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Pharmacokinetics of Codeine-an UHPLC coupled MS analysis of the metabolic products of codeine in urine

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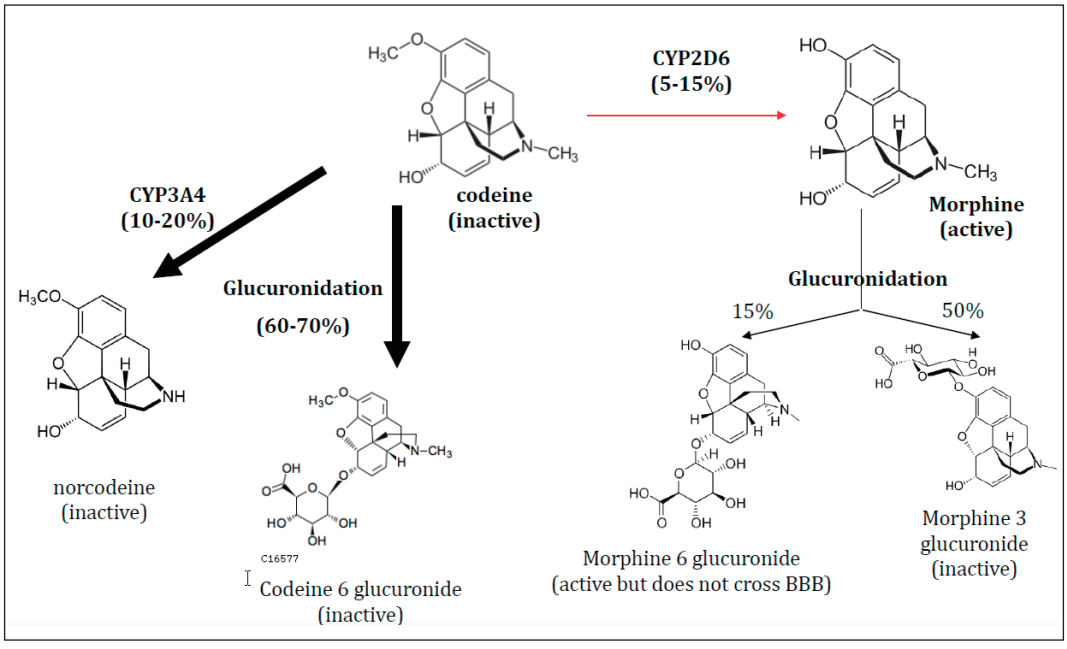
Introduction

Codeine is a naturally occuring opium alkaloid which is used to relief mild to moderate pain. For the analgesic effect codeine has to be converted in the liver to morphine by the enzyme CYP2D6. Nevertheless there are significant individual genetic variations in the actvity of this enzyme which results in variable effectiveness of the pain reliever. Slow metabolizer are unable to convert enough codeine to morphine needed for the analgesic effect. On the other hand fast metabolizer may be at risk to poisen themselves, because of the augmented metabolism of codeine to morphine. Therefore we developed a clinical assay to monitor the pharmcokinetics of codeine with a triple quadrupole mass spectromter coupled to a UHPLC system.



Codeine Metabolism

Materials and Methods

8 healthy volunteers took one tablet of Gelodina® (Pfizer) containing 30 mg of Codein. After certain time intervals their urine was taken, 10 times diluted and codeine and its metabolites morphine, morphine-3-glucuronide and codeine-6-glucoronide were analyzed via HPLC on a Shimpack XR-ODSII column on a Nexera-i UHPLC (Shimadzu) coupled to a LCMS 8050 triple quadrupole mass spectrometer. The amount of the metabolites was calculated using a calibration function of standards in urine. The values of the concentration vs time were used to calculate the peak concentration, the half-life, the morphine/codeine ratio and the elemenation rate of the metabolites.

