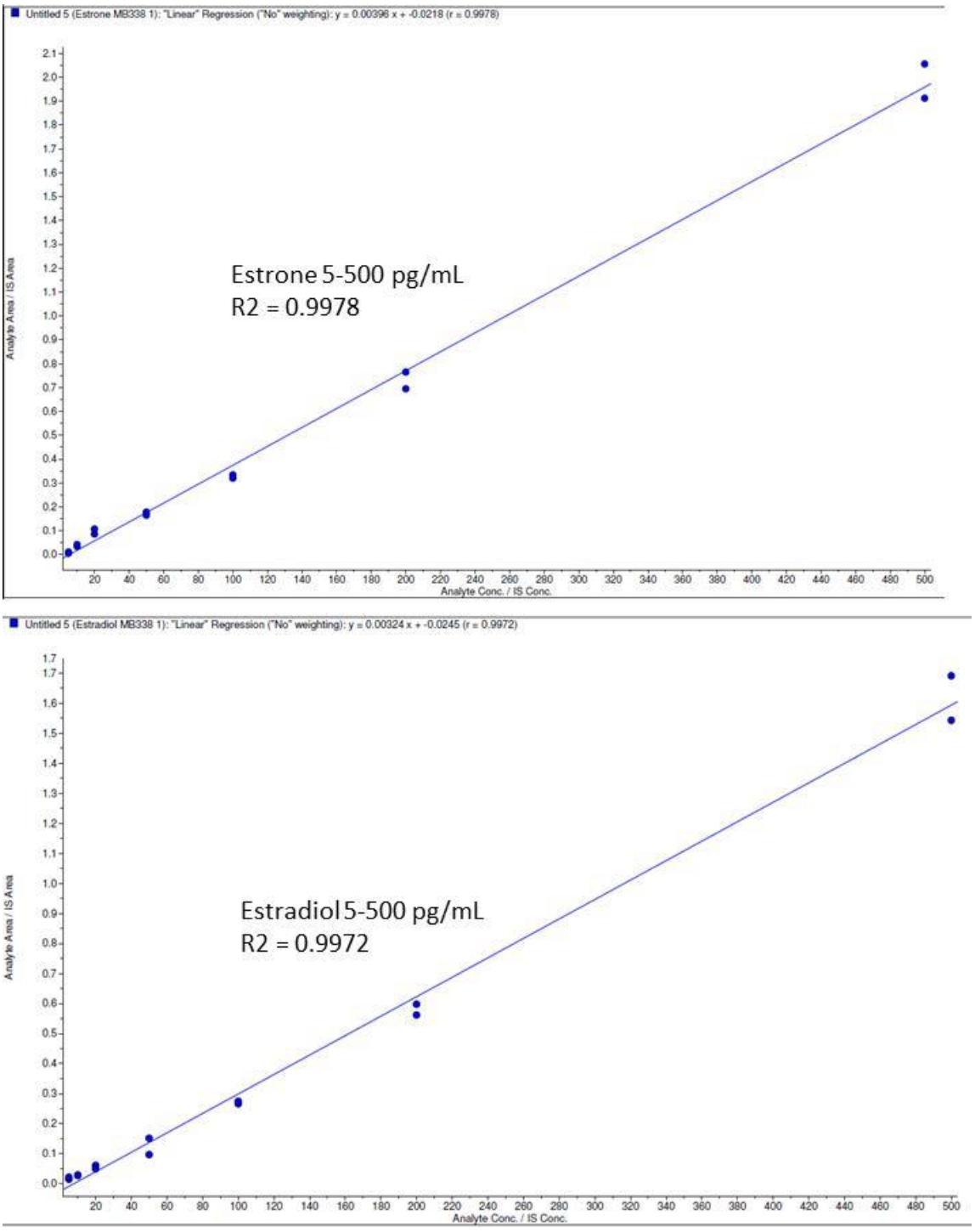

Figure 1. Standard Curves of Estrone and Estradiol.

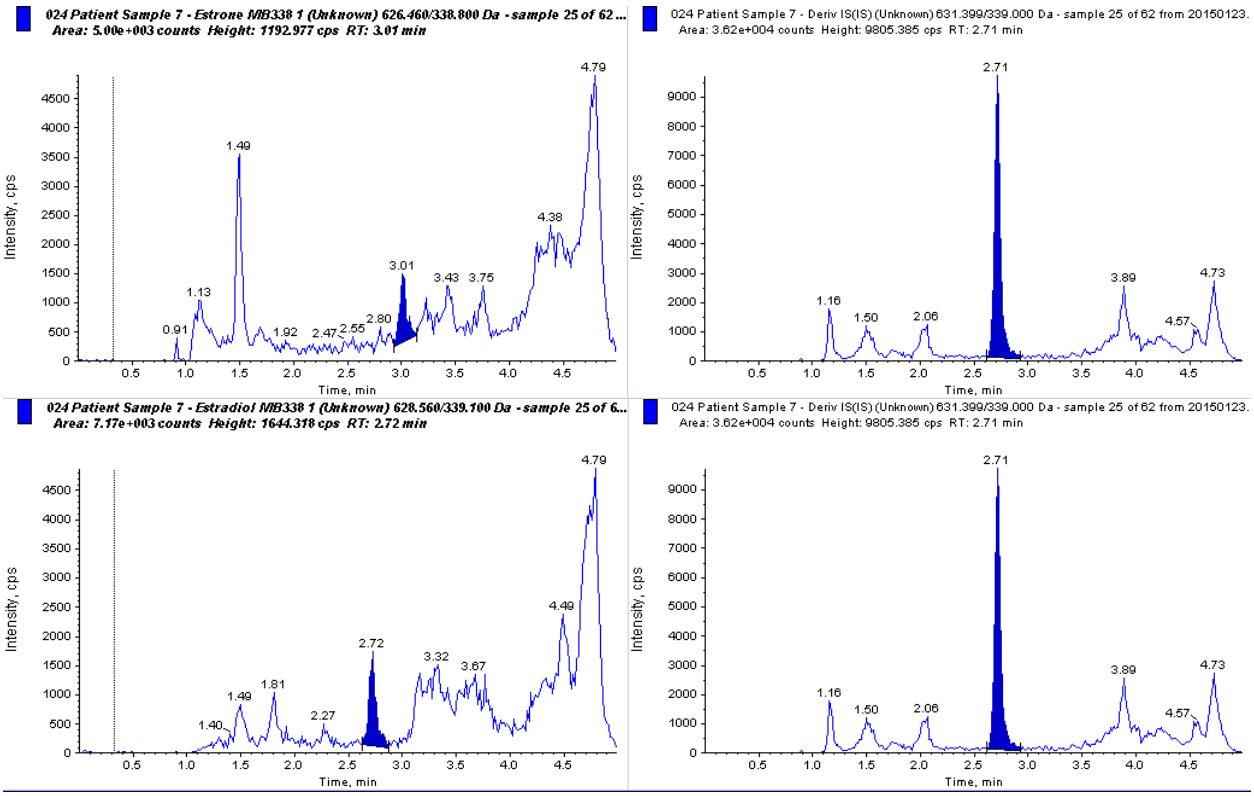

Figure 2. Analysis of a sample from a healthy female patient. Estrone and estradiol levels were quantified to be 40 and 68 pg/mL respectively.