

The Overlapping Thermodynamic Dissociation Constants of the Antidepressant Vortioxetine Using UV-VIS Multiwavelength pH-Titration Data

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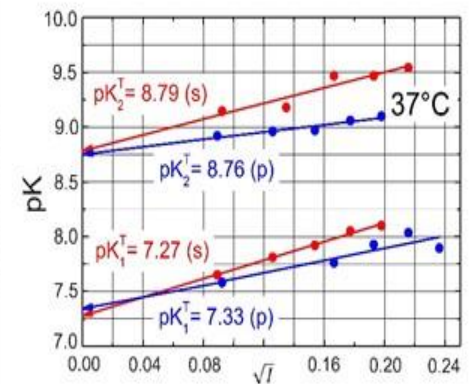
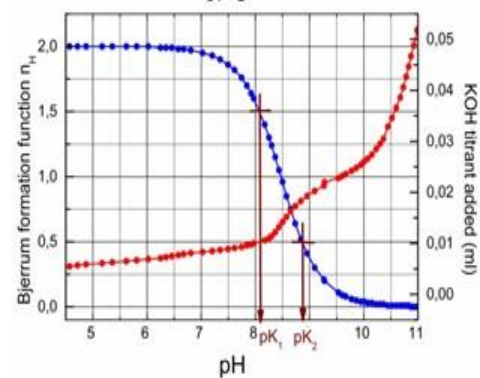
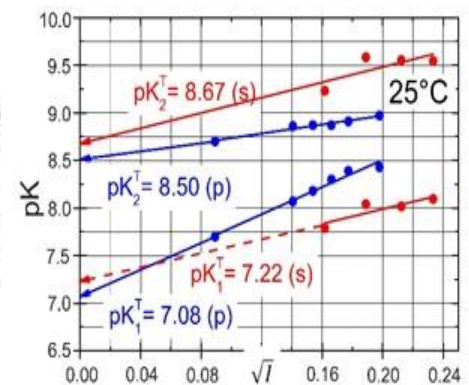
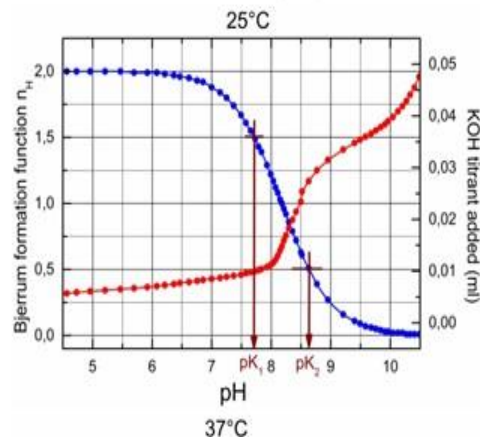
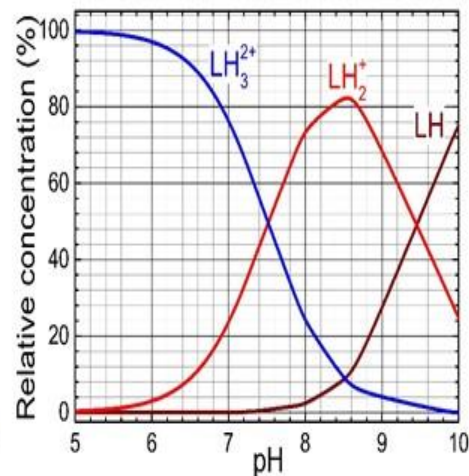
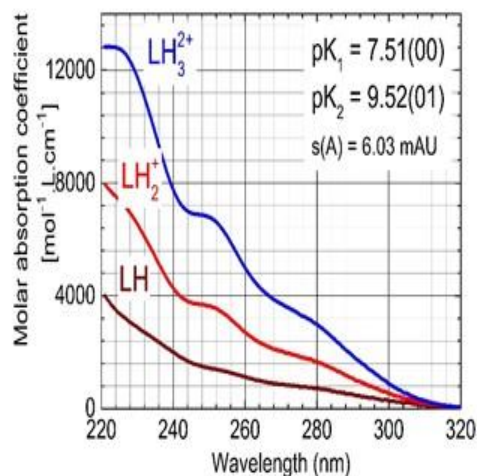
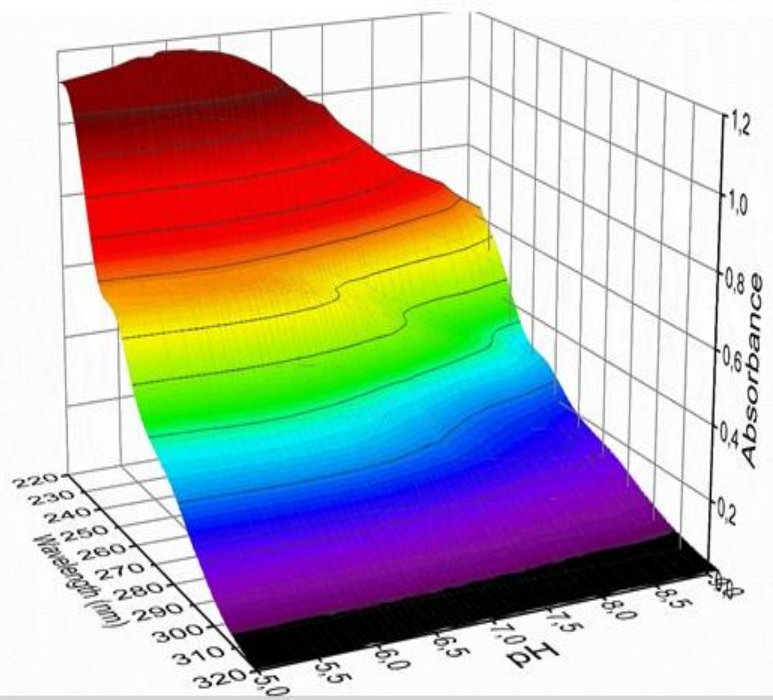
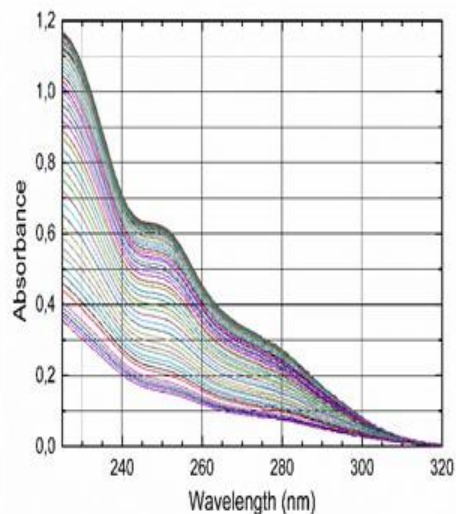
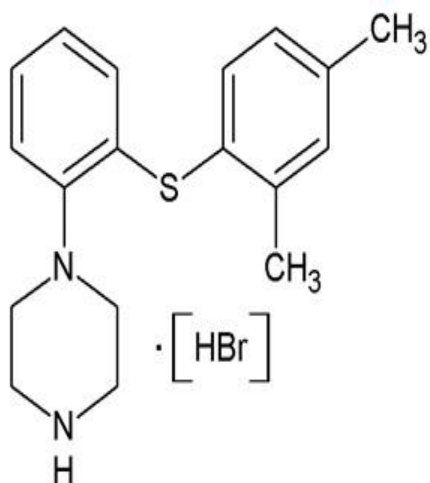
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Graphical abstract shows input (left) and output (right)

