Practical exercise in QSAR – determination of a dependence of activity or toxicity of local anesthetics on a hydrophobic structure parameter

We search the dependence

A = f (parameter) in the form $A = a_1x + b$, or $A = a_1x^2 + a_2x + b$

where A … relative infiltration anesthetic activity related to procaine (A(procaine) = 1); rel. surface anesth. activity related to cocaine, anesthetic index (*Carassius carassius* or *C. auratus*), acute toxicity as LD_{50}
x … a hydrophobic parameter (log P, log k´)

log k´ from HPLC: stat. phase: a column with $-(CH_2)_3C{\equiv}N$; mobile phase: methanol : 0.01 mol/l Na_2HPO_4 1 : 1, stream 0.6 ml/min., injection 20 μl solution of the mixture of anesthetics of the concentration of individual compounds around 0.02 mg/ml, also containing a substance for dead retention time t_0 determination (eg. $NaNO_2$), UV detection at 230 nm

$$\log k' = \log\left(\frac{(t_r - t_0)}{t_0}\right),$$

Calculation & results

- log P_{exp}, activities, toxicities: see attached material (in PDF)
- with values log P, and log k´ and others, perform a **linear regression** in MS Excel or an equivalent, or in a suitable statistics software; where
  - the activity, or toxicity (as it defined in the text of the task assignment) is a **dependent quantity (→y axis);**
  - log P,log k´ - **independent quantity (→ x axis)**
- compose the **equation** from resulted values, present also the linear correlation coefficient R, and the value of Fisher-Snedecor test F
- input data in the table and results the linear regression, and your calculation, put into the **report**
- upload your report into the subject homework vault **QSAR of local anesthetics** in IS **until June 5th**

Individual task assignment

1. Correlation of toxicity expressed as LD_{50} *i.p.* for the mouse with experimentally determined log P(octanol/water) value for the collection methyl 4-aminobenzoate, benzokcaine, propyl 4-aminobenzoate, butyl 4-aminobenzoate, *iso*-butyl 4-aminobenzoate, pentyl 4-aminobenzoate,bupicakaine, cinchocaine, lidocaine, procainamid, procain, tetracaine, and trapencaine. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of LD_{50} *i.p.* of prilocaine.
2. Correlation of toxicity expressed as LD_{50} *i.v.* for the mouse with experimentally determined log P(octanol/water) value for the collection articaine, bupicaine, ropivacaine, cinchocaine, cocaine, lidocaine, trimecaine, prilocaine, procainamide, procaine, tetracaine, trapencaine. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of LD_{50} *i.v.* of propyl 4-aminobenzoate.
3. Correlation of **the reversal value of relative infiltration anesthetic activity** with experimentally determined log P(octanol/water) value for the collection articaine, benzocaine, butyl 4-aminobenzoate, pentyl 4-aminobenzoate, bupivacaine, cocaine, lidocaine, trimecaine, prilocaine, procaine, tetracaine. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of activity of mepivacaine.
4. Correlation of **the logarithm of the relative surface anesthetic activity** with experimentally determined log P(octanol/water) value for the collection cinchocaine, cocaine, lidocaine, trimecaine, oxybuprocaine, procaine, tetracaine, trapencaine. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of activity of ropivacaine.

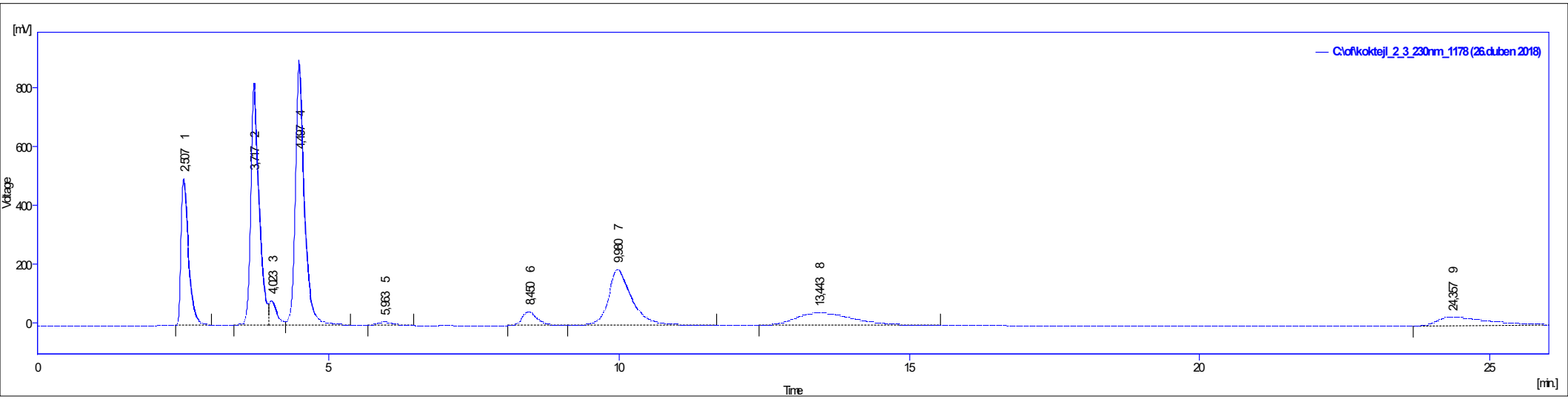
5. Correlation of the **anesthetic index** *Carassius carassius* with experimentally determined log P(octanol/water) values for the collection methyl 4-aminobenzoate, benzocaine, propyl 4-aminobenzoate, 2-propyl 4-aminobenzoate, butyl 4-aminobenzoate, *iso*-butyl 4-aminobenzoate, and pentyl 4-aminobenzoate. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of activity of procaine.

