Deconjugation of steroids in urine: is there an ultimate method for complete deconjugation of steroids in urine?

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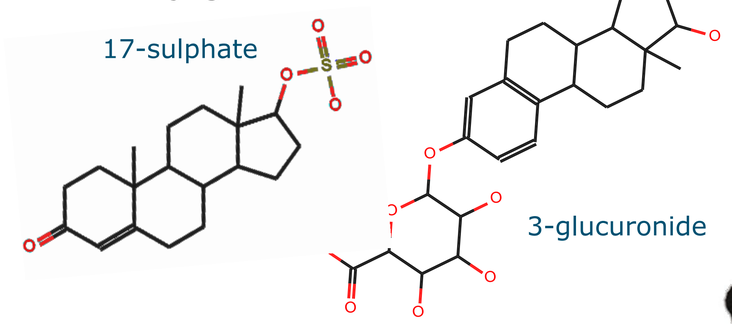
In memorial of Jeroen van den Brink who passed away in the summer of 2014. He performed this work as an intern at RIKILT.

What did we do

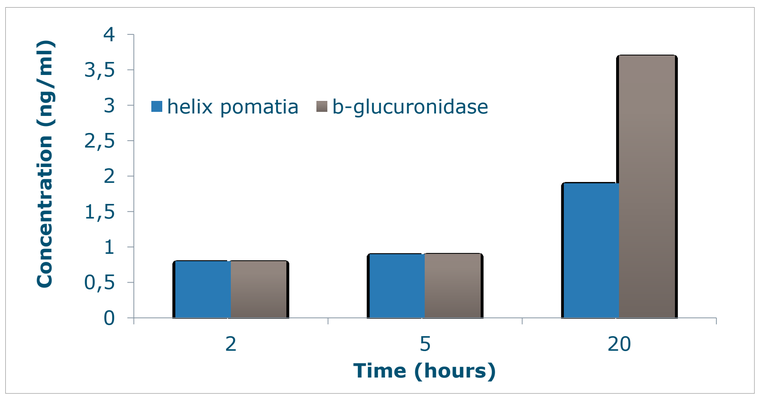
Phase II metabolites of steroids can be conjugated as a sulphate and/or glucuronide at the 3- and/or 17-position. For conjugation a hydroxy group should be present. Different deconjugations techniques were tested on 3 and 17 conjugated steroids



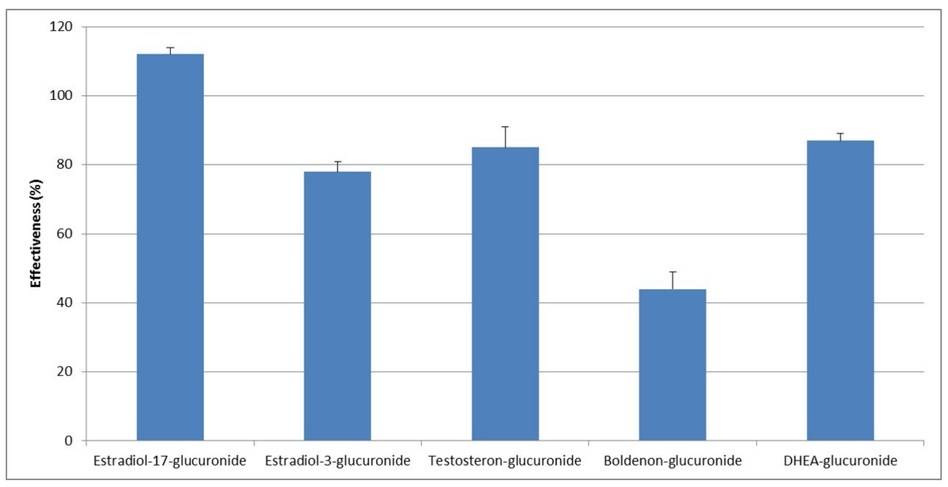
Goal of this study: improve deconjugation step during sample clean-up of steroids

Enzymatic deconjugation

Extracts of *Helix Pomatia* can convert steroids into other compounds eg. DHEA to androstenedione. The use should be limited. It is also less effective then beta-glucuronidase as can been seen in the figure below of a incurred sample of urine with methylboldenone.