Vortioxetine hydrobromide



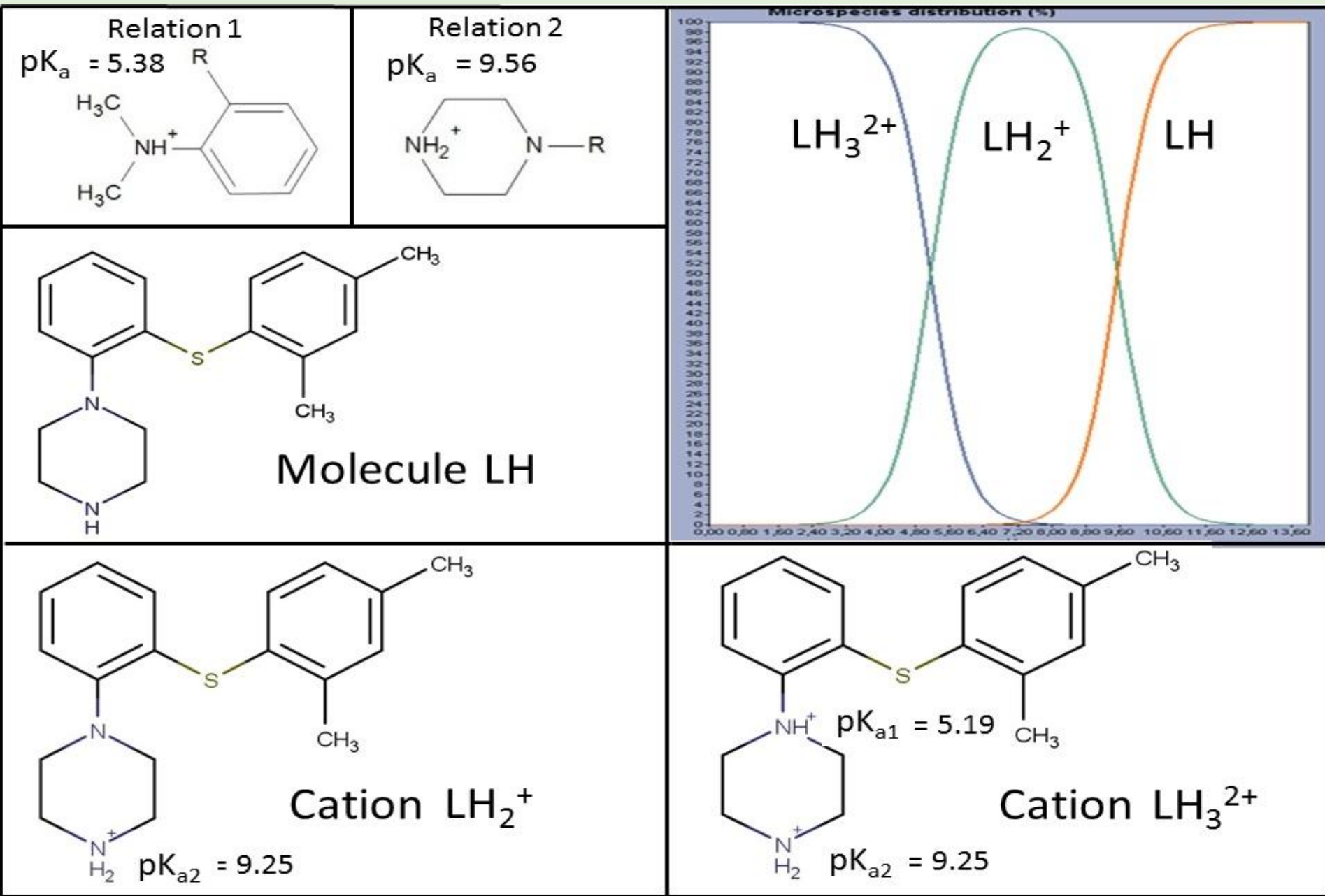
Abstract

Potentiometric and spectrophotometric pH-titrations of the antidepressant Vortioxetine for dissociation constants determination were compared. Vortioxetine is an atypical antidepressant, i.e. a serotonin modulator and stimulator. Depressive disorders are common mental health conditions thought to be caused by an imbalance in serotonin and norepinephrine in addition to multiple situational, cognitive, and medical factors.

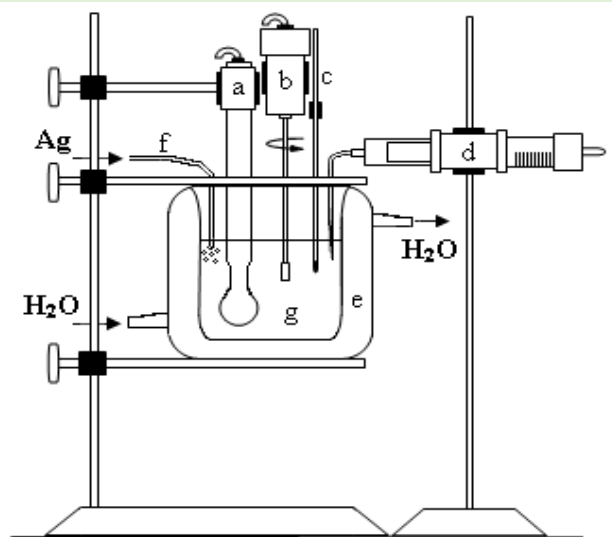
The nonlinear regression of the pH-spectra (REACTLAB, SQUAD84) and pH-titration (ESAB) determined two overlapping dissociation constants. Vortioxetine hydrobromide was capable of protonation to form the still soluble two cations LH_2^+ and LH_3^{2+} in pure water. Two thermodynamic dissociation constants were estimated $\text{p}K_{\text{a}1}^{\text{T}} = 7.22$ and $\text{p}K_{\text{a}2}^{\text{T}} = 8.67$ at 25°C and $\text{p}K_{\text{a}1}^{\text{T}} = 7.27$ and $\text{p}K_{\text{a}2}^{\text{T}} = 8.79$ at 37°C . The graph of molar absorption coefficients of protonated species on wavelength shows that the spectrum of species LH_2^+ and LH vary in colour, while protonation of chromophore LH_2^+ to LH_3^{2+} has less influence on chromophores in Vortioxetine hydrobromide molecule. Two thermodynamic dissociation constants of Vortioxetine were determined by a regression of potentiometric titration curves $\text{p}K_{\text{a}1}^{\text{T}} = 7.08$ and $\text{p}K_{\text{a}2}^{\text{T}} = 8.50$ at 25°C and $\text{p}K_{\text{a}1}^{\text{T}} = 7.33$ and $\text{p}K_{\text{a}2}^{\text{T}} = 8.76$ at 37°C .

A prediction of the $\text{p}K_{\text{a}1}^{\text{T}}$ and $\text{p}K_{\text{a}2}^{\text{T}}$ of Vortioxetine was carried out with MARVIN and ACD/Percepta programs and two dissociation constants were theoretically proposed.

