Determination of Estradiol Valerate Content in Grey Market HRT Tablets

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Chem 134

Introduction

- Estradiol is a feminizing drug used in Hormone Replacement Therapy (HRT) (with a variety of pro-drugs[1], one of which is Estradiol Valerate which we consider here)
- Hormone Replacement Therapy (HRT) is a regimen involving the administration of “sex hormones” for, among other things, aligning secondary sexual characteristics, mental health benefits and increased life expectancy.
- Even for individuals that have access to conventional healthcare and are able to obtain prescriptions for feminizing HRT related drugs, injectable pro-estrogens have experienced multiple shortages in American and European pharmacies[2]. These factors, among many others, have driven many transgender individuals by choice or not to use grey market alternatives[3].
- No investigation or organized effort has ever considered the purity or variability in grey market HRT drugs[4].

We aimed to fill this research and humanitarian need by looking at tablet Estradiol Valerate purchased from an easily accessible venue (eBay) and by developing a general purpose procedure. Here, we determined how much Estradiol Valerate was contained in our samples, the deviation from the reported amount and the variance in these measures across tablets.

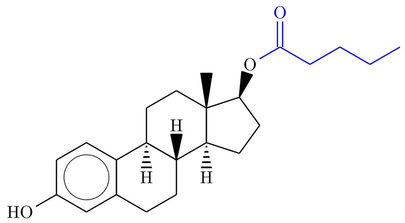

Figure 1. Estradiol Valerate
Estradiol valerate is a prodrug metabolized by enzymatic hydrolysis into the fatty acid (shown in blue) and Estradiol (shown in black) the active component in feminizing HRT.

Methods

Progynova tablets presumably containing estradiol valerate were purchased via eBay from gtxl7863 (a now shutdown seller, eBay does not permit the selling of pharmaceuticals) after a brief interview with seven (7) trans individuals who had, or who knew someone that had, procured HRT outside of a doctors prescription to confirm eBay was a reasonable venue for realistic analysis. Each tablet was powdered and dissolved in methanol (methanol and tablet quantification via weighing by difference). The samples were then sonicated at 60℃ for 36 hours to completely dissolve the tablets before filtering through a 20µm filter to remove any debris. The samples were then analyzed primarily via GCMS for quantification and secondarily via UV/Vis and IR spectroscopy to identify future quantification avenues. Samples were run on an Agilent J&W DB-5ht nonpolar column ((5%-phenyl)-methylpolysiloxane, 30m, 0.25 mm. 0.10 µm, 7 inch cage) with a general purpose method consisting of a 7 minute hold at 160℃, a ramp to 260℃ at 10℃/min to hold for 4 minutes and a 10℃/min ramp to 300℃.

Figure 2. (Right)
Packaging (external)
Packaging (internal)
Tablet held for size reference

Results

We quantified ten estradiol valerate tablets. While their reported estradiol valerate content was 1 mg/tablet, we instead found their estradiol valerate content to be on average 1.36 mg/tablet with a variance of 0.0295 mg^2. We identified no correlation (R^2=0.25) between either tablet weight and estradiol content or peak area. See Figure 5 for tablet quantification distrbution.

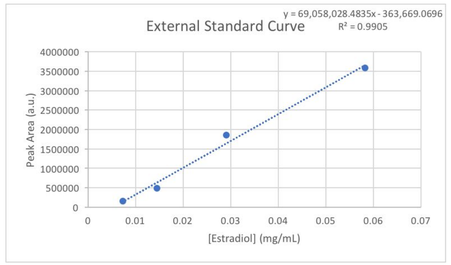

Figure 3. External estradiol standard

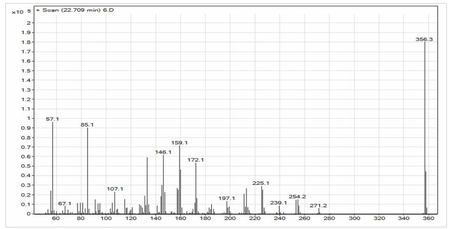

Figure 4. Estradiol valerate’s electron ionization fragmentation (M+ m/z = 356.3)

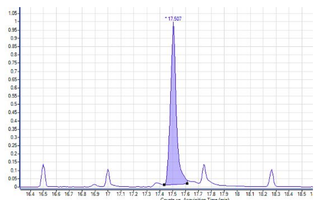

Figure 6. Estradiol chromatogram peak (RT = 17.507 min, M+ m/z = 272.2)

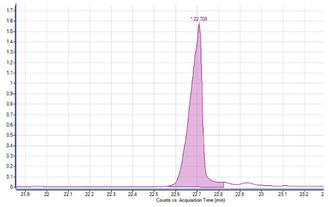

Figure 7. Estradiol valerate chromatogram peak (RT = 22.708 min, M+ m/z = 356.3)

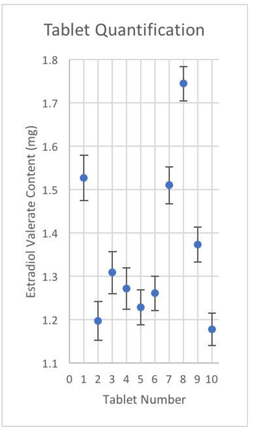

Figure 5. Tablet quantification with 95% confidence error intervals

We also briefly explored **UV/Vis and IR spectrometry** as an alternative analysis method as it is both cheaper and faster than GCMS. While IR showed no peaks for quantification of an estradiol standard, UV/Vis spectroscopy did, with a peak at 289 nm having a linear dependance on solution concentration (n=4, R^2=0.9958). However, the same absorbance peak was not identified in estradiol valerate nor did we have enough estradiol sample to analyze a real world example for a proof-of-concept.

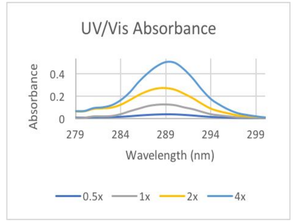

Figure 8. UV/Vis peaks at varying concentrations of estradiol standard

Conclusions/Future Work

We have found the estradiol valerate content of grey market HRT tablets to greatly differ from their reported values. While it is still unclear as to the effects of varying dosage on transitioning, it is known that varying estradiol supplements in postmenopausal women increases risk for deep vein thrombosis which is not uncommonly fatal[5]. For these reasons, it is very important that further work is done to determine the risk associated with “DIY” HRT. We have found that UV/Vis spectroscopy is a promising avenue for continuing quantification of different grey market HRT alternatives that contain estradiol (but not estradiol valerate) without the cost and time associated with GCMS.

Works Cited

1. B. Duumlsterberg, M. Schmidt-Gollwitzer, and M. Huumlmath-semicolonmpel, “Pharmacokinetics and biotransformation of estradiol valerate in ovariectomized women,” Hormone Research, vol. 21, no. 3, pp. 145–154, 1985.
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3. C. Nast, “How an estrogen shortage is making life hell for trans femmes.” https://www.them.us/story/estrogen-shortage-estradiol-valerate.
4. Coleman et, al. “Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7,” International Journal of Transgenderism, vol. 13, pp. 165–232, Aug. 2012.
5. M. Townsend, H. Jaffer, and L. Goldman, “Adverse health outcomes in transgender people,” Canadian Medical Association Journal, vol. 189, pp. E1046–E1046, Aug. 2017.