Degradation of caffeine in urine and saliva

Authors: Stijn Stevens, Vincent Van Den Broeck, Senne Steukers, Robbe Vranken, Ruben Reynders, Arthur Vande Velde

Introduction

The most known and consumed drug in the world is caffeine. Caffeine belongs to the drug category 'stimulants'. It accelerates the activity between the brain and the nervous system. It will block the adenosine receptors which will make the consumer feel more alert and awake.

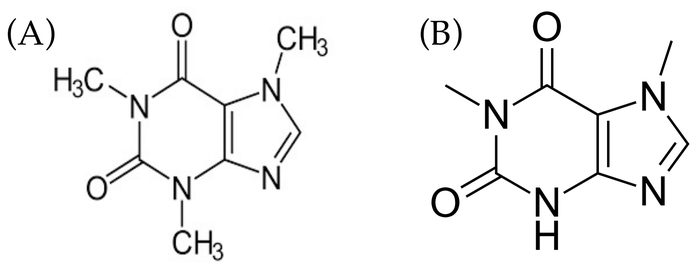

Figure 1: chemicale structure (A) Caffeine (B) paraxanthine

The absorption of caffeine takes place in the gastrointestinal tract after which it will reach the bloodstream and reach a peak height after 30 to 60 minutes. Since only 17 % of the caffeine can bind with the plasma in the bloodstream, the remaining excess will migrate through different biological membranes because of its hydrophobic characteristics. Because of this caffeine will be spread throughout the body. The metabolization of caffeine mainly happens in the liver and kidneys. In the liver and saliva, caffeine will be degraded into several metabolites by a group of iso-enzymes called 'cytochrome P450'. The main metabolite is paraxanthine. This metabolization will differ from person to person because of several physical factors (smoking, age, weight, gender, diseases etc.). In this project we will follow the degradation of caffeine in different test persons over time and link the gathered results to these physical factors of these test persons.

Materials and methods

The High Performance Liquid Chromatography (HPLC) method was used to determine caffeine and paraxanthine in the saliva and urine samples. HPLC is a separation technique. Different HPLC devices were used for the determination of caffeine and paraxanthine in urine than for saliva. Gradient elution was used for the determination in urine, different gait phases are used or the composition of the gait phases are changed while the chromatogram is recorded. Specific use was made of 2 pure mobile phases, which were mixed in the device itself. This allows the composition of the mobile phase to be changed as a function of time.

For the saliva samples isocratic elution was used. Isocratic elution is that all chromatograms are measured at the same running phase.

Saliva samples were pre-treated, the test person uses a Salivette® to collect the saliva. After this the Salivette® is centrifuged for 2 minutes at 1000 G. 4 mL of ethyl acetate is added to 1 mL of saliva to extract the caffeine and to divide the organic layer and the aquatic layer. The layers get mixed again with a vortex for 5 minutes and after this it is centrifuged again for 10 minutes at 4000 rpm. The organic layer is heated to 70°C until everything evaporated and a tiny amount of precipitate remains. This is added to 200 µl of eluent. The urine is collected in a cup and can be directly used for analyses.