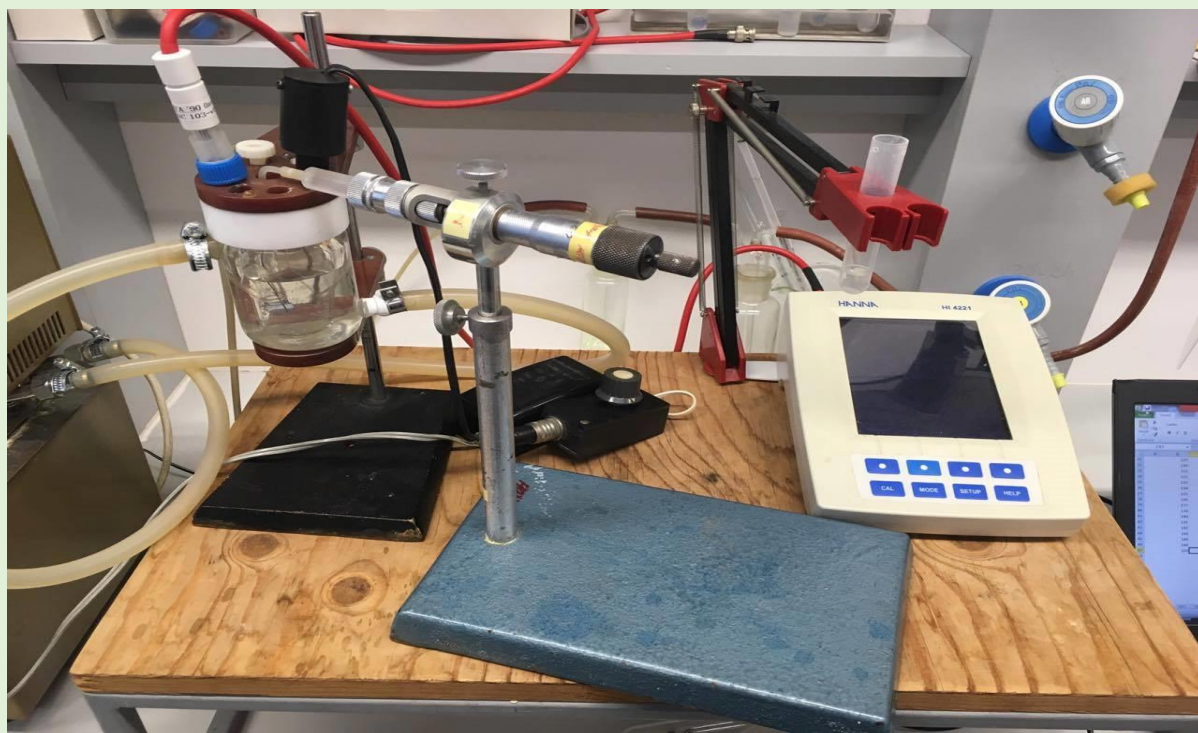
Prediction of protonation model and diagram of two pK_a



Experimental equipment

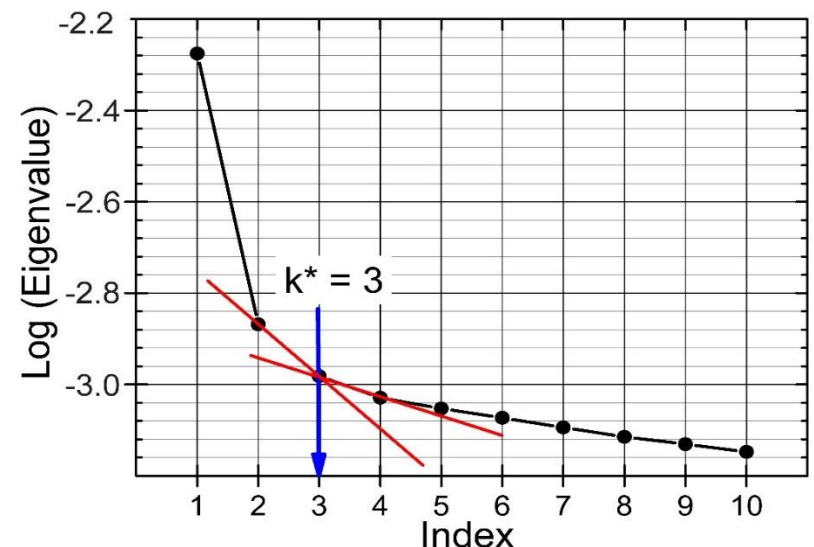
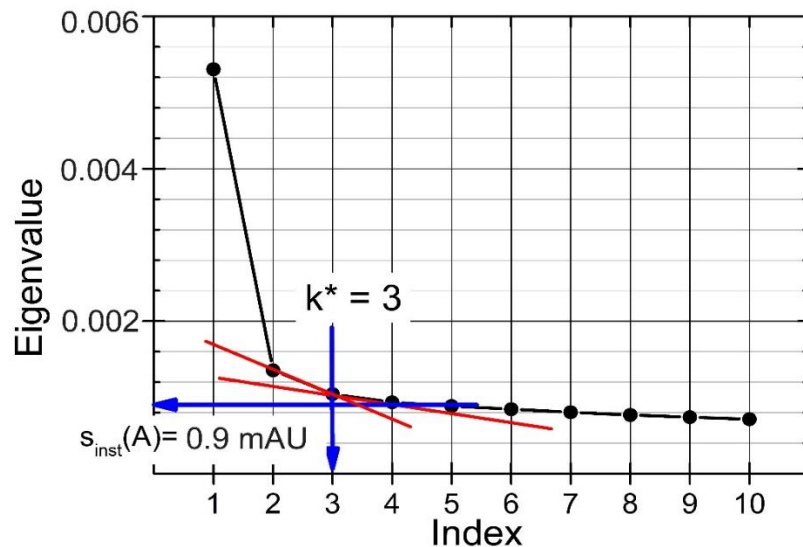
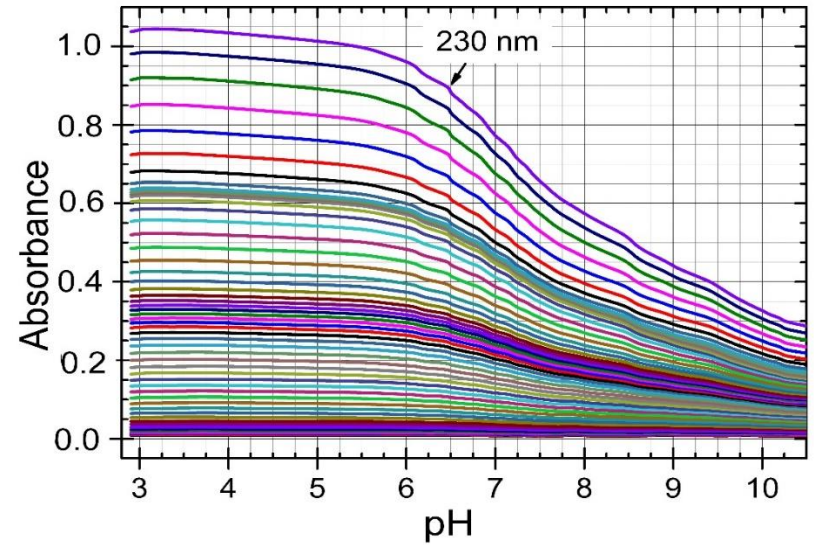
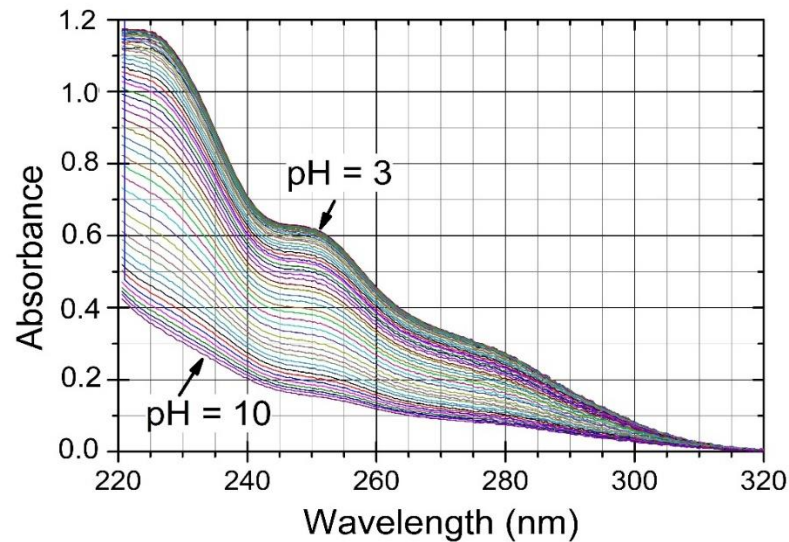