6. Correlation of the **anesthetic index** *Carassius auratus* with experimentally determined log P(octanol/water) values for the collection methyl 4-aminobenzoate, benzocaine, propyl 4-aminobenzoate, butyl 4-aminobenzoate, pentyl 4-aminobenzoate, hexyl 4-aminobenzoate, and heptyl 4-aminobenzoate. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of activity of lidocaine.

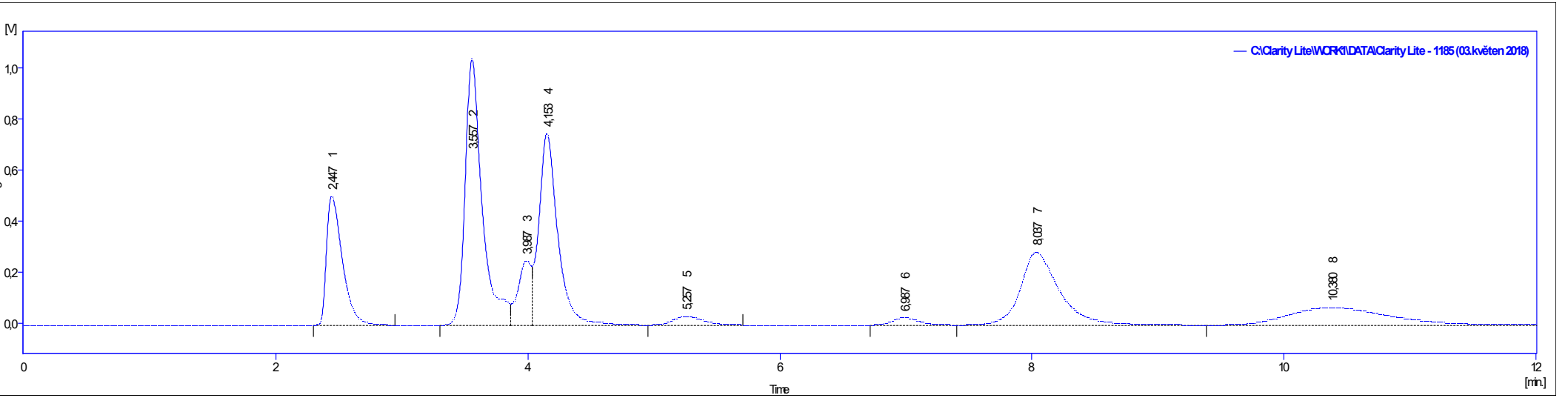
7. RP-HPLC: Correlation of the **logarithm of relative infiltration activity** with log k´ for a „coctail“ of local anesthetics C1, which contains benzocaine(2), trimecaine(4), articaine (3), tetracaine(6), cinchocaine(7), carbizocaine(8), trapencaine(9), $NaNO_2$ (1). Numbers in brackets mean numbers of peaks at the chromatogram. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of activity of pentyl 4-aminobenzoate(5).

8. RP-HPLC: Find, if a correlation of the **cosinus of relative infiltration activity** with log k´ for a “coctail” of local anesthetics C2, which contains benzocaine(2), articaine(3), lidocaine(4), procaine(6), tetracaine(7), cinchocaine(8) , and $NaNO_2$(1), exist. Numbers in brackets mean numbers of peaks at the chromatogram. If the correlation will be confirmed ($r \geq 0.6$), calculate the estimated value of activity of pentyl-4-aminobenzoate(5).

Chromatogram for the task No. 7



Chromatogram for the task No. 8



Procedure of calculation in Excel (no problem with use of other software)

- data of activity and a structure parameter along into two adjascent columns of the table
- select a rectangle of 2 x 4 cells at other place other than the input data are
- select the function LINEST (linear regression)
- select the column of the **structure parameter** as **data x**
- column of **activity** as **data y**
- type B = true, Stat = true
- “perform calculation”(OK)
- you´ll get the results in the table placed likewise in the table at the bottom, if not, the value **a** appears in the top left corner cell of the selected rectangle; press F2, and then CTRL+ SHIFT + ENTER
- now, the required results should appear; you can compose now the equation in the form y = ax + b (eg. log (1/MIC) = a.logP + b); r^2 … square of the linear correlation coefficient; s_y … standard deviation of the estimate, F … Fischer-Snedecor test value

a	**b**
s_a	s_b
$\mathbf{r^2}$	$\mathbf{s_y}$
F	d_f

Excel, however, does´nt enable the linear regression in the quadratic form, i.e. interleaving a parabola. For such a purpose, eg. **QCExpert** can be used

•30 days fully functional trial version to download at trilobyte.cz, together with the manual