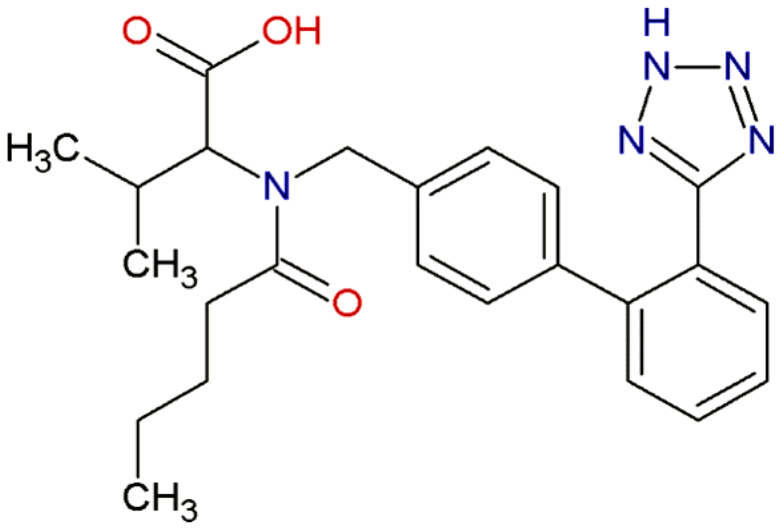
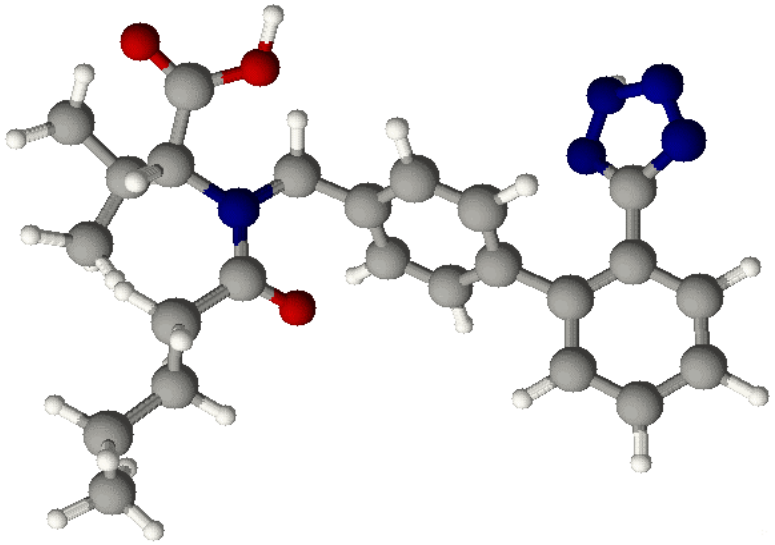
UV-Metric and pH-Metric Determination of Thermodynamic Dissociation Constants and Thermodynamic Parameters of the Valsartan

*Milan Meloun[1], Lucie Pilařová[1], Aneta Pfeiferová[1] and Tomáš Pekárek[2]
[1]Department of Analytical Chemistry, University of Pardubice, CZ 532 10 Pardubice, Czechia,
[2]Zentiva k.s., U Kabelovny 130, CZ 102 37 Prague, Czechia,
***Corresponding author:** milan.meloun@upce.cz, **Phone:** +420466037026, **Fax:** +420466037068,

Valsartan, $C_{24}H_{29}N_5O_3$





Abstract

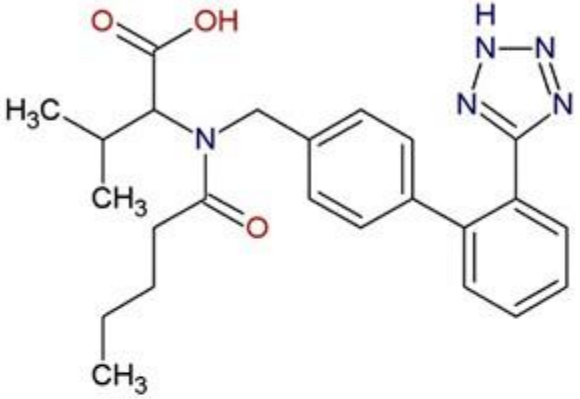
Valsartan is used for treatment of high blood pressure, congestive heart failure, and to increase the chances of living longer after a heart attack and to reduce death for people with left ventricular dysfunction after having had a heart attack.

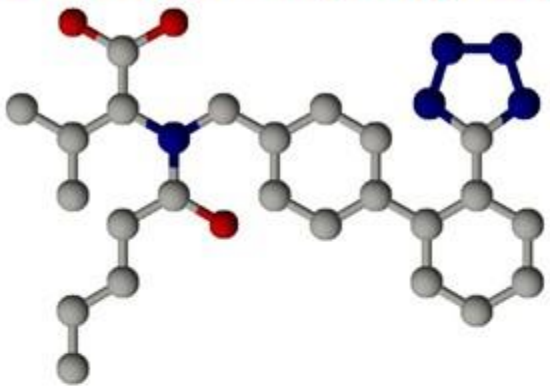
The nonlinear regression of the *A*-pH spectra with REACTLAB and SQUAD84 and of the pH-titration curve with ESAB determined two close pK_a in 11 steps of the proposed procedure. Prediction by MARVIN, PALLAS and ACD/Percepta shows the protonation sites.

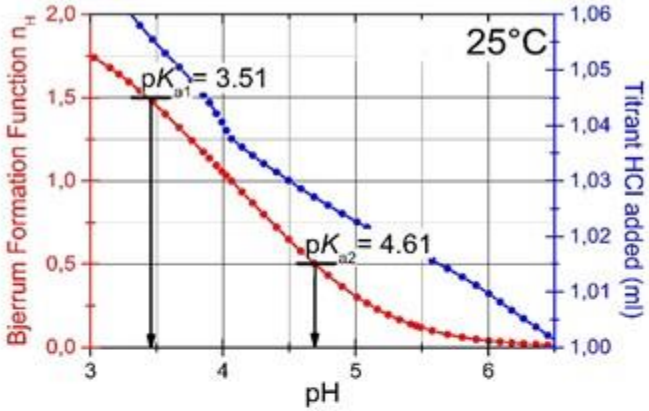
(UV-metric spectra) pK^T_{a1} = 3.70 ± 0.12, pK^T_{a2} = 4.82 ± 0.08 at 25°C
and pK^T_{a1} = 3.44 ± 0.08, pK^T_{a2} = 4.67 ± 0.02 at 37°C.
(pH-metric titration) pK^T_{a1} = 3.51 ± 0.00, pK^T_{a2} = 4.63 ± 0.00 at 25°C
and pK^T_{a1} = 3.44 ± 0.03, pK^T_{a2} = 4.51 ± 0.03 at 37°C