- Glass electrode HC103 (THETA '90) – high precision
- Digital pH-metr HANNA HI 3220 (measurement pH in a range -2.00 to 20.00 with the precision ± 0.002 pH)
- Thermostat ED-5 (JULABO), thermometer
- Input of argon using polyethylene tube to keep carbondioxide-free solution
- Piston microburette for very precise dosing of KOH solution or HCl ($\pm 0,1\mu\text{L}$)



Factor analysis of spektra in UV-metric spectra analysis

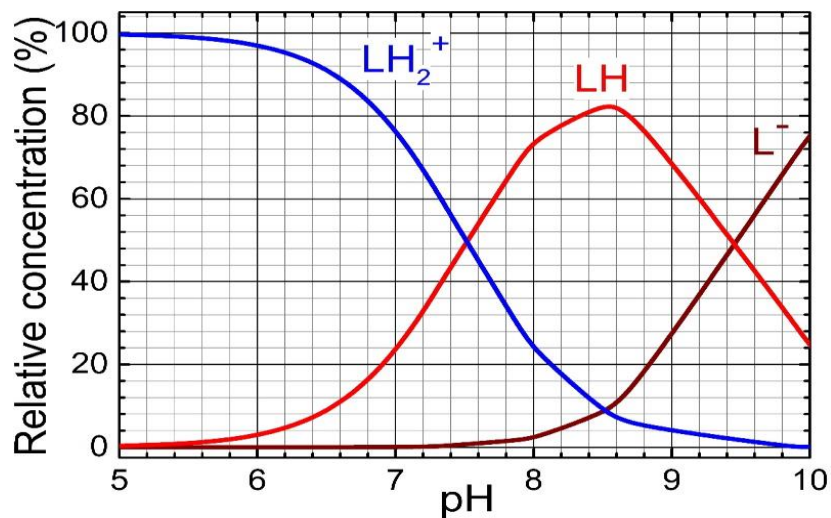
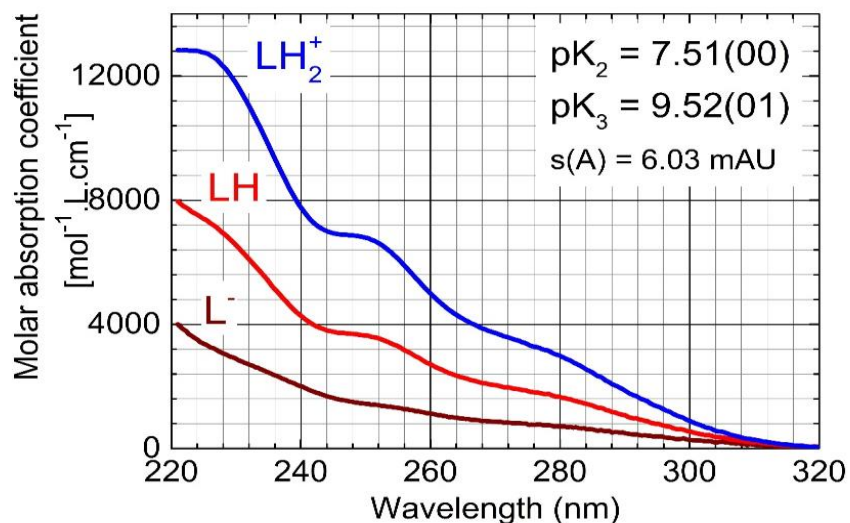
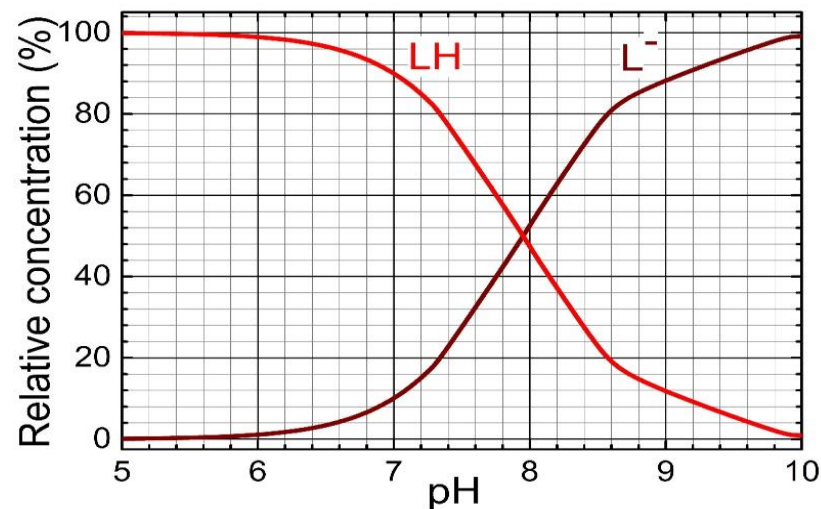
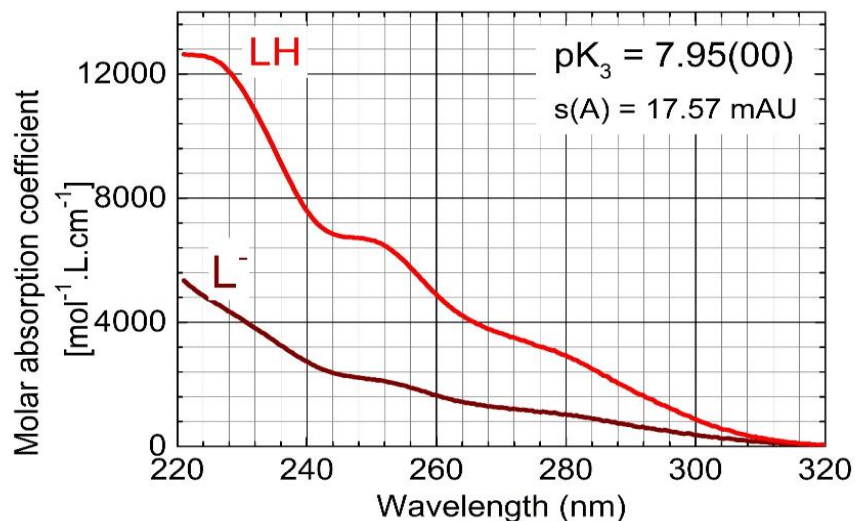
In a factor analysis the rank of the absorbance matrix is estimated by the Cattell graph (Scree plot). The rank is equal to the number of light-absorbing species in a mixture.