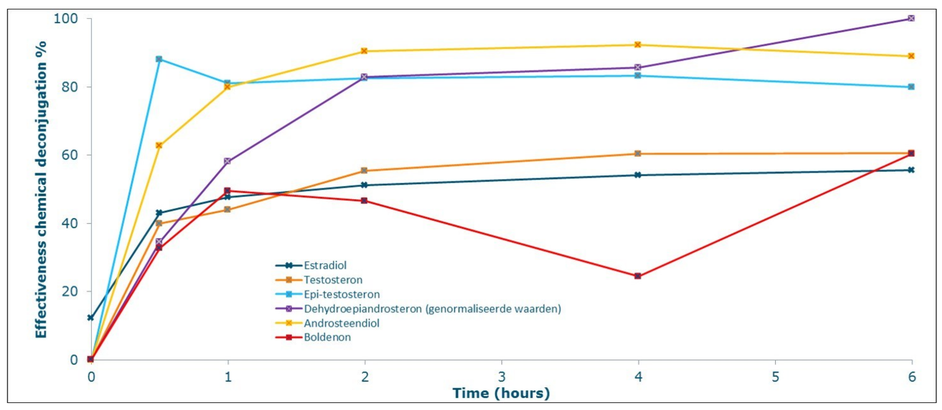
The effectiveness of beta-glucuronidase to remove the glucuronide moiety is determined for several conjugated compounds. See figure below for the results.



For most glucuronide-conjugates deconjugation with beta-glucuronidase is effective.
Beside beta-glucuronidase also arylsulfatase was tested to remove sulphate groups. This was found not to be effective.

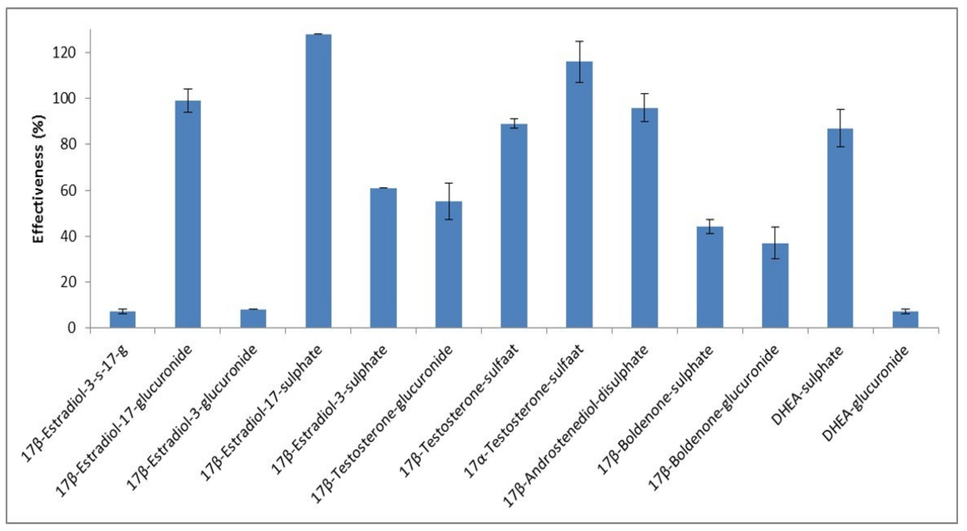
Chemical deconjugation

Chemical deconjugation is capable te remove glucuronide and sulphate moieties. In the figure below the deconjugation with methanolic hydrochloric acid (1M) at 60°C over time is shown. Compounds tested are conjugated at 3- and 17-position with a sulphate or glucuronide group.



Optimisation chemical deconjugation

Chemical deconjugation was optimised and optimal conditions were found to be 60°C with methanolic hydrochloric acid of 1M for 2 hours. Using these conditions the effectiveness of the chemical deconjugation on the individual components was determined and is shown in figure below.



The results shows that the deconjugation of 3- and 17-sulphates and 17-glucuronides is effective with this chemical deconjugation method. It is striking that the deconjugation on the 17-position is better than steroids with a substituent on the 3-position. A good example is 17β-Estradiol-17-sulphate which has a higher deconjugation rate than 17β-estradiol-3-sulphate. The same is true for DHEA, the deconjugation of the sulphate on the 3 position is effective, the deconjugation of the glucuronide on the 3 position is not effective at all.

Conclusions

The enzymatic deconjugation has shown that β-glucuronidase of *Escherichia coli* works effectively for glucuronide conjugates. The enzymatic deconjugation of sulphate conjugates is not possible. It is concluded that chemical deconjugation is effective for sulphates at the 3- and 17-position and for glucuronides at the 17-position and is not very effective for glucuronides at the 3-position

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