

Steroids Analysis of Human Saliva: Testosterone a New Way to Identify Gender

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Abstract

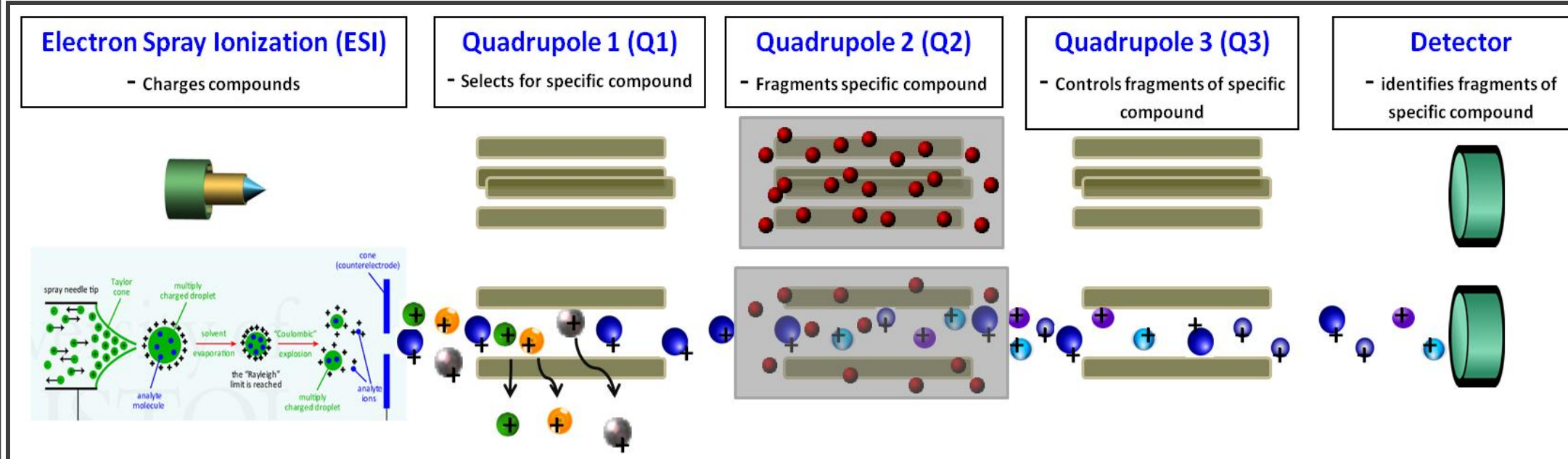
Identification of gender in a forensic cases can significantly narrow down the identity of individuals involved. Steroid hormones are a group of naturally occurring compounds that vary in concentration depending on gender. Because of the blood stream connection to saliva production, steroids from the blood circulation enter saliva. We hypothesize that steroid hormones in saliva can be used to determine gender. To investigate steroid hormones levels in saliva, we used Ultra Performance Liquid Chromatography with Electron Spray Ionization Triple-Quadrupole Mass Spectrometer (UPLC-ESI-TQ-MS). We collected six human saliva samples and used UPLC-ESI-TQ-MS to look for four steroid hormones: progesterone, testosterone, estrone, & estradiol. Testosterone was detected in males, but was not detected in females. However, progesterone, estrone, and estradiol are found in both males and females. These results demonstrate that testosterone can be used to identify gender. In summary, our preliminary findings suggest testosterone could be a potential candidate in forensic science to help identify the gender of an individual without the use of DNA.

Methods

Saliva samples were collected from six different human subjects; 3 men and 3 women. The saliva samples were labeled with numbers to blind the gender of the subjects. Saliva was next concentrated, filtered, and run on a UPLC-ESI-TQ-MS. The samples were then analyzed for four steroid hormones: Progesterone, Testosterone, Estrone, & Estradiol. After steroid analysis was completed, samples were un-blinded.