MRM transitions

One of the first steps during this automated process is the precursor ion selection, followed by the m/z adjustment of the precursor. The collision energy is optimized for the most abundant fragments and finally the fragment m/z adjustment. The result of these automated steps was the automatic generation of a final MRM method as seen in the table below.

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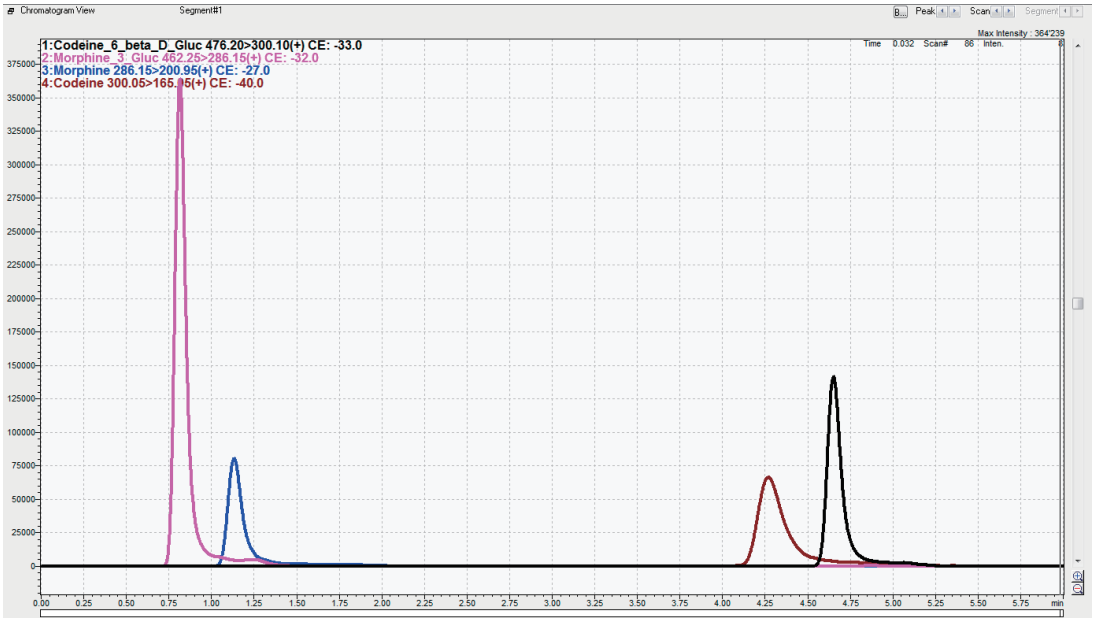
Compound	Precursor [m/z]	MRM [m/z]	RT [min]	Elemental composition
Codeine-6-β-D-Glucuronide	476.2	300.1	4.69	C24H30NO9
Codein-6-β-D-Glucuronide	476.2	69.05	4.69	C24H30NO9
Morphine-3-Glucuronide	462.25	286.15	0.88	C23H28NO9
Morphine-3-Glucuronide	462.25	164.9	0.88	C23H28NO9
Morphine	286.15	200.95	1.14	C17H20NO3
Morphine	286.15	153.1	1.14	C17H19NO3
Codeine	300.05	165.05	4.30	C18H21NO3
Codeine	300.05	183.11	4.30	C18H21NO3



Results

We found one slow metabolizer lacking the CYP2D6 enzyme activity. In the liver only a very little amount of codeine is metabolized to morphine and further to morphine-3-glucuronide. The codeine is mainly glucuronidated to codeine-6-glucoronide. The peak concentration of codeine-6-glucoronide was much higher compared to the normal and fast metabolizer. In all samples the peak concentration of codeine was reached 1 h after consumption of the tablet.

We found one fast metabolizer. The codeine was converted to morphine, reaching the peak concentration 0.5 h after consumption. The peak concentration was around 50% higher compared to the normal metabolizer. 2 h after consumption most of morphine was further metabolized to morphine-3-glucuronide. Using a one phase decay model we calculated the average half life for codeine with around 2-2.5 h and the average half life of morphine with around 3 h.

