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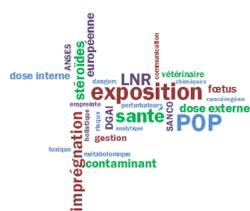
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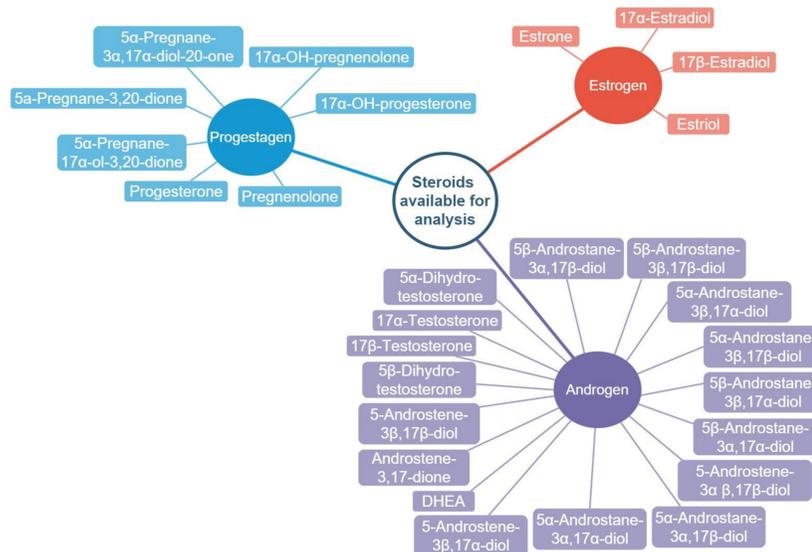
Introduction

Steroids hormones are synthesized in several tissues (e.g. adrenal glands, brain, skin) and regulate essential biological functions including growth and reproduction. Disruptions in steroid homeostasis can lead to serious physiological problems. It is a real challenge to be able to quantify them rapidly in various matrices.

In this work, protocols have been developed to measure steroids in various human matrices such as follicular liquid or periprostatic adipose tissue with GC-MS/MS. These methods are very efficient in terms of sensitivity but remain long and expensive. Thus, a new method was developed aiming at providing a faster and cost effective method that allows to enlarge the panel of monitored steroids available for analysis with the detection of molecules belonging to the corticoid family by HPLC-HRMS with online SPE system. These two techniques are discussed and evaluated.

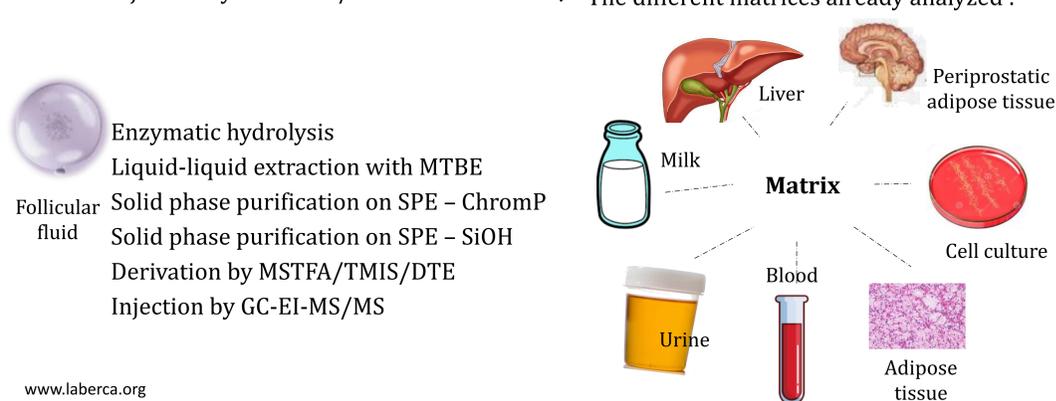
Sample preparation

Steroids are synthesized in several tissues and play a critical role in several biological processes. In order to be able to quantify steroids in various matrices, our sample preparation methods are systematically re-evaluated according to the matrix in order to adapt to the constraints of expected concentration, quantity of matrix available in order to have access to the thirty or so steroids available for analysis.

