



JACOBS
UNIVERSITY

LAB COURSE ANALYTICS

Handout Fall 2012 – **Photometer and TLC Experiment**

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TLC and UV- Experiment

Aim of the experiment :

Comparison between the caffeine content of different Energie drinks using HPLC , TLC and UV – Method. (Photometer) –
Extraktion with Dichloromethane- Generation of a Calibration Curve –
Separation of a Dichloromethane solution by TLC. Determine the Extinction coefficient to calculate the caffeine concentration using the Lambert - Beer law.

After the Lambert-Beer law the extinction is with a given wavelength and layer thickness proportionally to the concentration. (**for clear solutions – ideal dilutions and monochromatic light**)

$$E = \lg \frac{I_0}{I}$$

The extinction is calculated by the natural logarithm of the source light intensity, divided by the light intensity after penetrating of the sample. The following formula arises from it.

The extinction E is depending on :

ϵ = the molar extinction coefficient (material constant for given wavelength and for a given solvent.)

d = the layer thickness of the Küvette

c = concentration of the sample

The Lambert Beer law consists of these components as follows : **$E = \epsilon * d * c = \epsilon' * c$**

Sample preparation :

Extrakt the caffeine with 50 ml Dichloromethane. Give the two layers time enough to separate into two layers.

Dilute the extract 1: 20 in Methanol and Inject 100 μ l on the HPLC. For calculating the amount of caffeine you don't need to multiple by 20. (**think of the distribution coefficient - you need to dilute because it is a non ideal dilution**)

For the TLC - experiment apply 20 μ l using a glas-syringe on a TLC plate – leave for a few seconds for drying.

Also apply 20 μ l 100 mg/l caffeine standard in dichloromethane as a reference the same way.

Put the tlc-plate`s in a chamber (beaker etc.) containg solvent mixture TBME and Aceton (8 : 2 provided) and leave for a few minutes until the solvent layer reached 2 cm below the upper edge of the tlc-plate. Take out of the chamber and again leave a few minutes for drying.

Mark the spots using UV- Light (254 nm) with a pencil and scratch into a Eppendorf Cup – dilute with 1ml Dichloromethane and centrifuge for 2min.

Measure the absorbance using the maximum wavelength from caffeine.

Dilute the dichloromethanextract 1:20 in dichloromethane and determine the absorbance.

(Compare with the HPLC – Result)

Kalibration of Caffeine using the photometer :

Four different caffeine concentrations are being provided.

Take the 10 mg / L solution to find the maximum wavelength.

Measure each of the different concentrations at that wavelength.

Prepare a calibration curve – **calculate the mg/l in mol/l (x -axis)**

- Absorbance to the **y - axis**

Because the extinction coefficient is given by the equation **$E = e * d * c = e' * c$**

you read directly from the slope of the graph. ($Y=a+b*x$)

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