A search of protonation model in UV-metric analysis

Graph of the molar absorption coefficients of all variously protonated Vortioxetine species of proposed protonation model using non-linear regression of the spectra

Distribution diagram of the relative concentration of all variously protonated Vortioxetine species



UV-spectra deconvolution

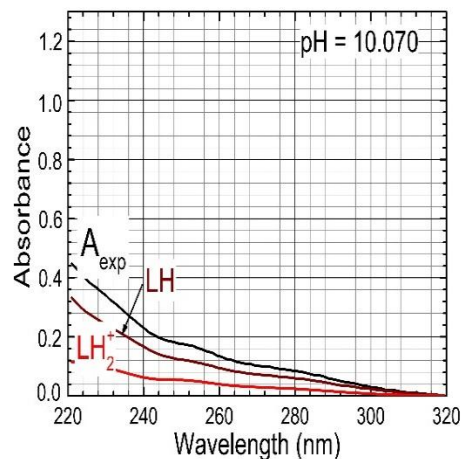
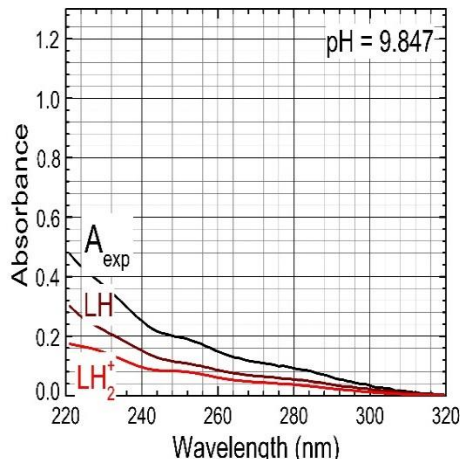
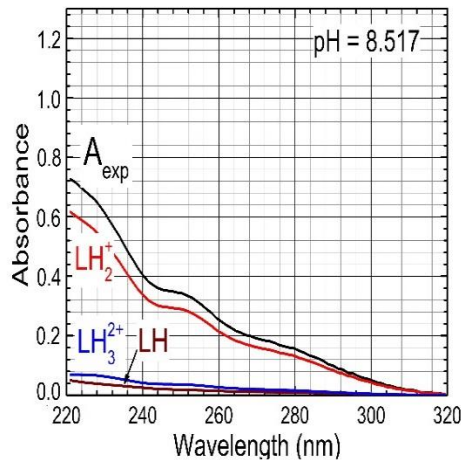
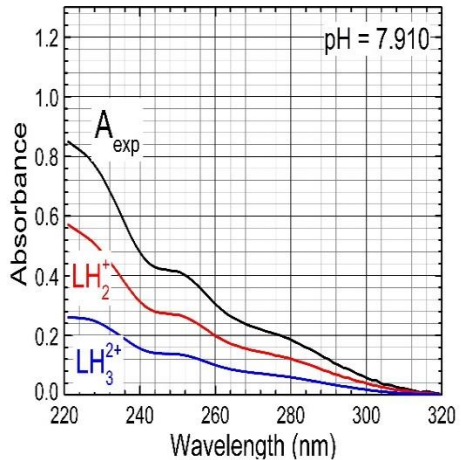
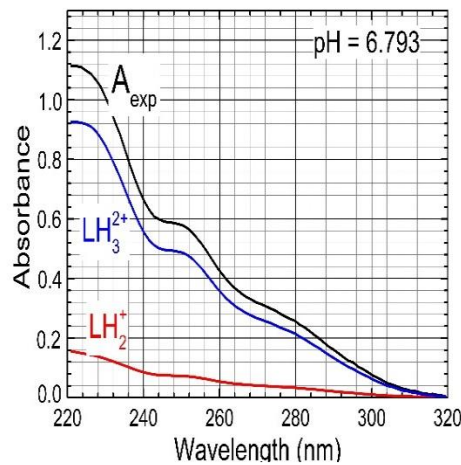
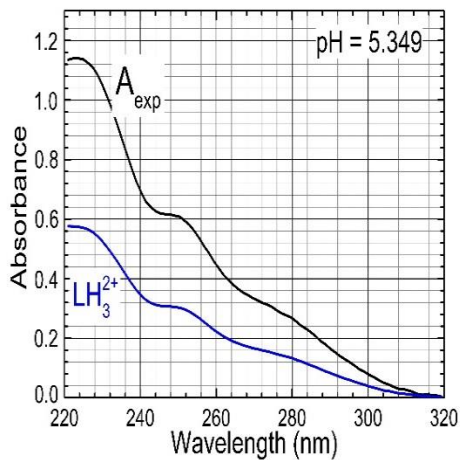
Six following graphs show the consecutive deprotonation response in spectra: each experimental spectrum was decomposed into the spectra of variously protonated species in a mixture of Vortioxetine:

At **pH = 5.35** the cation LH_3^{2+} predominates in the solution.

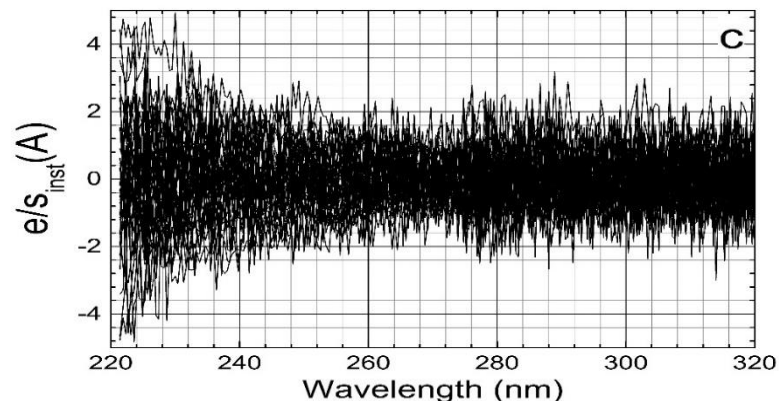
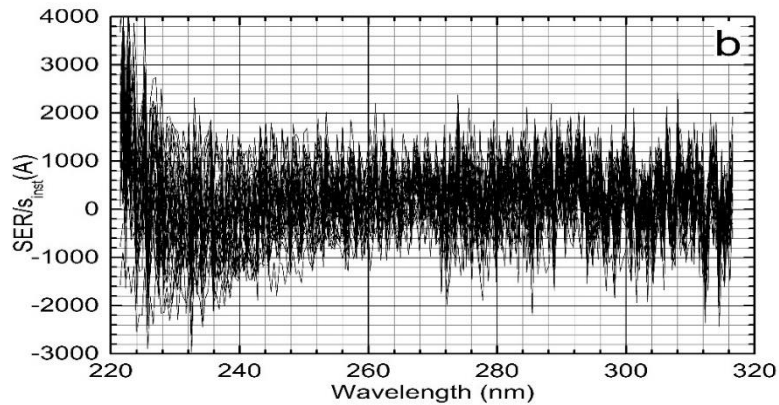
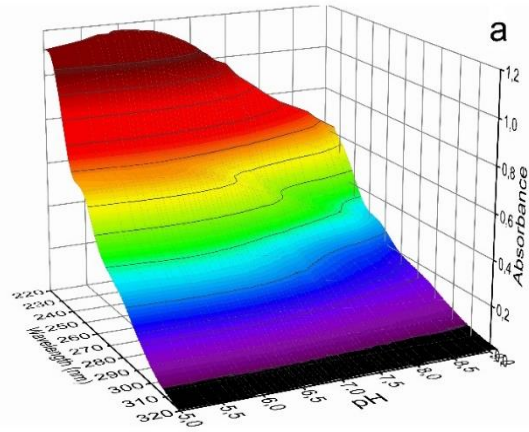
At **pH = 6.79** together with the cation LH_2^+ one dominant species LH_3^{2+} exhibits an absorption band at the same wavelength of the absorption maximum λ_{max} .

