



JACOBS  
UNIVERSITY

## LAB COURSE ANALYTICS

Handout Fall 2012 – **HPLC**

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### **Determination of caffeine in soft drinks by High Performance Liquid Chromatography**

#### Sample Preparation :

Take 1 ml of the drink and dilute 1:10 in MeOH/Water 1:1 ( provided ) – Do not leave the lid off the container for longer than it is necessary to transfer you sample.

The rest remaining in the tin transfer into a 250 ml Separatory funnel and shake three times not too vigorously – because of the foam ( vent in between ) to remove the carbon dioxide. You need it for the TLC and the UV Experiment.

Inject 100  $\mu$ l into the HPLC. ( overloading the loop at least five time to inject 20  $\mu$ l accurately. )

Calculate the amount of caffeine in mg/l using the following method.  
Don't forget to multiply the result times 10.

Discuss the result considering that the allowed concentration for caffeine in energy drinks in Germany is 320 mg / L.

## **Generation of a Calibration Curve.**

In analytical chemistry, a calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.

The calibration curve is a plot of how the instrumental response, the so-called "analytical signal," changes with changing concentration of analyte (substance to be measured). The operator will create a series of standards across a range of concentrations near the expected unknown concentration. One must take care that these concentrations are in the working range of the technique they are using.

To find the best-fit straight line we use linear regression (least square method) analysis.

**Formular :  $Y = A + B \cdot X$**

Y = Result (concentration)

a = y-achsenabschnitt (schnittpunkt mit der konzentrationsachse)

x = variable value (peak area)

b = slope

## **Calibrating Caffeine using the external standard method**

Three different caffeine concentrations -2,5 – 5 and 10 mg/L (provided) are being injected to the HPLC at least three times. (Injection volume 100 µl – overloading the loop at least five times to inject 20 µl accurately.)

**Method:** C18 – Column, 35 % MeOH / 0.005 % Formic acid, 1.0 ml / min, 254 nm

(The method is already existing on the HPLC-Computer – just open with "monitor baseline" and let the column equilibrate for 15 min.)

Prepare a calibration curve with achieved data.

Inject the sample in the same way (100 µl) and calculate the concentration using the upper formula.

# EXAMPLE

N	A	B	R	Correlation coefficient
9	0.542168675	2.560240964	0.997414903	0.994836489

## Caffein calibration curve