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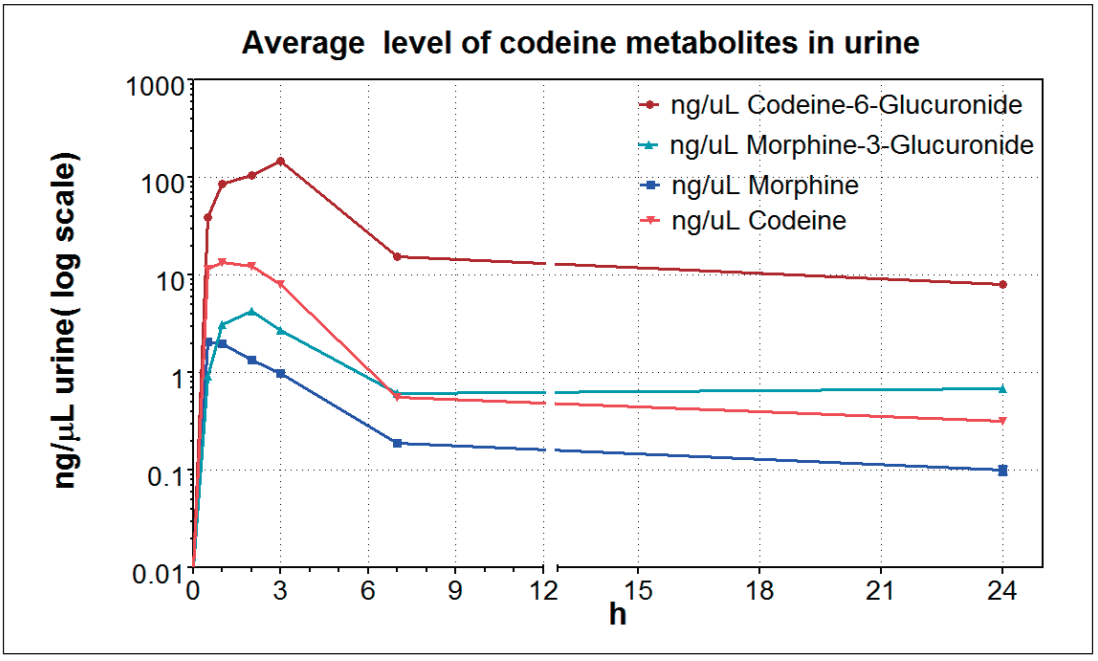
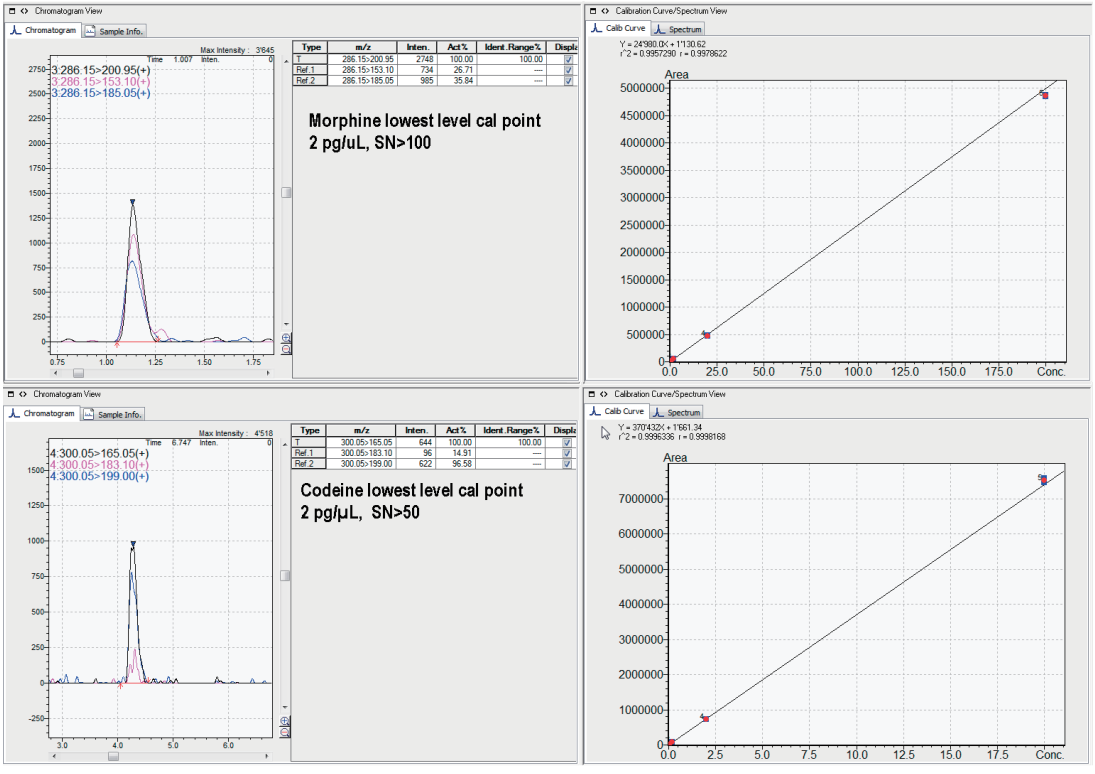