Ultra performance liquid chromatography (UPLC) uses a column and solvents to separate and identify chemicals based their polarity. The time it takes a given chemical to pass through the column is a known retention time (RT).

UPLC has 2 phases that control a chemical by its polarity:
Stationary phase → Column that is packed with non-polar beads
Mobile phase → polar liquid (solvent) that moves through the column



Electron Spray Ionization:

The process begins with ionization of the chemicals: the samples are pumped through a highly charged capillary. The capillary will help create positive droplets of solvent enclosed chemicals. The solvent will then be evaporated with the use of infused nitrogen gas, leaving just the charged chemical. The positively charged chemicals are attracted to the negatively charged cone and travel into the mass analyzer.

Triple-quadrupole Mass Analyzer:

The machine will then transfer the positively charged chemicals into the triple-quadrupole mass spectrometer. The triple-quadrupoles in the mass spectrometer work by utilizing electrostatic attraction and ion size.

The first quadrupole in the mass spectrometer selects ions based on electrostatic attraction. Electrostatic attraction is created by combining direct and alternating currents which constantly change the quadrupoles charge from positive to negative. The selected positively charged compound will spiral through the quadrupole, while other unwanted compounds will be filtered out. The second quadrupole is filled with argon gas which will fragment the selected compound, creating a unique splitting pattern. The third quadrupole contains all the fragments of the selected compound and sends them to the detector to be identified.