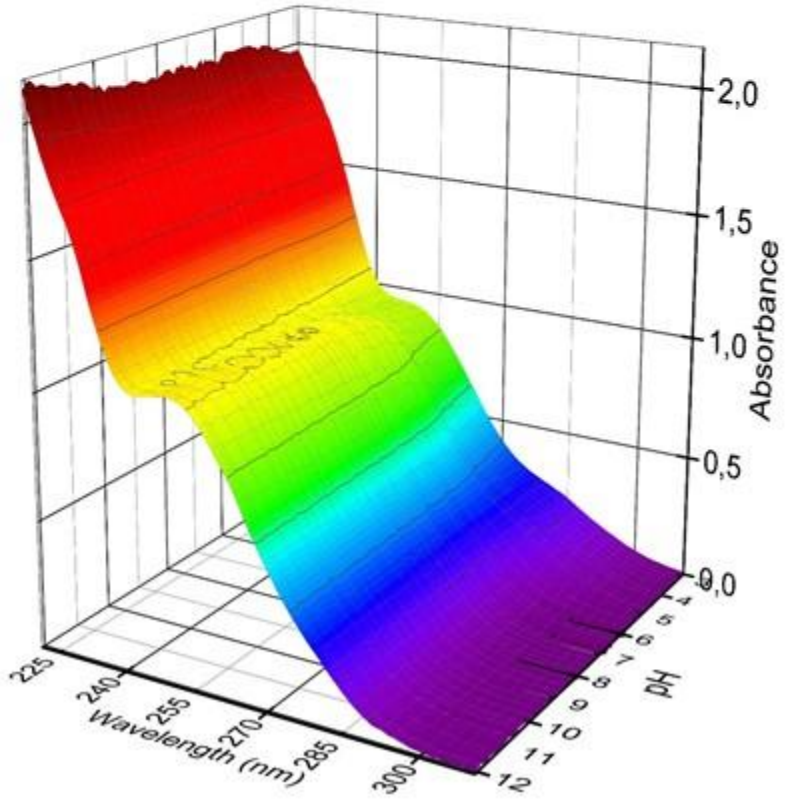
Positive enthalpy values ΔH^0 at 25°C show the dissociation process is endothermic and is accompanied by absorption of heat.
Positive value of the Gibbs free energy ΔG^0 at 25°C and 37°C indicated that the dissociation process was not proceeded spontaneously.
Negative entropy value of dissociation process ΔS^0 at 25°C and 37°C indicated the dissociation process was reversible.

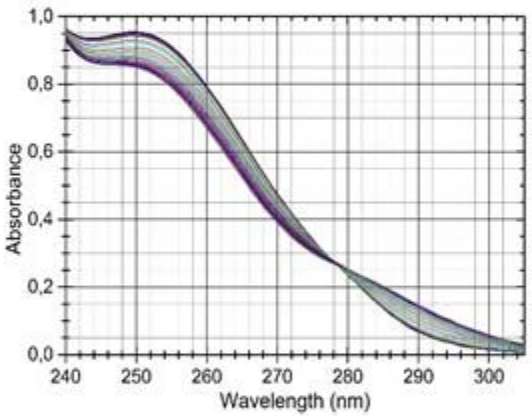
Valsartan is for treatment of hypertension, cardiac insufficiency, myocardial infarction, and diabetic nephropathy.