At **pH = 7.91** and **8.52** the experimental spectrum is decomposed into two absorption bands concerning the cation LH_3^{2+} and LH_2^+ .

At **pH = 9.85** the neutral molecule LH occurs with cation LH_2^+ , and the concentration of LH in the solution increases up to pH = 10.07.



Signal-to-error ratio in analysis of small spectra changes



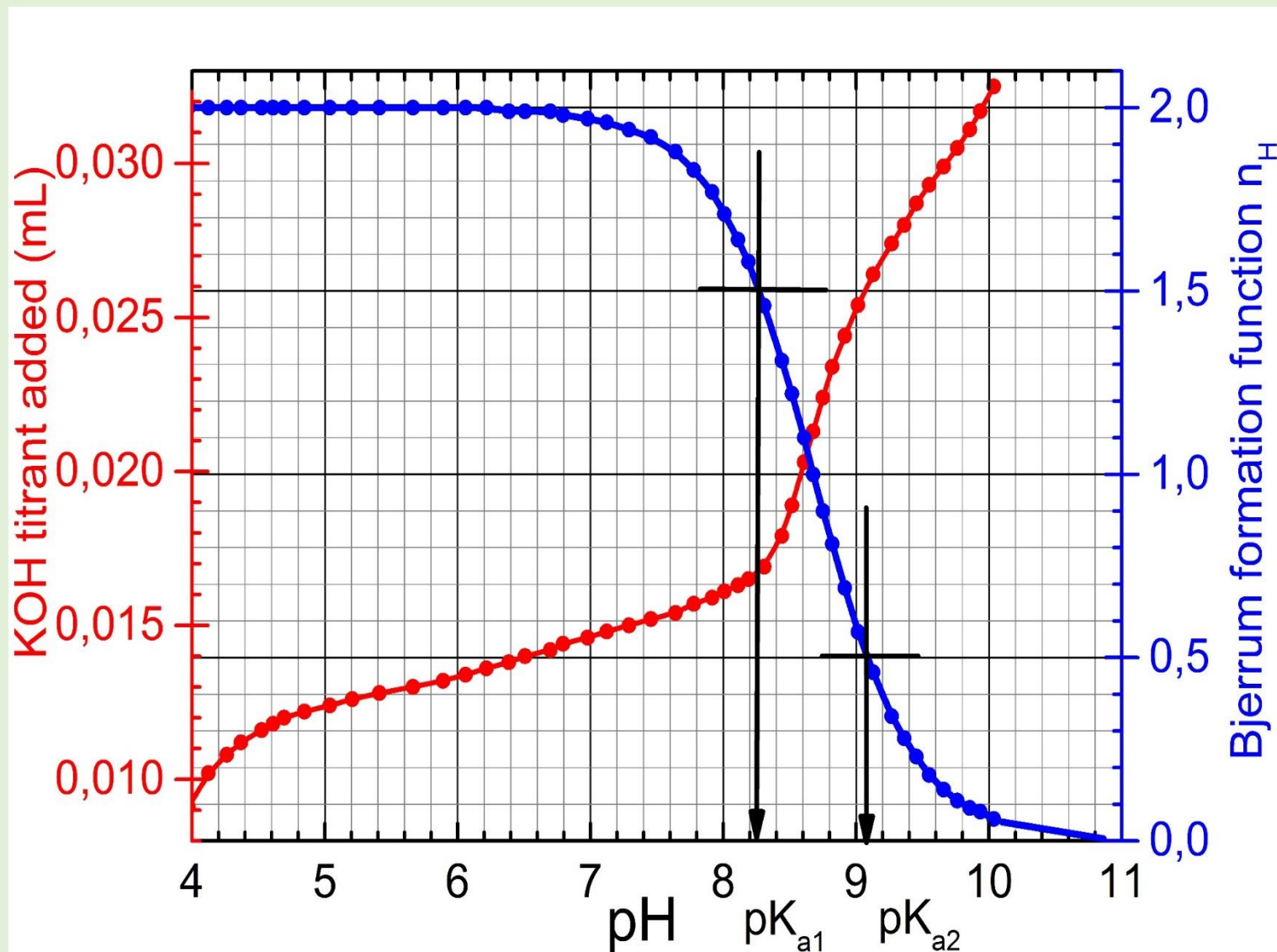
(a) 3D-plot of the absorbance-response-matrix is analyzed.

(b) The resulting ratio of the normalized spectra changes $SER = \Delta/s_{\text{inst}}(A)$ are plotted according to wavelength λ for all absorbance matrix elements. The SER ratio is then compared to the limiting SER value and to test if the small absorbance changes are still significantly larger than the instrumental noise. When the SER value is greater than 10, a factor analysis is able to predict the correct number of light-absorbing components in the equilibrium mixture. To prove that the non-linear regression can analyze such spectral data, the residuals set was compared to the instrumental noise, $s_{\text{inst}}(A)$.

(c) Figure shows a comparison of the ratio of the residuals of spectra normalized against instrumental noise, $e/s_{\text{inst}}(A)$, according to wavelength for the measured Vortioxetine. It is clear that most of the residuals are of the same magnitude as the instrumental noise and the ratio $e/s_{\text{inst}}(A)$ is less than 2.