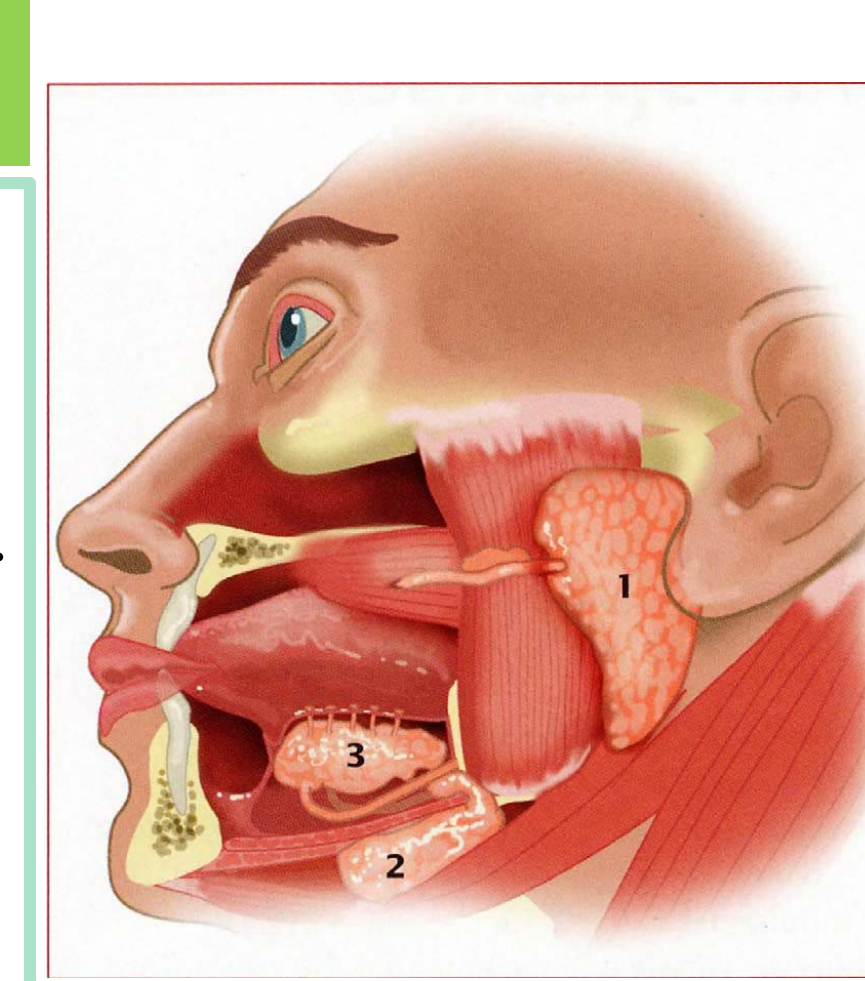