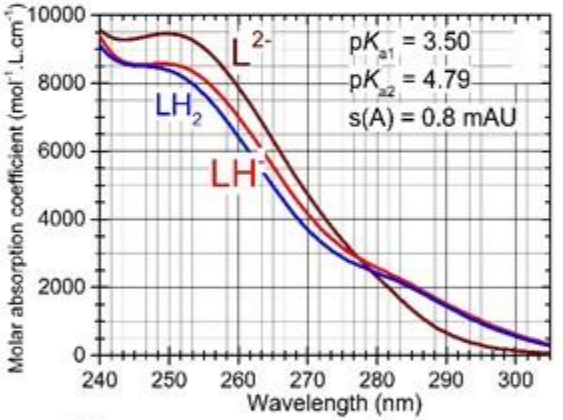


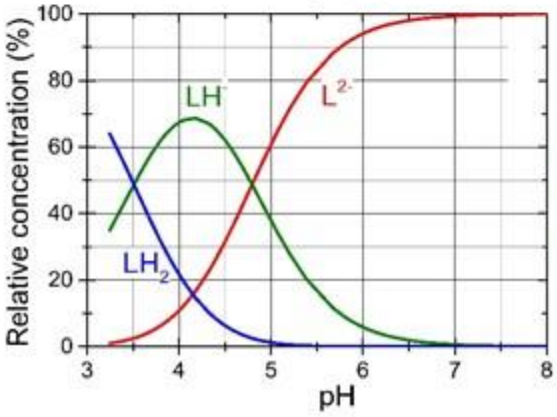


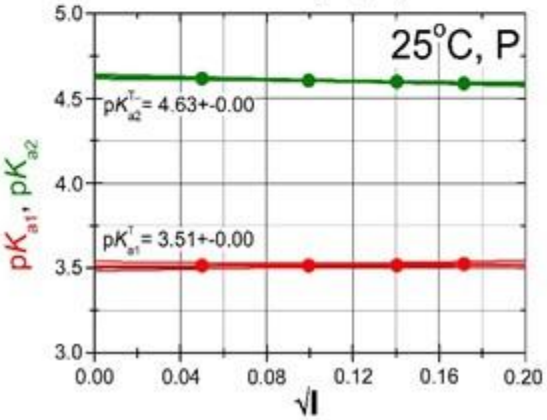






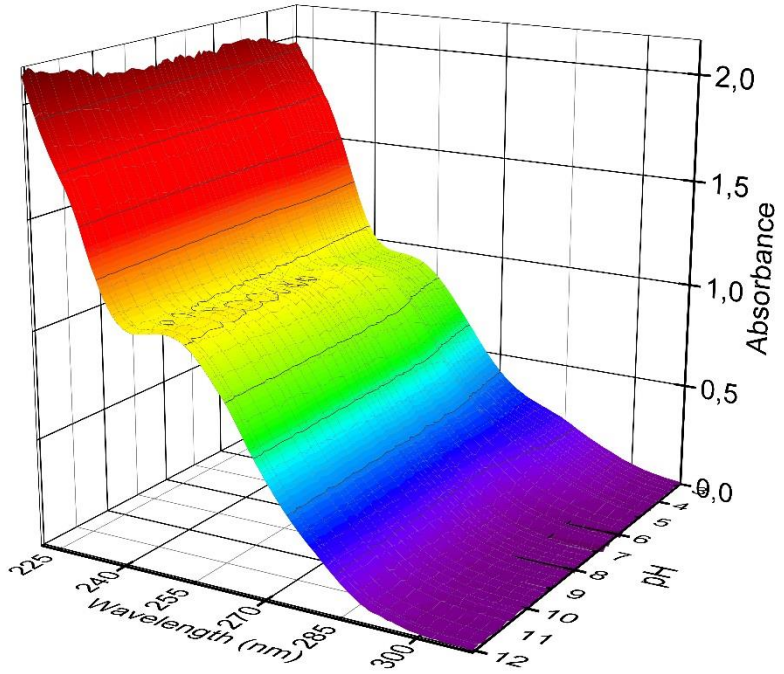






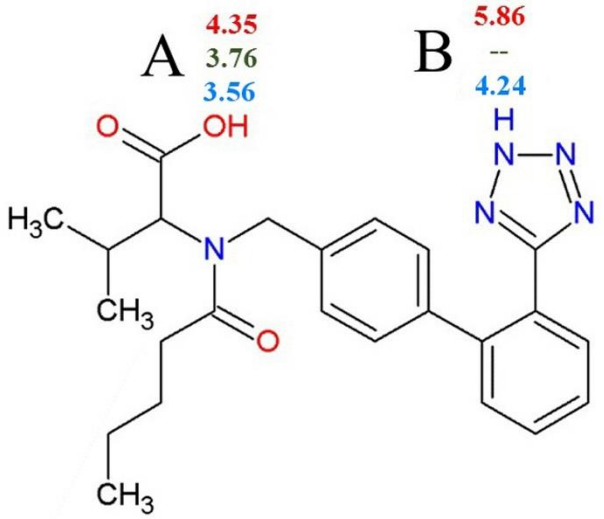
Prediction of pK_a

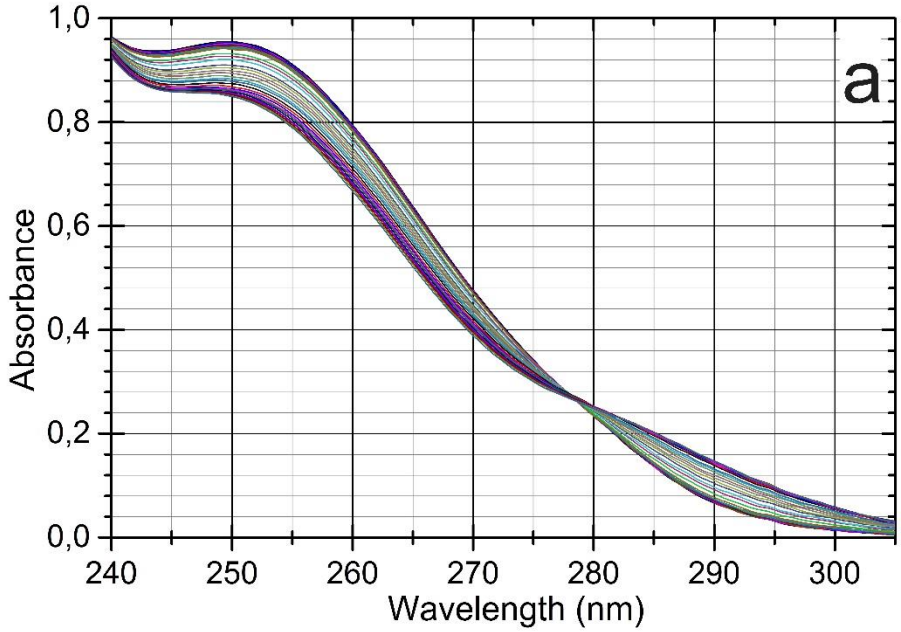
Molecular structure of Valsartan (insert) with two highlighted protonation centers A and B and predicted pK_a values using programs MARVIN, PALLAS and ACD/Percepta.