Results and discussion

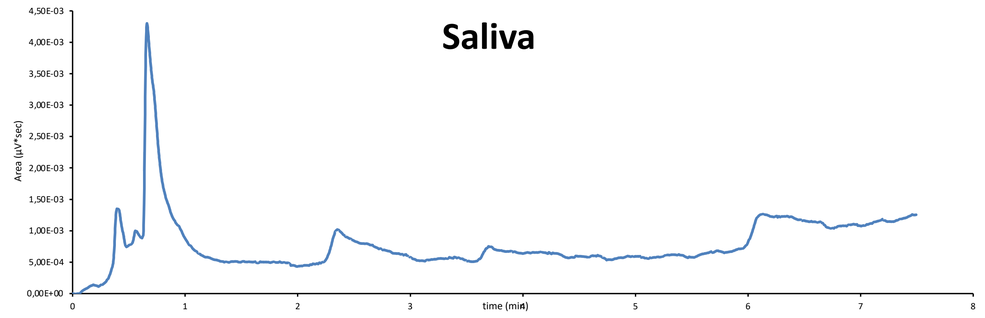

Figure 2: Chromatogram of saliva

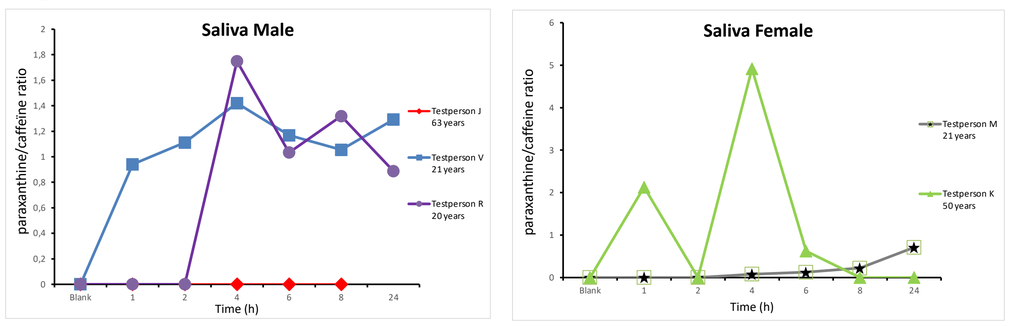

Figure 3: Paraxanthine/Caffeine rate in saliva left for men right for women

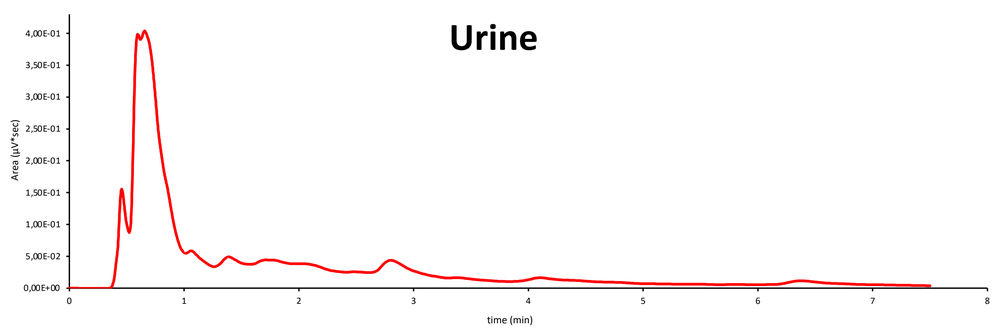

Figure 4: Chromatogram of urine

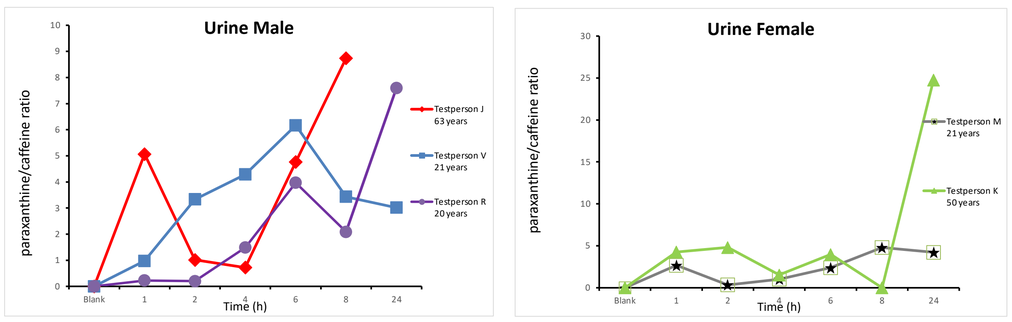

Figure 5: Paraxanthine/Caffeine rate in urine left for men right for women

Conclusion

The degaradation of caffeine was determined in saliva and urine in 5 different people. From the results we see that older people process caffeine faster than younger people. Older people have more metabolite in their system then people after 24 hours. Older people process caffeine faster than younger people. The living conditions of the person determines how fast caffeine is degraded. Smokers appear to break caffeine down faster than non-smokers. A smoker will need more caffeine to establish the same effect als a non-smoker

[1] ScienceDirect. Pharmacokinetics of caffeine and its metabolites in plasma and urine after consuming a soluble green/roasted coffee blend by healthy subjects [internet]. [geraadpleegd op 6/12/21] beschikbaar op https://www.sciencedirect.com/science/article/pii/S0963996914003585#:~:text=Caffeine%20(CF%2C%201%2C3,(TP%2C%201%2C3%2D

[2] S.Arickx.instrumentele analytische chemie: theorie en oefeningen deel 1: Chromatografie . UCLL. Maken cursustekst werk vele jaren. 76p

Promotor research: Natalie Chiaverini
Programma Technologie, campus Gasthuisberg, Herestraat 49, 3000 Leuven