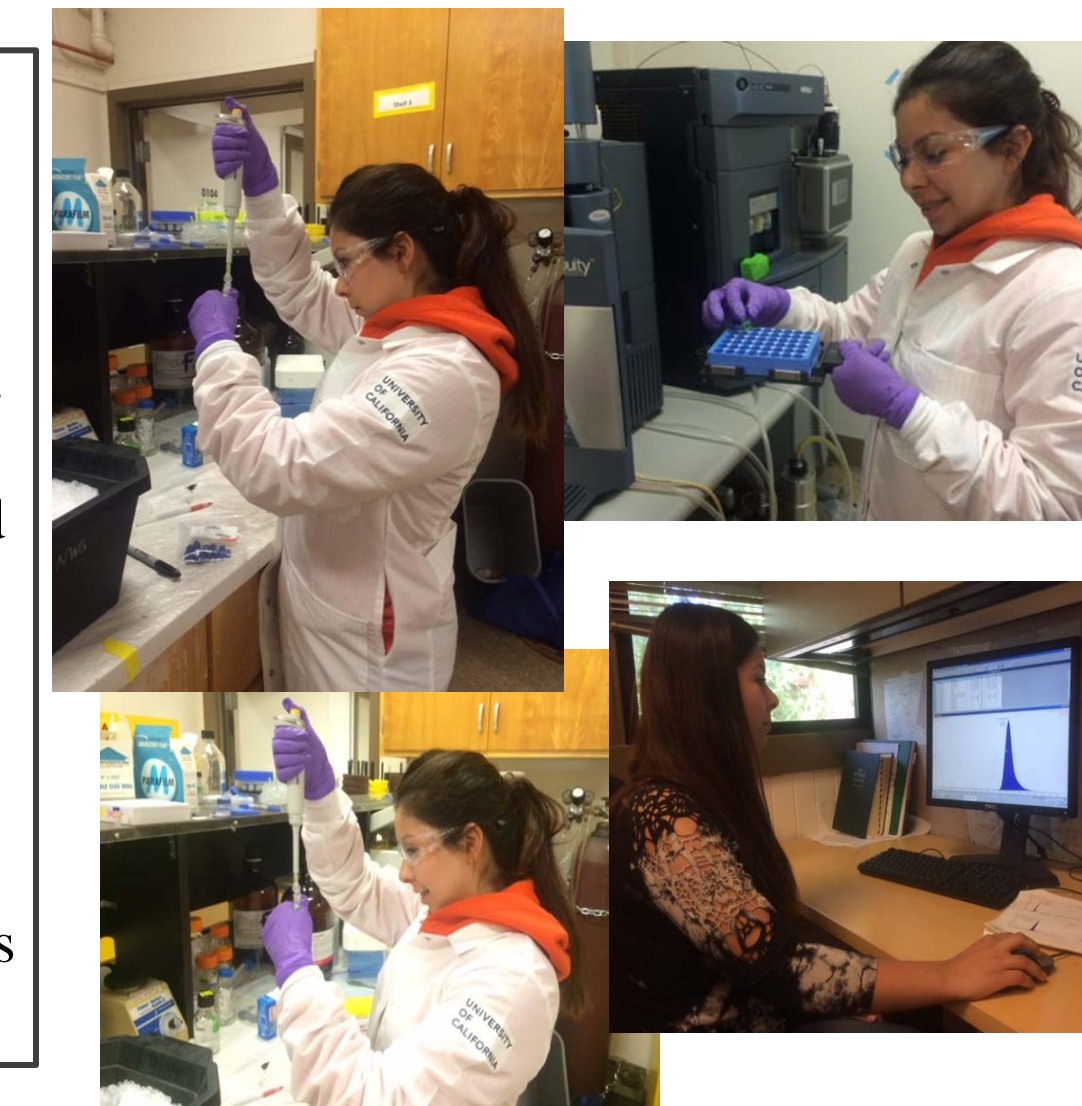