Predicted pK_{pred} of Valsartan with

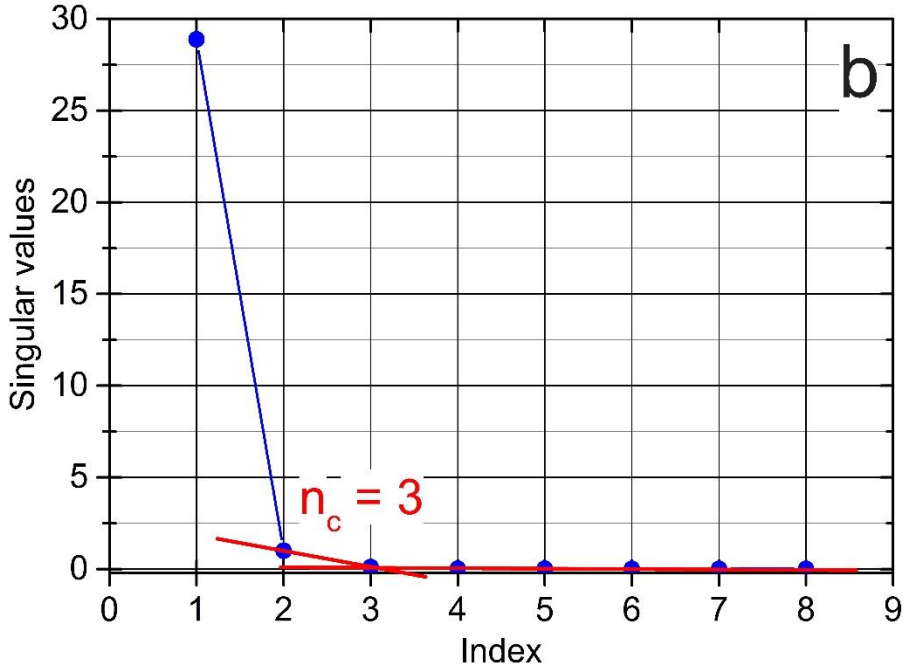
MARVIN, PALLAS, ACD





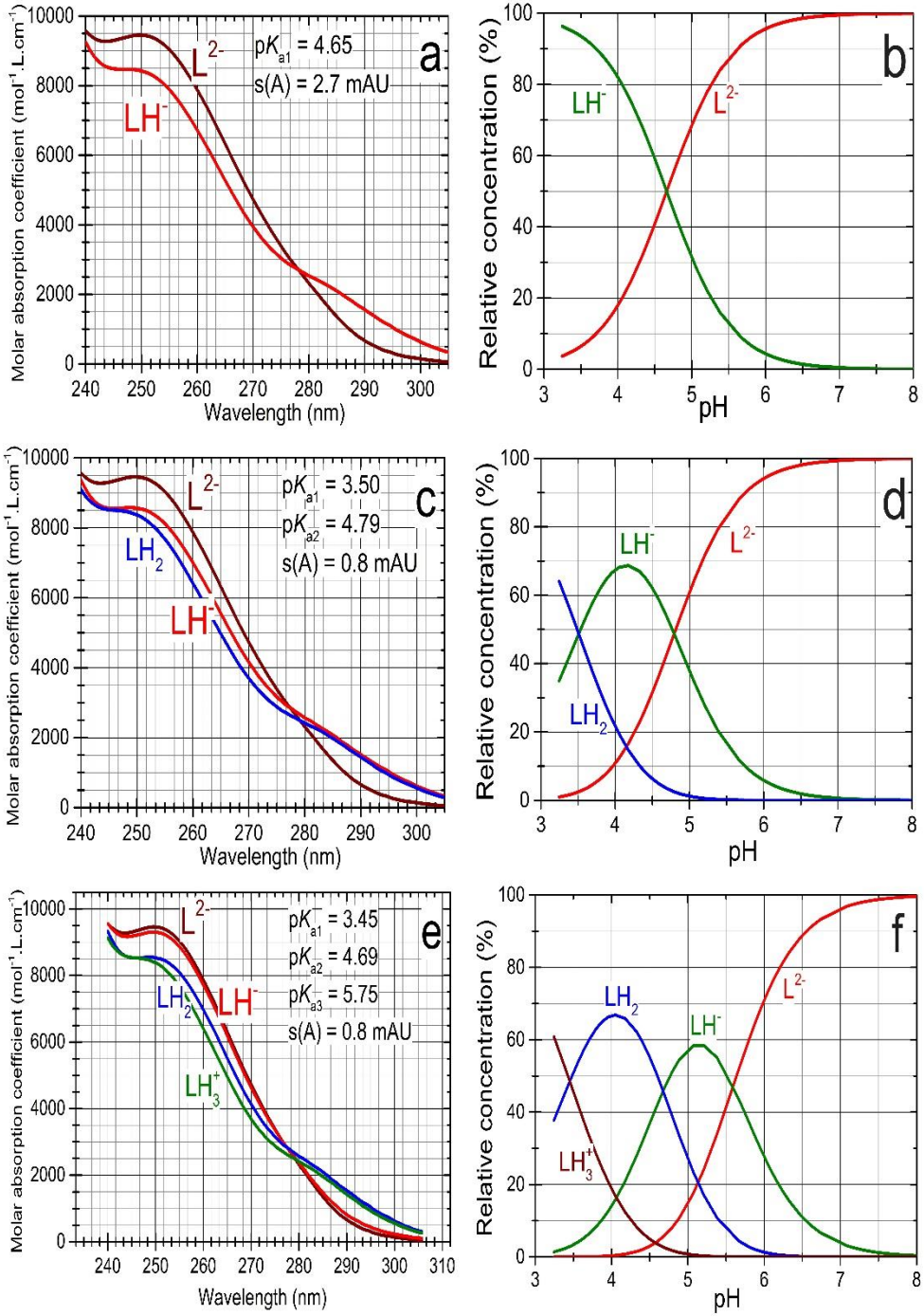
The absorbance responce surface

Graph a: The absorbance responce plot of Valsartan in dependence on pH.



Number of species with FA

Graph b: Using Cattel index graph with the residual standard deviation $s_k(A)$, the rank of the absorbance matrix is k^* = 3 for Nilotinib hydrochloride or the number of species is equal to n_c = 3. (INDICES in S-PLUS), [42].



The search in protonation model building and testing

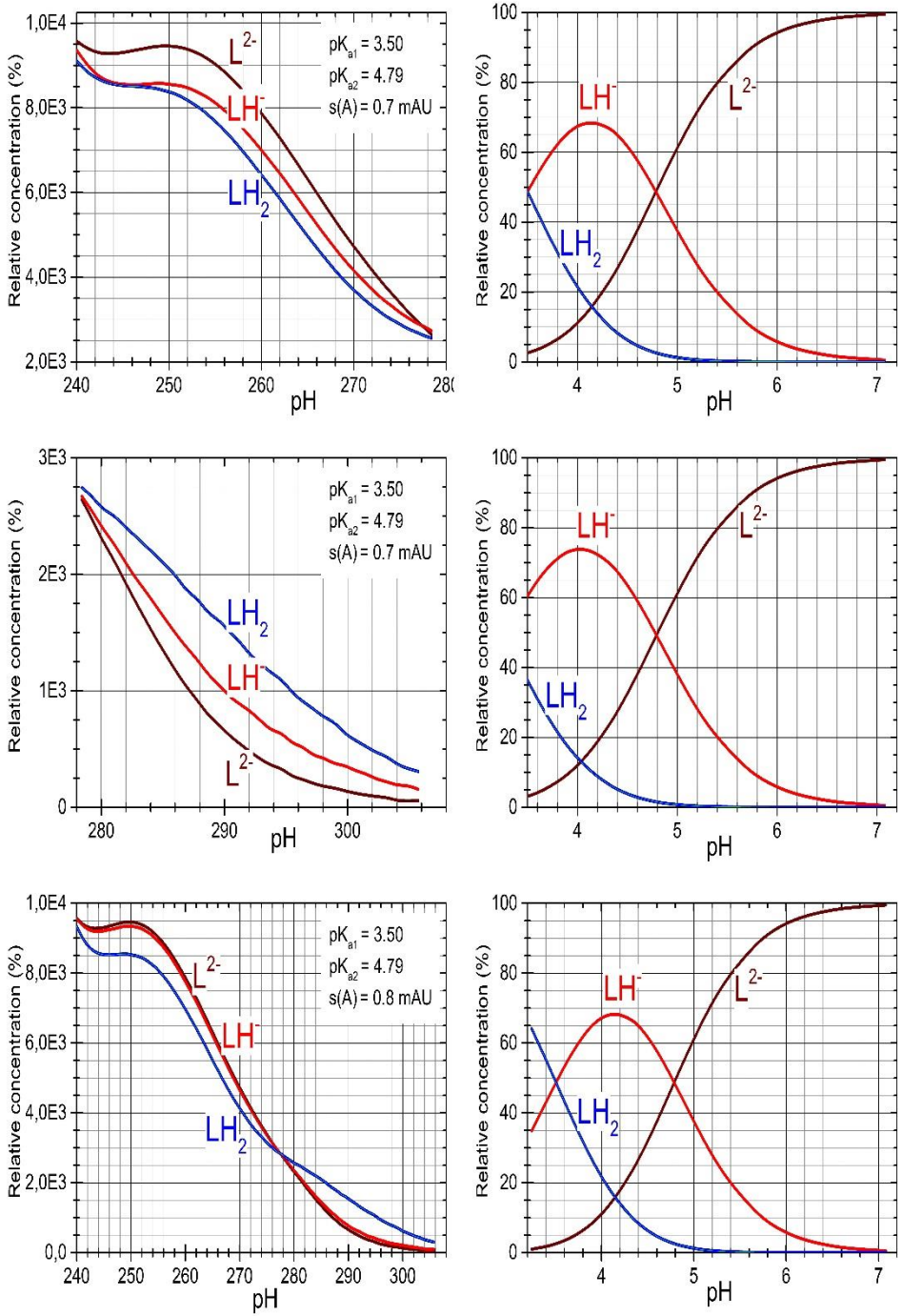
Building and testing of the best protonation model of Valsartan in the pH range of 2 to 7

for one pK_a (***Upper 2 graphs a,b***),
for two pK_a´s (***Middle 2 graphs c, d***),
for three pK_a´s (***Lower 2 graphs e, f***).

The spectra analysis of 1.0×10^{-4} mol. dm^{-3} Valsartan at 25°C.

Left graphs: Pure spectral profiles of molar absorption coefficients versus wavelength (nm) for all the variously protonated ions of Valsartan.

Right graphs: The distribution diagram of the relative concentrations of all of the variously protonated species in dependence on pH, (REACTLAB, ORIGIN 9).