The main product of the codeine metabolism was codeine-6-glucuronide showing an average peak concentration of around 150 nanogram/µL three hours after consumption of codeine. Using a weighted calibration curve (1/c ^{2}) the limit of quantitation for codeine and morphine in urine was estimated around 2 picogram/µL. This equals around 5-6 attomol (10 $^{-15}$ Mol) absolute amount of metabolite per injection on the HPLC column.



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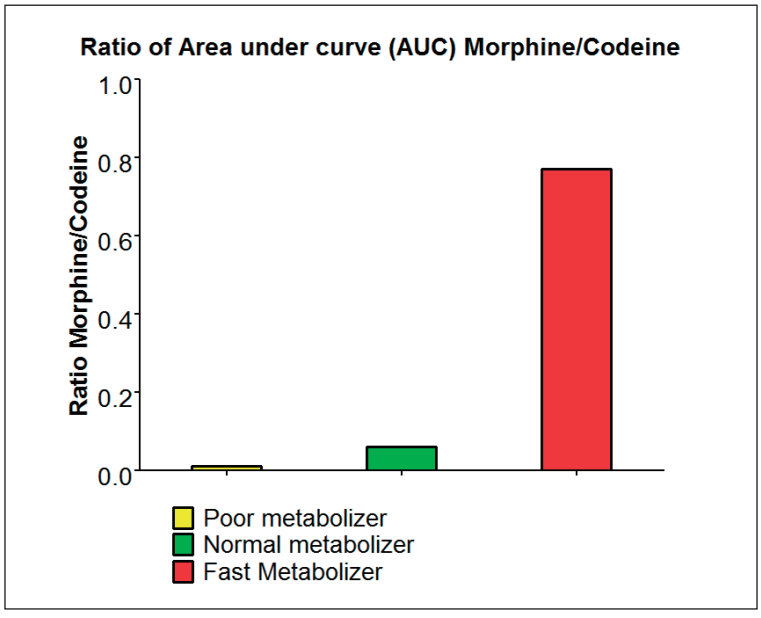
The ratio of the area under curve (AUC) morphine/codeine showed big differences between poor, normal and fast metabolizer as shown in the figure below. The fast metabolizer had a 10 times higher ratio than the normal metabolizer. This individual is able to bioactivate codeine to morphine very rapidly and might be at higher risk for opioid intoxication or developing codeine addiction.



Conclusion

Using a LCMS system we were able to develop an assay that detected metabolites of codeine in urine even in the low attomol amounts.

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