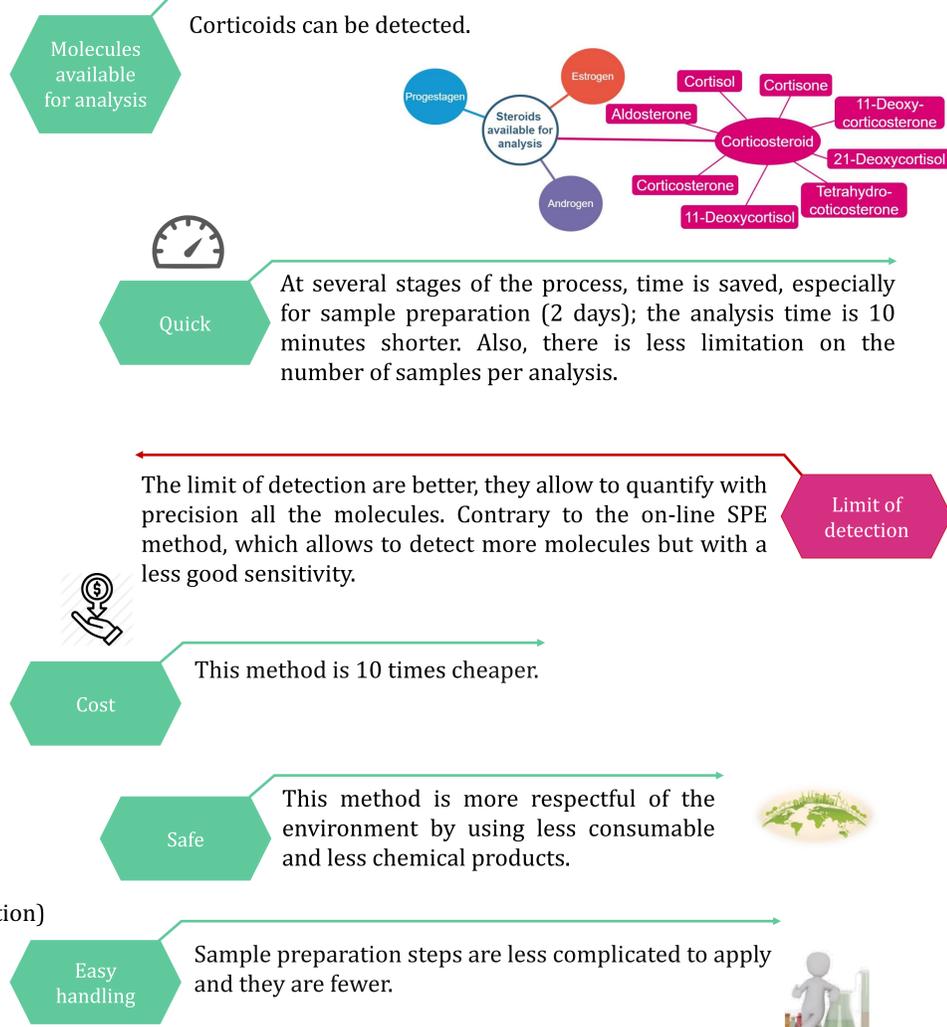
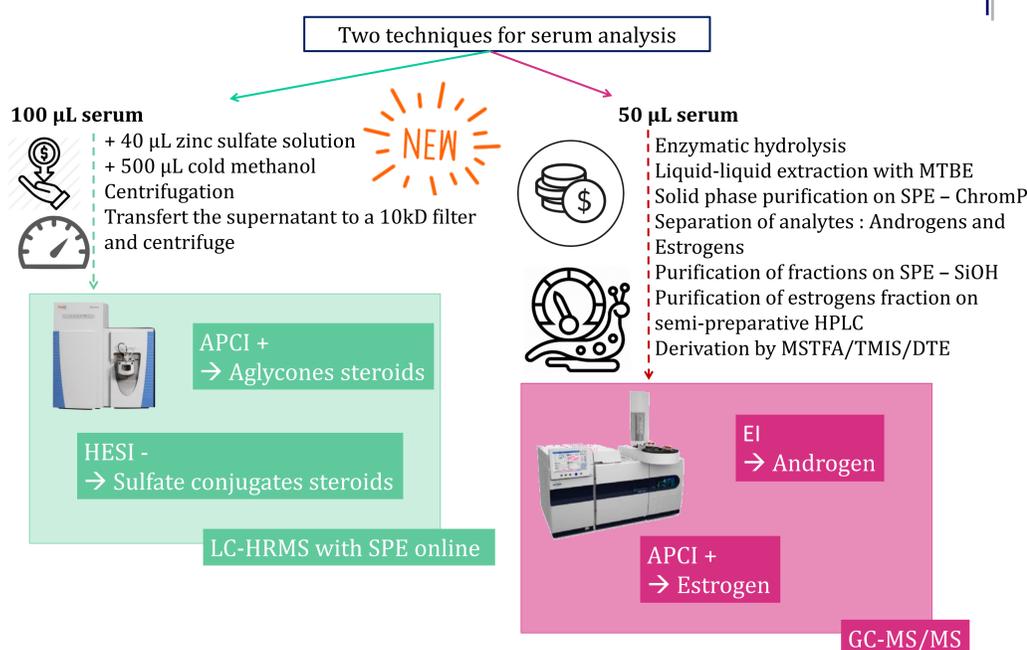


✓ Several matrices are studied including, for example, follicular fluid and adipose tissue :

- Adipose tissue:
 - Grinding of the adipose tissue in chloroform : methanol
 - Phase separation by adding 0.5% NaCl solution
 - Organic phase is taken and evaporated
 - Removal of lipids : addition methanol : water (centrifugation/separation of fat layer/evaporation)
 - Gel Permeation Chromatography (GPC)
 - Derivation by MSTFA/TMIS/DTE
 - Injection by GC-EI-MS/MS
 - Follicular fluid:
 - Enzymatic hydrolysis
 - Liquid-liquid extraction with MTBE
 - Solid phase purification on SPE - ChromP
 - Solid phase purification on SPE - SiOH
 - Derivation by MSTFA/TMIS/DTE
 - Injection by GC-EI-MS/MS
- ✓ The different matrices already analyzed :



Comparison of two methods



Conclusion

Several sample preparation procedures have been developed to answer to the need of different studies.

The new proposed method appears to correctly answers several requirements in term of high throughput capacity and cost effectiveness. The perspectives would be to transfer this new method on a more sensitive instrumentation and allowing to realize MS/MS for better sensitivity and specificity performances.