Search for the effective wavelength range

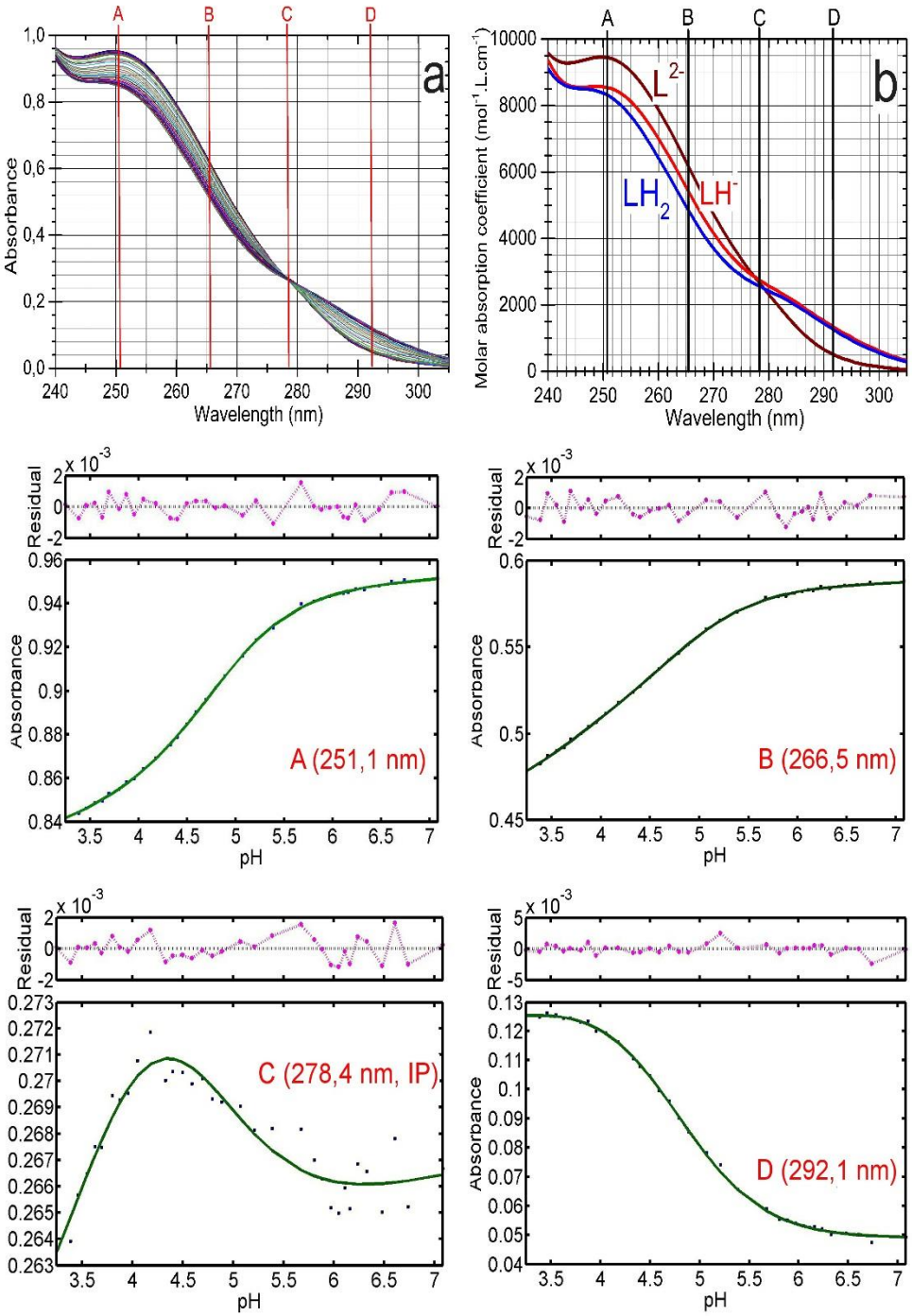
Search for the effective wavelength range to examine the position of ionizable groups and chromophores to find a sufficient absorbance change in spectrum for adjusted pH which allows a reliable determination of dissociation constants.

The protonation model of two dissociation constants was analyzed using

1) either 240 – 280 nm spectrum (***Upper 2 graphs***)
2) or by two separate absorption bands (***Middle 280 – 308 nm*** and ***Lower 2 graphs 270 – 308 nm***).

The best fitted spektra: in the 280 - 308 nm range, although pK_a estimates were similar for all three wavelength ranges.

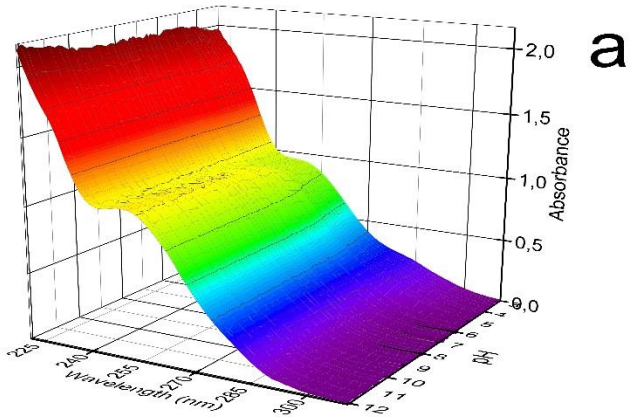
The efficient wavelength range

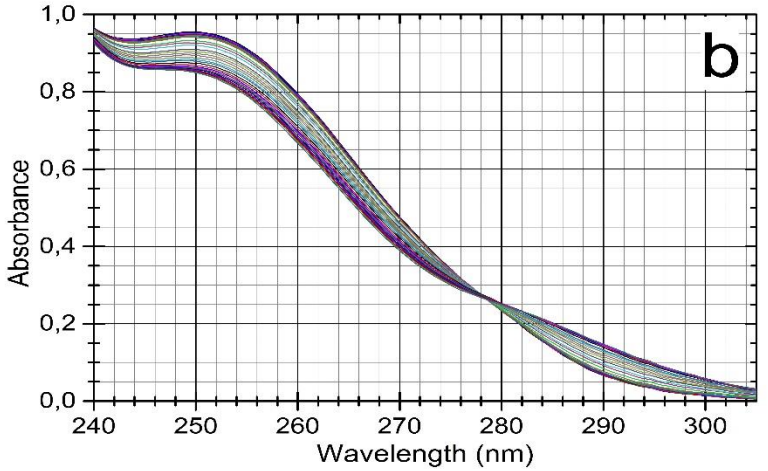


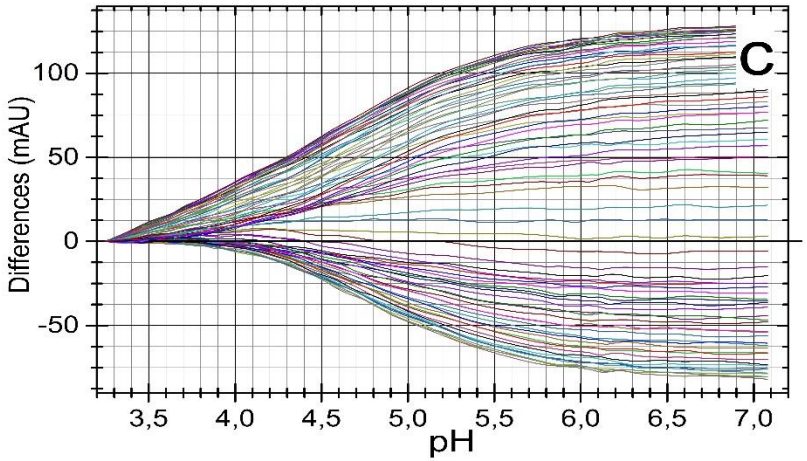
The adjusted pH did not cause the same absorbance change in the Valsartan spectrum as some chromophores were slightly affected by pH:

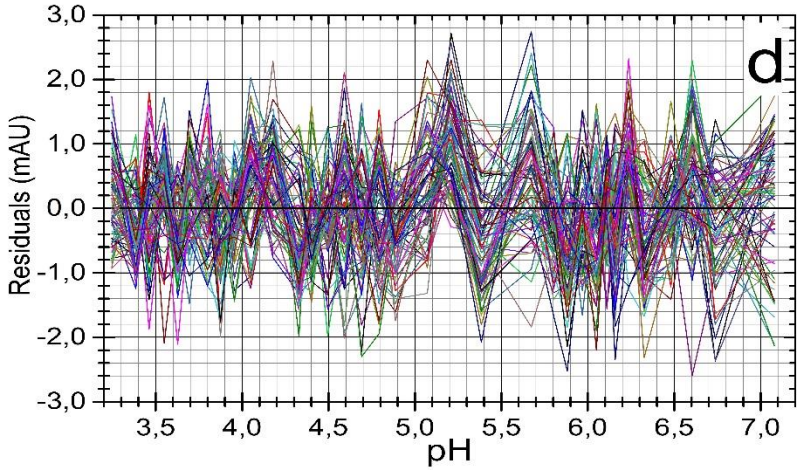
Upper graph: The spectrum of the molar absorption coefficient contains positions of four wavelengths **A** through **D** for which the A-pH curves were analyzed.

Graphs A through F: The sensitivity of chromophores in the Valsartan molecule to pH is analyzed.









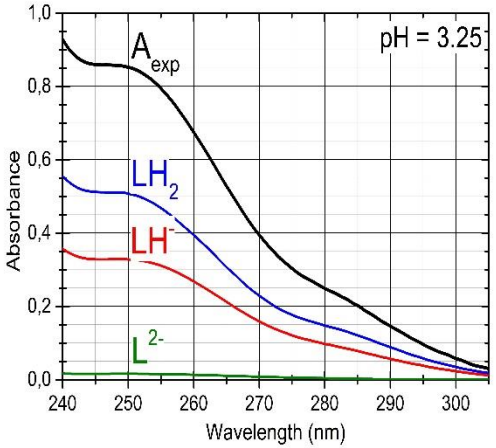
Analysis of the small changes in spectra at adjusted pH

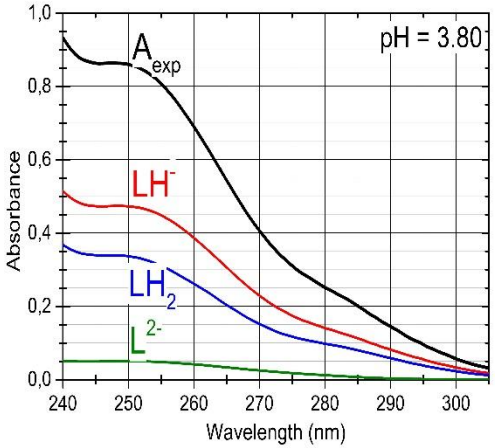
Graph a: 3D-graph of the absorbance change in the Valsartan spectrum at pH-titration.

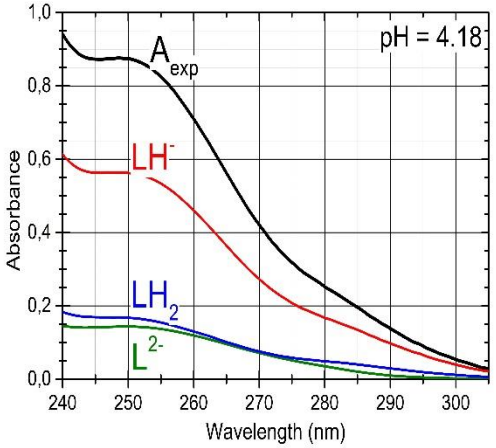
Graph b: 2D-graph of the absorbance change in the spectra set from pH_1 = 3.2 to pH_n = 7.1,

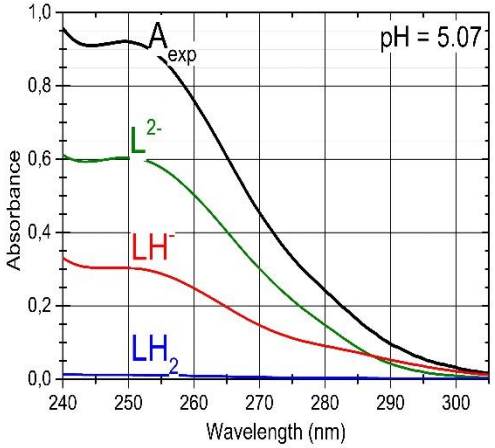
Graph c: The absorbance Differences D_{ij} [mAU] in the Valsartan spectrum from 240 to 308 nm within pH titration.

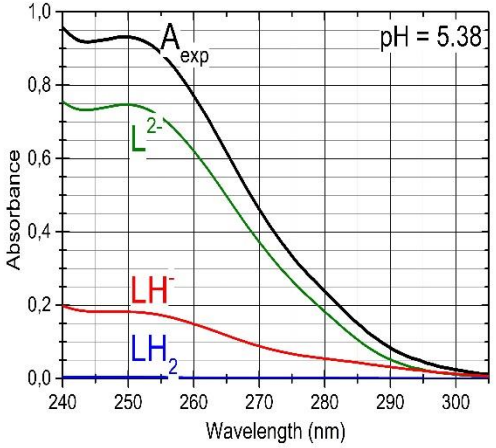
Graph ***d:*** Residuals ***e*** [mAU] show whether they were of the same size as the instrumental noise $s_{inst}(A)$, (REACTLAB, ORIGIN 9).