Figure 1. Salivary glands. Saliva is a biological fluid produced by salivary glands. Saliva is 99% water, but also contains steroid hormones, and other compounds^{1,2}. Saliva is produced from 3 main pairs of salivary glands: the parotid (1), submaxillary (2), and sublingual (3) glands³. Adults will approximately produce 500-1,500 mL of saliva per day. Because of the blood streams connection to saliva production, steroid hormones will diffuse into the saliva⁴.



Results

In this study we used UPLC-ESI-TQ-MS to determine the presence of four different steroid hormones. Our chromatography results showed that progesterone is present in samples W1, W2, and M1, but it was not present in W3, M2, or M3. Testosterone, a down stream metabolite of progesterone, was detected in M1, M2, and M3, however it was not detected in W1, W2, or W3. Estrone, a type of estrogen, was present in W1 and M2, but it was not detected in W2, W3, M1, or M3. Estradiol, a metabolite of testosterone, was detected in W2, M1, and M2 but not in W1, W3, or M3. These results indicate that in saliva testosterone can be used to identify a humans gender.

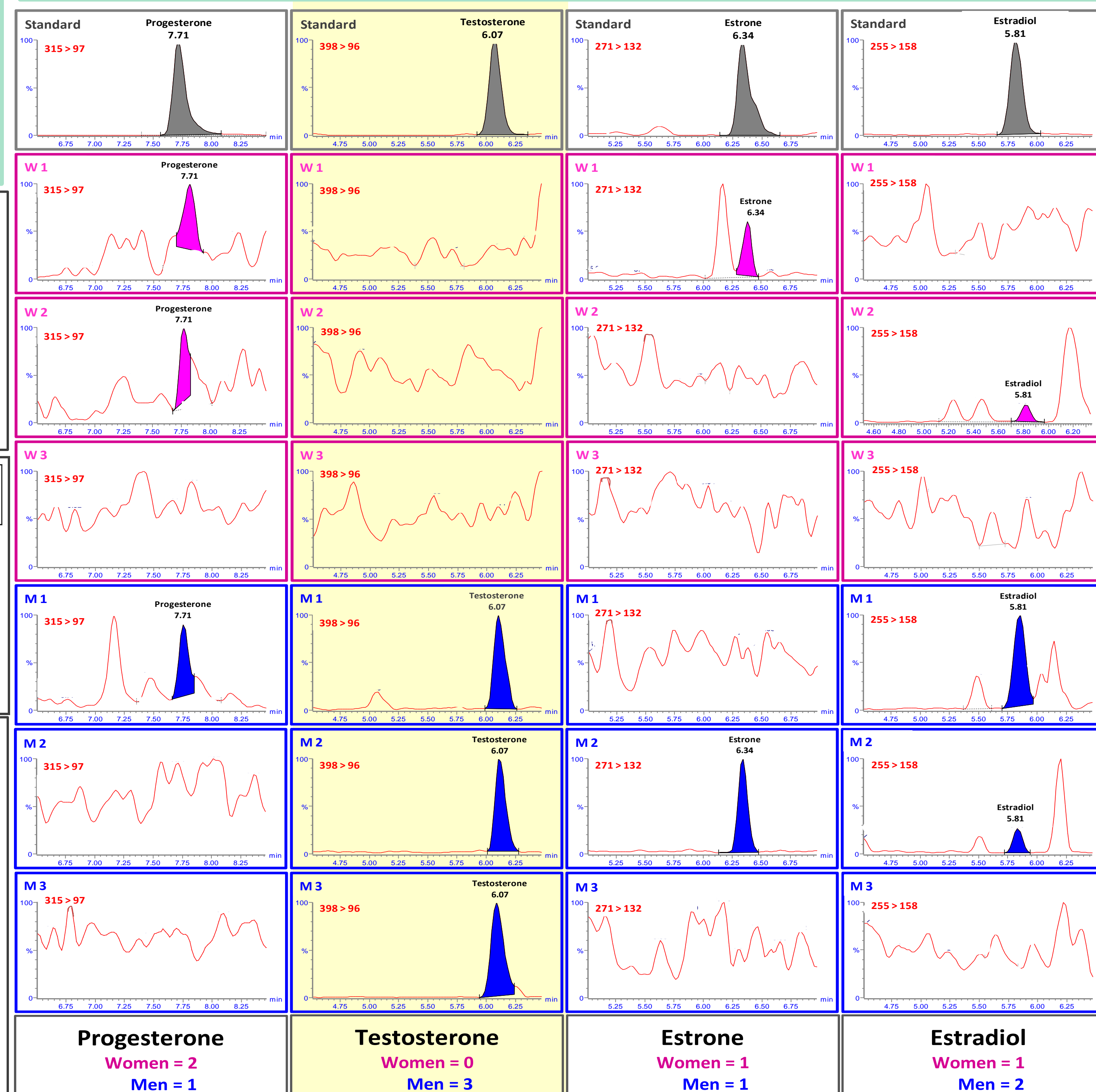


Figure 2. UPLC-ESI-TQ-MS chromatograms of the 6 saliva samples. Progesterone, estrone, and estradiol were detected in both males and female saliva. Testosterone was only present in male saliva, revealing that it can be used to identify gender.

My Experience:

- I chose this project because it combines science with real life applications. My goal is to become a scientist and this project helped introduce me to this career.
- The hardest aspect about the project was learning about the UPLC-ESI-TQ-MS, due to the fact that it is a complex machine. However, my mentor worked with me and now I have a basic understanding of the machine.
- My favorite part about the project was that I participated in the entire experiment. I was able to create this poster with minimal help from my mentor. Collectively, this was a great learning experience.
- What I learned in this project will be beneficial for me in college because I will be able to work in a lab and be confident in my work. In addition, LC-MS is used across many different scientific fields, so my experience will open doors to many careers.

		Progesterone	Testosterone	Estrone	Estradiol
Men	Production	adrenal glands	testis	fat tissue	testis
	Function	testosterone synthesis in the testis	development reproductive organs	aids in reproductive function	prevents sperm cell death
Women	Production	corpus luteum & placenta	ovaries & adrenal glands	ovaries	ovaries
	Function	pregnancy maintenance	bone strength & muscle development	regulates menstrual cycle	bone strength & regulates menstrual cycle

Figure 3. Production and function of steroid hormones in humans.