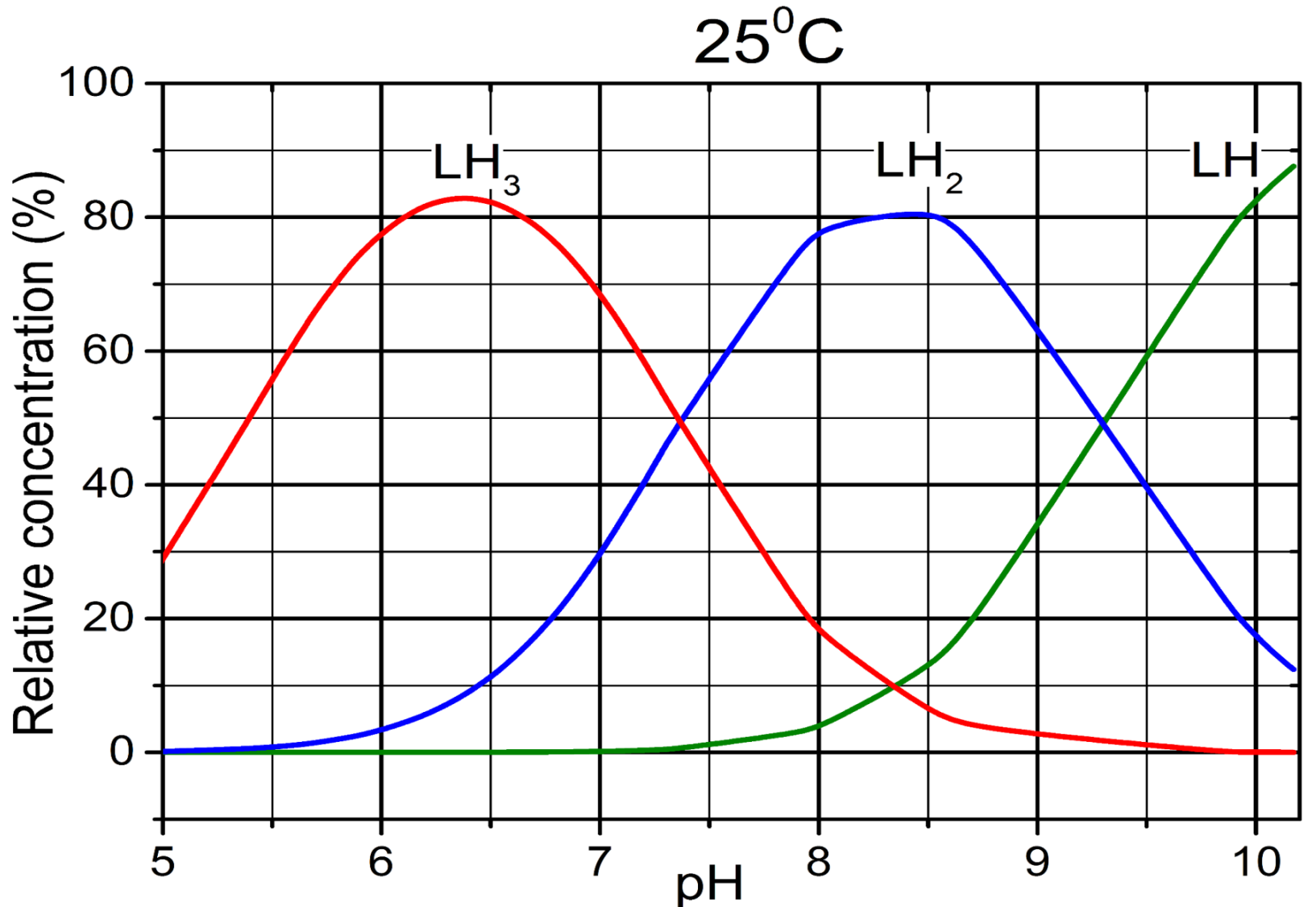
pH- metric data analysis

The potentiometric titration curve of dibasic Vortioxetine (red curve) acidified with HCl is titrated with KOH and is plotted together with Bjerrum's protonation curve function (blue curve).



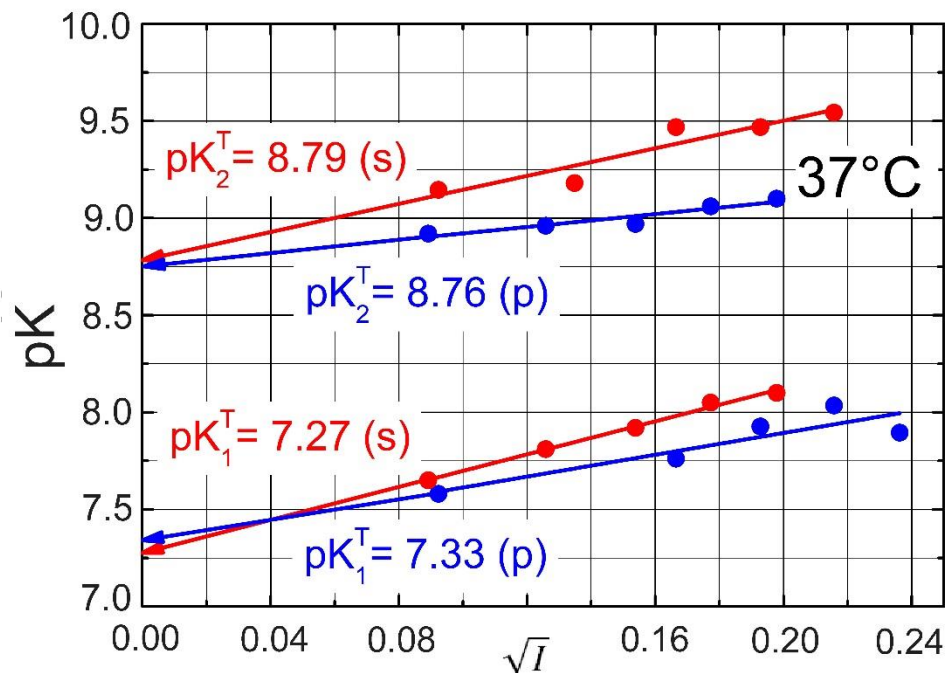
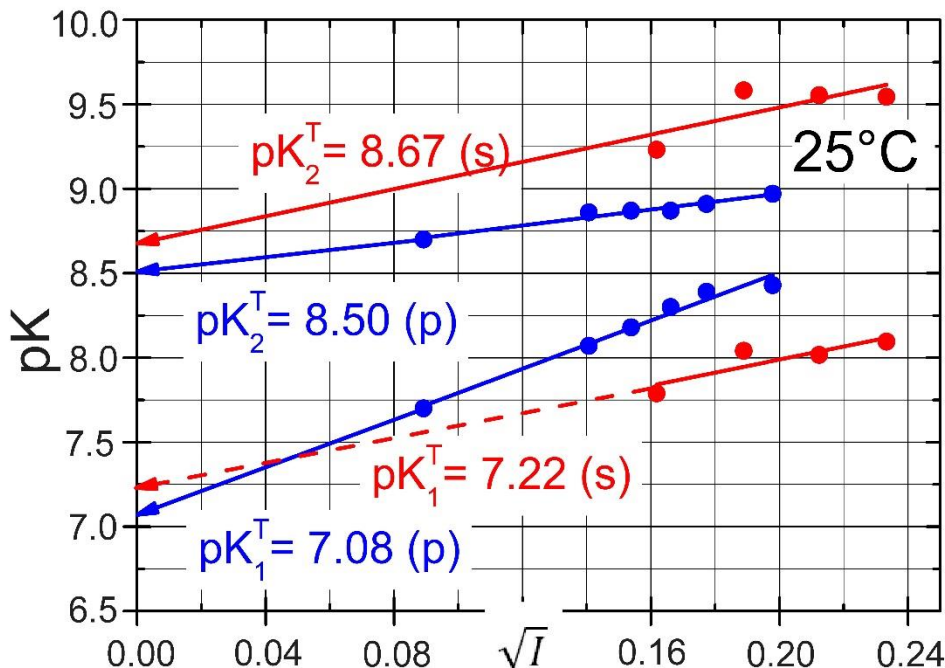
Distribution diagram of the relative concentration

Distribution diagram of variously protonated species concerns the Vortioxetine protonation model.



Comparison of pK estimates from A-pH spectra to pH titration

Overview of two thermodynamic dissociation constants pK_a determined spectrophotometrically (s) and potentiometrically (p).



The extrapolation of the mixed dissociation constants to the zero value of ionic strength according to the limited Debye-Hückel law for the protonation model of two dissociation constants at temperatures 25°C and 37°C.