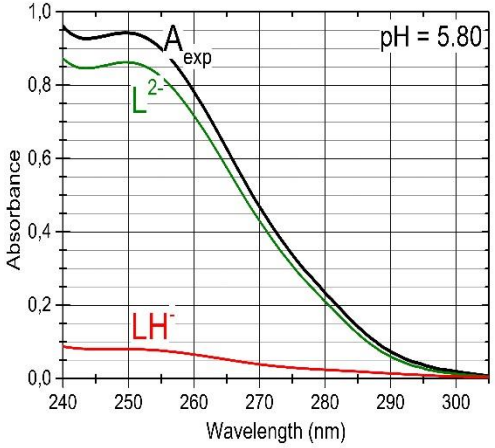










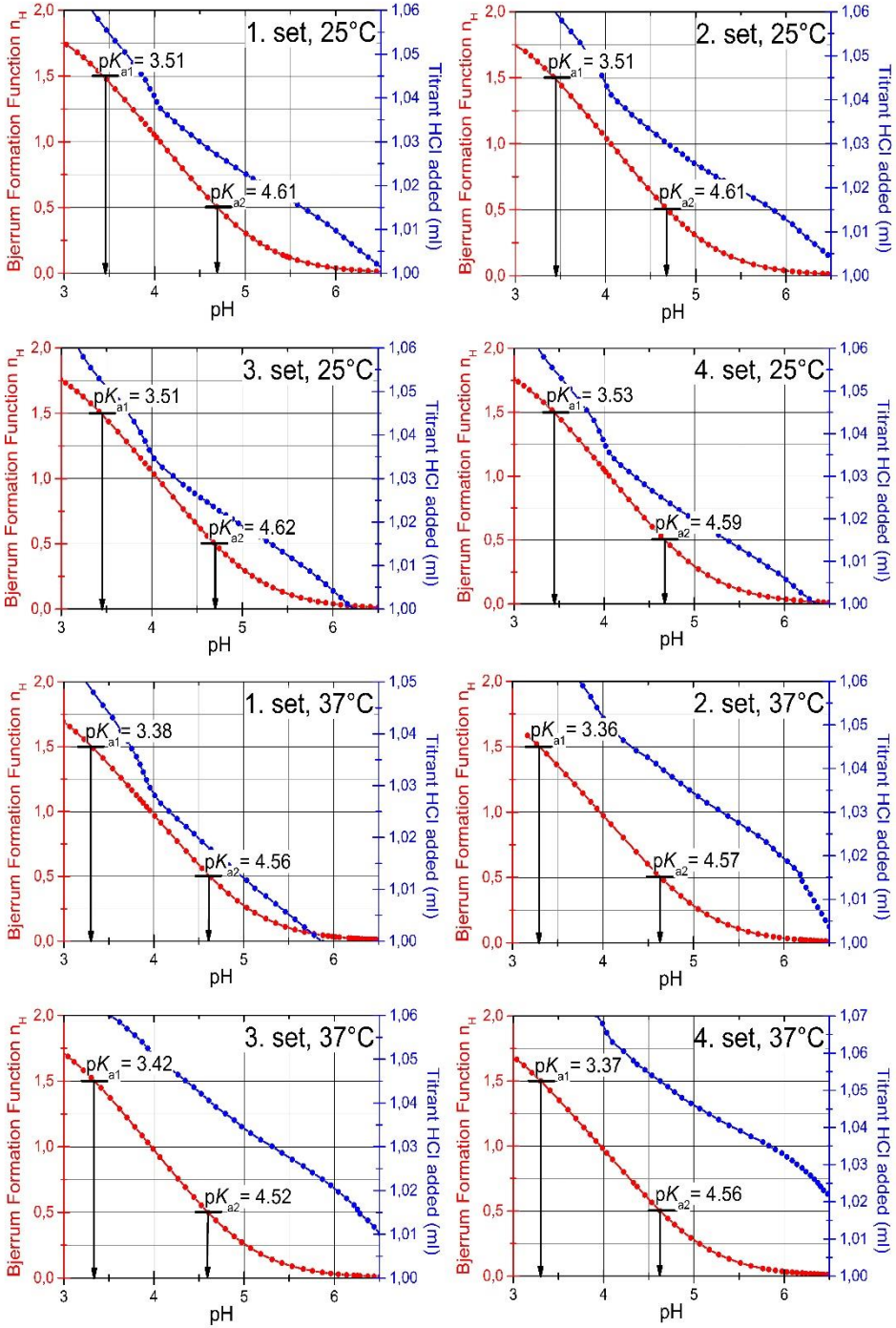


Spectra deconvolution

Deconvolution of each experimental spectrum A_{exp} of 1.0×10^{-4} mol. dm^{-3} Nilotinib hydrochloride at I = 0.0026 and 25°C into the spectra of the individual differently protonated ions L, LH^+, LH_2^{2+}, LH_3^{3+}, LH_4^{4+} in mixture for selected pH values:

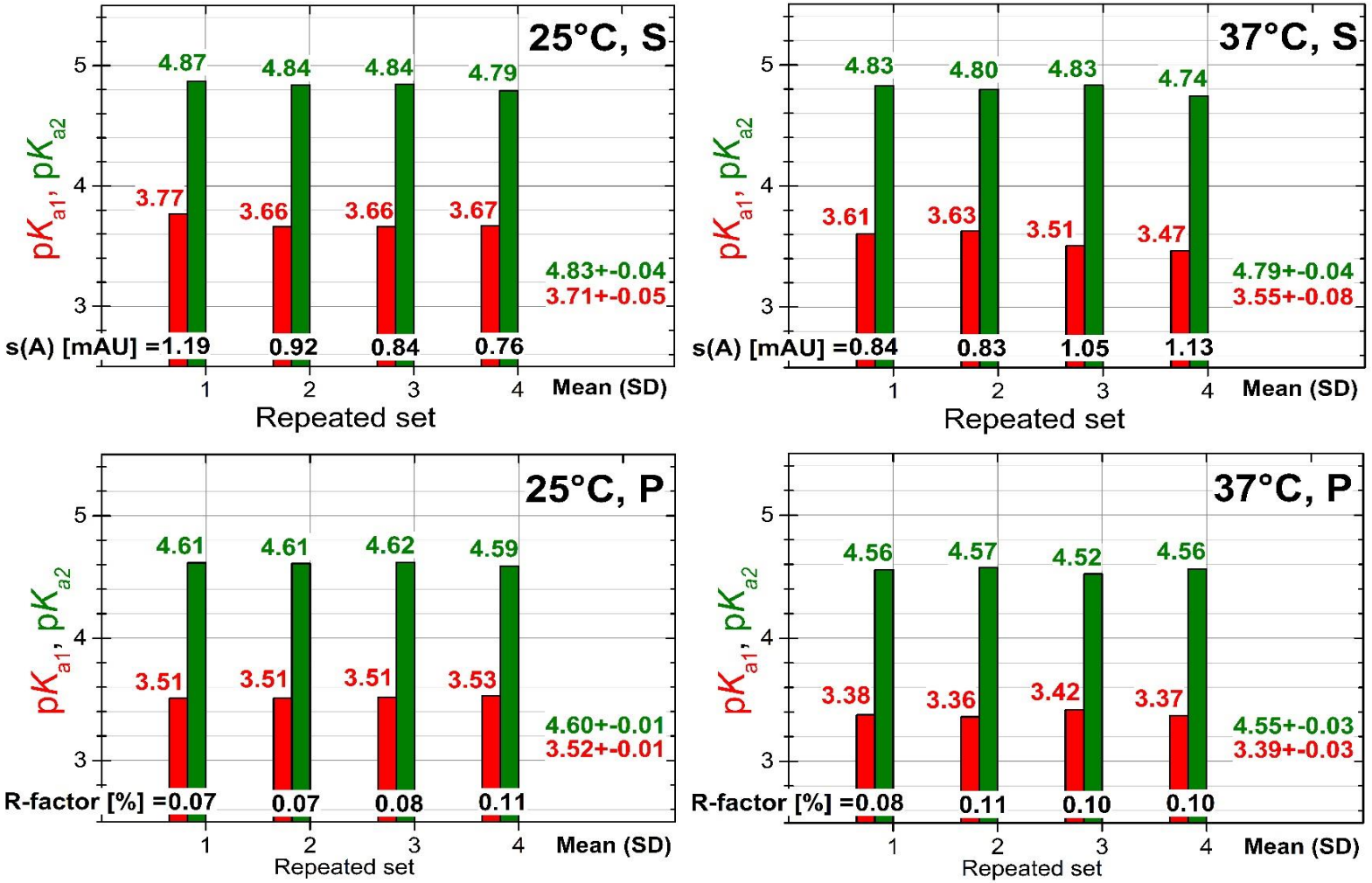
3.25,
3.80,
4.18,
5.07,
5.38,
5.80

calculated by SQUAD84.