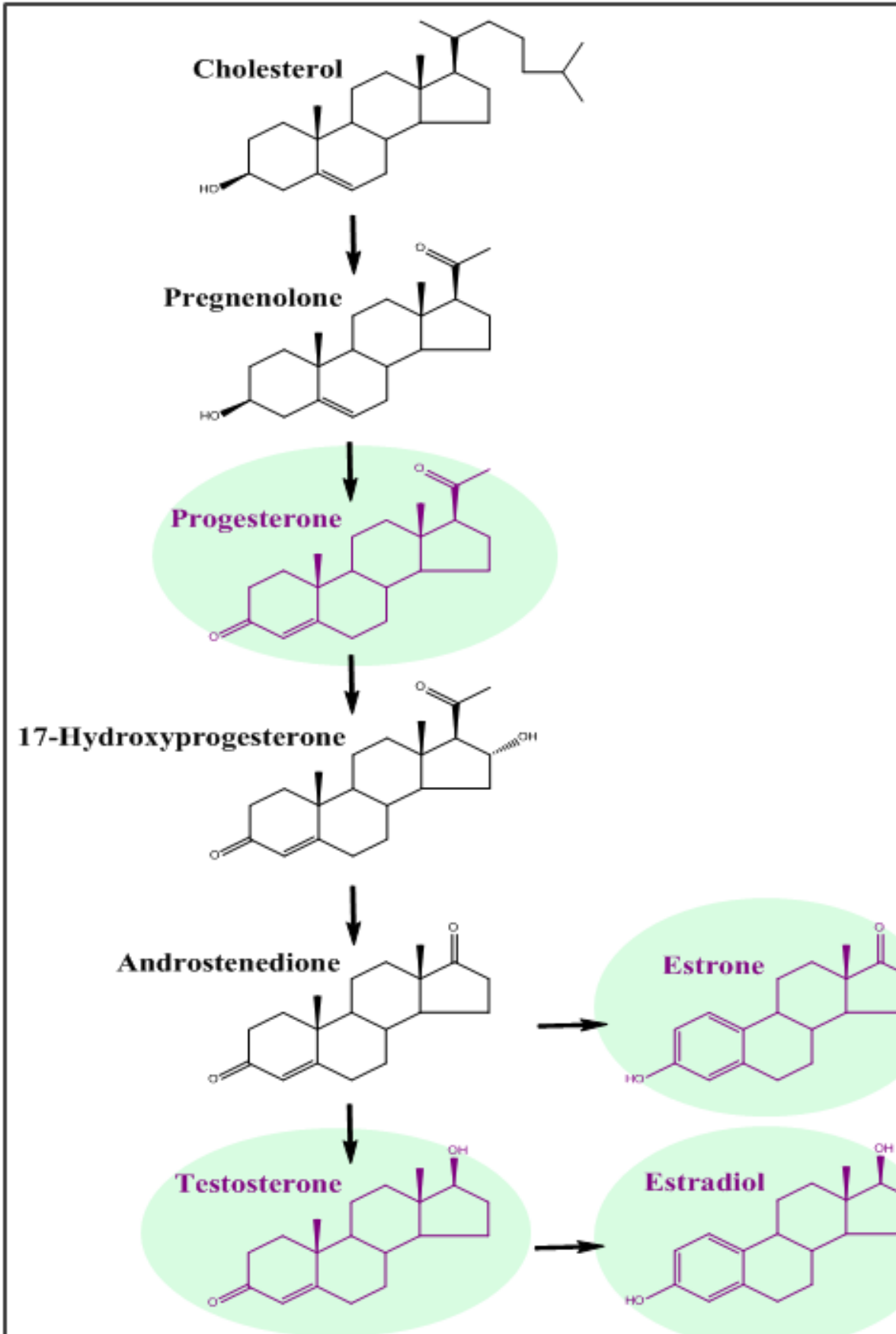


Figure 4. Simplified steroid metabolism in humans. Progesterone, testosterone, estrone, & estradiol are highlighted in green to indicate targeted steroids analyzed in this study.

Conclusion

- Our research suggests that testosterone can be used to identify gender in saliva.
- The first report of gender identification through saliva in humans.
- Testosterone could be used as an alternative to DNA gender identification, which would be beneficial in forensic science.
 - preliminary test to provide further characteristic of the evidence.
 - Potentially faster method to determine gender.
- Progesterone, estrone, and estradiol were found in males and female, therefore they can not be used to determine gender through saliva.

What's next?

- Further studies with larger sample sizes are needed to confirm these findings.
- Identify gender using saliva left on food and drinks.

Citations

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- Mrs. Evert for introducing to this great opportunity

Collected Saliva samples

Blinded 6 saliva samples

Analyzed saliva samples for 4 steroid hormones

Ultra-Performance-Liquid-Chromatography-Electron-Spray-Ionization-Triple-Quadrupole-Mass-Spectrometry (UPLC-ESI-TQ-MS)

Chromatograms for 4 steroid hormones

Testosterone can determine sex from saliva

The same compound will do the same thing, under the same conditions. RT, splitting patterns are unique to a compound, just like a finger print is unique to a human.