Statistical analysis of residuals of pH-absorbance matrix

The best reliability criterion of the regression (protonation) model found is the statistical analysis of residuals to examine a fitness of the calculated absorbance response area by experimental points of the spectra set.

Ionic strength		0.0085	0.0181	0.0277	0.0372	0.0466	0.0558
Cattel's scree plot indicating the rank of the absorbance matrix (INDICES)							
Number of spectra measured, n_s		46	53	49	50	49	47
Number of wavelengths, n_w		234	117	117	117	117	117
Number of light-absorbing species, k^*		2	2	2	2	2	2
Residual standard deviation, $s_k^*(A)$ [mAU]							
Estimates of dissociation constants in the searched protonation model							
$pK_{a1}(s_1), LH_4^{3+} = H^+ + LH_3^{2+}$	SQUAD84	7.58(02)	7.50(02)	7.77(02)	7.93(04)	8.08(06)	7.89(02)
	REACTLAB	7.58(00)	7.50(01)	7.76(00)	7.93(01)	8.03(01)	7.89(01)
$pK_{a2}(s_2), LH_3^{2+} = H^+ + LH_2^+$	SQUAD84	9.15(01)	9.19(01)	9.49(01)	9.48(03)	9.66(05)	9.38(02)
	REACTLAB	9.14(01)	9.18(01)	9.47(01)	9.47(02)	9.54(03)	9.38(01)X
Goodness-of-fit test with the statistical analysis of residuals							
Mean residual $E \bar{e} $ [mAU]	SQUAD84	68.50	4.56	6.11	10.9	15.61	8.01
	REACTLAB	2.29	5.09	4.27	7.62	10.69	7.98
Standard deviation of residuals $s(\hat{e})$ [mAU]	SQUAD84	16.30	9.65	11.80	24.70	35.25	19.70
	REACTLAB	4.58	6.01	7.48	15.71	22.27	9.54
Sigma from ReactLab [mAU]	REACTLAB	19.90	9.47	11.63	24.23	34.45	19.25
Hamilton R-factor from SQUAD84 [%]	SQUAD84	0.028	0.019	0.021	0.043	0.058	0.029

ESAB refinement of common and group parameters for a pH-metric titration of Vortioxetine hydrobromide with HCl and KOH: the estimated dissociation constants pK_{a1} , pK_{a2} of Vortioxetine when their standard deviations in last valid digits are in parentheses at 25°C. The reliability of parameter estimation is proven with a goodness-of-fit statistics: the bias or arithmetic mean of residuals $E(\hat{e})$ [mL], the mean of absolute value of residuals, $E|\hat{e}|$ [mL], the standard deviation of residuals $s(\hat{e})$ [mL], the residual skewness $g_1(\hat{e})$ and the residual kurtosis $g_2(\hat{e})$ proving a Gaussian distribution and Jarque-Berra normality test.
Common parameters refined: pK_{a1} , pK_{a2} . **Group parameters refined:** H_0 , H_T , L_0 . **Constants:** $t = 25.0$ °C, $pK_w = 13.9799$, $V_0 = 20.22$ mL, $s(V) = s_{inst}(y) = 0.0001$ mL, I_0 adjusted (in vessel), $I_T = 0.9304$ (in burette KOH) or 1.0442 (in burette HCl).

25°C					
Ionic strength I_0 [mol/L]	0.0080	0.0158	0.0237	0.0314	0.0391
Estimates of the group parameters H_0 , H_T and L_0 in the searched protonation model					
Number of points n	52	57	53	52	56
$H_0 \times 1E+04$ [mol/L]	5.08(02)	5.08(01)	5.25(02)	4.36(05)	4.86(06)
H_T [mol/L]	0.9729(0396)	0.9668(0208)	0.94081(0306)	0.9304	0.9375(0104)
$L_0 \times 1E+04$ [mol/L]	3.16(02)	3.26(01)	2.60(01)	2.98(05)	2.99(06)
Estimates of the common parameters i.e. dissociation constants in the searched protonation model					
pK_{a1}	8.39(06)	8.48(05)	8.82(09)	8.70(09)	8.82(09)
pK_{a2}	8.99(09)	8.96(07)	8.79(09)	8.65(08)	8.54(08)
Goodness-of-fit test with the statistical analysis of residuals					
Arithmetic mean of residuals $E(\hat{e})$, [mL]	-1.77E-20	-9.51E-21	-1.89E-06	3.34E-20	-3.04E-05
Hoaglin limit LL	-0.0002	-0.0002	-0.0001	-0.0002	-0.0002
Hoaglin limit LU	0.0002	0.0002	0.0001	0.0002	0.0002
Mean of absolute value of residuals, $E \hat{e} $, [mL]	0.0005	0.0005	0.0004	0.0005	0.0006
Residual standard deviation, pH (ESAB)	0.1271	0.1175	0.0907	0.1296	0.1456
Residual standard deviation, $s(\hat{e})$, [mL]	0.0006	0.0006	0.0004	0.0006	0.0007
Residual skewness $g_1(\hat{e})$	0.11	0.16	-0.18	-0.02	0.06
Residual kurtosis $g_2(\hat{e})$	2.63	2.36	2.32	2.38	2.45
Jarque-Berra test of normality: p,	0.894,	0.818,	0.780,	0.974,	0.952.
Normality is	Accepted	Accepted	Accepted	Accepted	Accepted

Conclusion

(1) The sparingly soluble neutral molecule LH of Vortioxetine capable of protonation to form the still soluble two cations LH_2^+ and LH_3^{2+} occurs in pure water. The graph of molar absorption coefficients of variously protonated species according to wavelength shows that the spectrum of species LH_2^+ and LH slightly vary in colour, while protonation of chromophore LH_2^+ to LH_3^{2+} has greater influence on chromophores in Vortioxetine molecule.

(2) We have proven that in the range of pH 4 to 10 two dissociation constants can be reliably estimated from the spectra when concentration of Vortioxetine is about $9.2 \times 10^{-5}\text{M}$. Although the change of pH somewhat less affected changes in the chromophore, two overlapping thermodynamic dissociation constants can be reliably determined with SQUAD84 and REACTLAB reaching the similar values with both programs, $\text{pK}_{\text{a}1}^{\text{T}} = 7.22$, $\text{pK}_{\text{a}2}^{\text{T}} = 8.67$ at 25°C and $\text{pK}_{\text{a}1}^{\text{T}} = 7.27$, $\text{pK}_{\text{a}2}^{\text{T}} = 8.79$ at 37°C .

(3) Two overlapping thermodynamic dissociation constants of Vortioxetine in a potentiometric concentration of $3 \times 10^{-4} \text{ mol.dm}^{-3}$ were determined by the regression analysis of potentiometric titration curves using ESAB, $\text{pK}_{\text{a}1}^{\text{T}} = 7.08$, $\text{pK}_{\text{a}2}^{\text{T}} = 8.50$ at 25°C and $\text{pK}_{\text{a}1}^{\text{T}} = 7.33$, $\text{pK}_{\text{a}2}^{\text{T}} = 8.76$ at 37°C .

(4) Prediction of the dissociation constants of Vortioxetine was performed using the MARVIN program to specify protonation locations and using the ACD/pK program. In comparing two predictive with two experimental techniques it may be concluded that the prediction programs often vary in estimating pK_{a} .