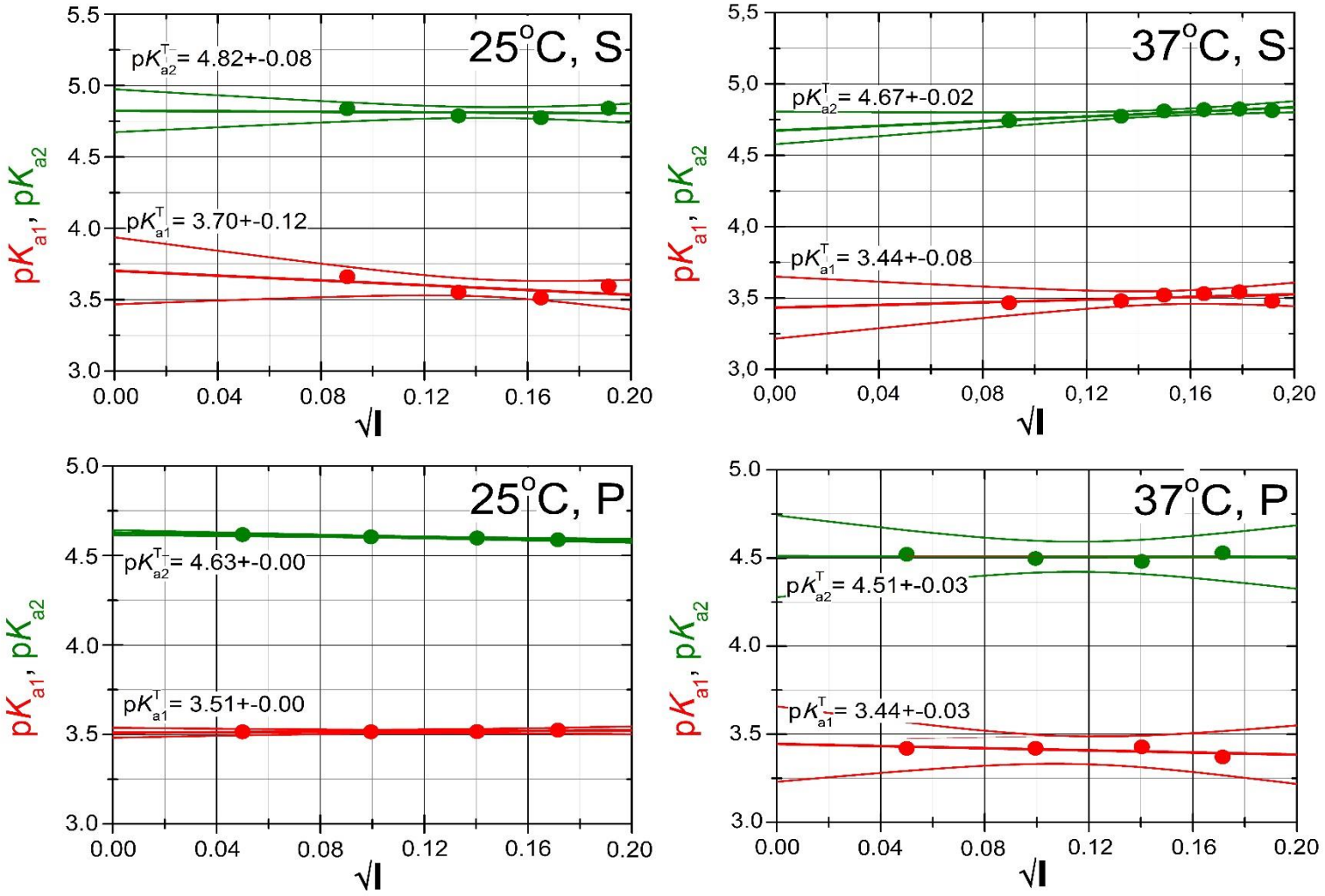


Bjerrum formation function:

Reproducibility in the search for the protonation model analysing four pH potentiometric titration curves at 25°C and 37°C. Acidified Nilotinib hydrochloride was titrated with KOH and the Bjerrum protonation curve was plotted for four pK_a. The residual graphs show the best curve fitting of titration curves (ESAB, ORIGIN).



The reproducibility of Valsartan dissociation constants of the four reproduced UV-metric spektra analysis (***graph 25°C, S*** and ***graph 37°C, S***) and four reproduced pH-metric titration curves (***graph 25°C, P*** **and** ***graph 37°C, P***) were in an agreement. Reproducibility of protonation model estimates with two dissociation constants was compared. The arithmetic mean of dissociation constants with the confidence interval was expressed on the base of the standard deviation *s*(*A*) and *s*(*V*), (REACTLAB, SQUAD84, ESAB, ORIGIN 9).



Thermodynamic dissociation constant pK^{T}_{a}**:** Dependence of the mixed dissociation constants of Valsartan on the square-root of the ionic strength for the two dissociation constants leading to the pK^{T}_{a} using the spectra analysis (***graph 25°C, S*** and ***graph 37°C, S***) and pH-metric technique (***graph 25°C, P*** and ***graph 37°C, P***). The straight lines are drawn with their Working-Hotteling confidence bands (QCEXPERT).

Conclusion

(1)Spectrophotometric and potentiometric pH titration allowed measurement of up to two close dissociati on constants of Valsartan.

(2) Valsartan was capable of protonation to produce in pure water four soluble species LH^-, LH_2. The graph of the molar absorption coefficients of differently protonated species in relation to wavelength indicated that the spectrum of ε_L, ε_{LH}, ε_{LH2} were for two pairs of species correlated and values in each pair were almost the same.

(3) In the range of pH 2 to 7, four dissociation constants could be reliably estimated from the spectrum when the concentration of the sparingly soluble Nilotinib with SQUAD84 and REACTLAB pK^T_{a1} = 3.60±0.04, pK^T_{a2} = 4.42±0.07, pK^T_{a3} = 4.71±0.04, pK^T_{a4} = 4.84±0.03 at 25°C and pK^T_{a1} = 3.61±0.11, pK^T_{a2} = 4.29±0.18, pK^T_{a3} = 4.49±0.02, pK^T_{a4} = 5.05±0.03 at 37°C.

4) The four thermodynamic dissociation constants of Nilotinib hydrochloride were determined by regression analysis of potentiometric titration curves at a potentiometric concentration of 3×10^{-4} mol. dm^{-3} with ESAB, pK^T_{a1} = 3.74±0.01, pK^T_{a2} = 4.05±0.01, pK^T_{a3} = 4.25±0.01, pK^T_{a4} = 4.91±0.20 at 25°C and pK^T_{a1} = 3.63±0.03, pK^T_{a2} = 3.96±0.03, pK^T_{a3} = 4.18±0.03, pK^T_{a4} = 4.81±0.05 at 37°C.

(5) Prediction of the dissociation constants of Nilotinib hydrochloride was performed by the programs MARVIN, PALLAS and ACD/Percepta to determine protonation sites.

(6) **Positive enthalpy values ΔH^0** at 25°C show that the dissociation process is endothermic and is accompanied by absorption of heat. **Positive value of the Gibbs free energy ΔG^0** at 25°C and 37°C indicated that the dissociation process was not proceeded spontaneously. **Negative entropy value ΔS^0** of dissociation process ΔS^0 at 25°C and 37°C means the dissociation process is reversible.