Multiple Dissociation Constants of the Intepirdine Hydrochloride Using Regression of Multiwavelength Spectrophotometric pH-Titration Data

*Milan Meloun[1], Lucie Pilařová[1], Filip Bureš[2] and Tomáš Pekárek[3]

[1]Department of Analytical Chemistry, University of Pardubice, CZ 532 10 Pardubice, Czechia,
[2]Institute of Organic Chemistry and Technology, University of Pardubice, CZ 532 10 Pardubice, Czechia,
[3]Zentiva k.s., U kabelovny 130, CZ 102 37 Prague, Czechia,

Abstract

UV/VIS-metric of the Neurotransmitter Intepirdine for three pK_a were estimated pK^T_{a1} = 5.64, pK^T_{a2} = 7.31, pK^T_{a3} = 8.85 at 25°C and pK^T_{a1} = 5.51, pK^T_{a2} = 7.15, pK^T_{a3} = 8.77 at 37°C.

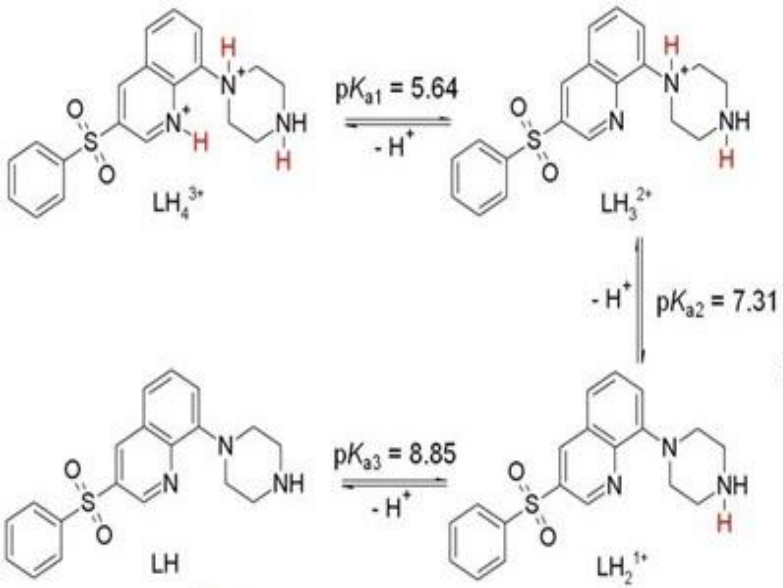
A sparingly soluble molecule LH was protonated to form still soluble three cations LH_2^+, LH_3^{2+} and LH_4^{3+} in pure water.

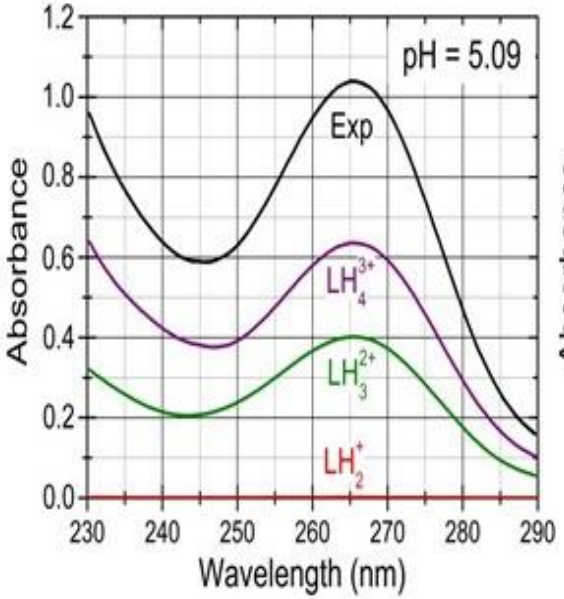
Three multiple thermodynamic dissociation constants of 3×10^{-4} M Intepirdine were determined by the regression analysis of pH-metric titration curves pK^T_{a1} = 5.14, pK^T_{a2} = 8.38, pK^T_{a3} = 9.33 at 25°C and pK^T_{a1} = 5.17, pK^T_{a2} = 8.31, pK^T_{a3} = 9.07 at 37°C.

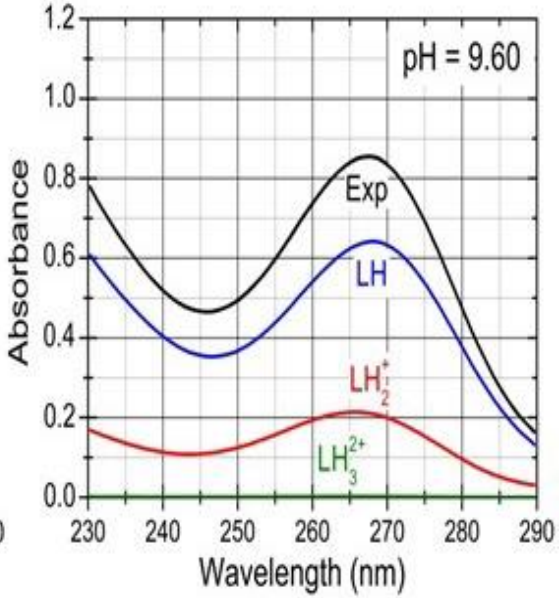
The macro-dissociation constants were predicted with MARVIN and ACD/Percepta programs.

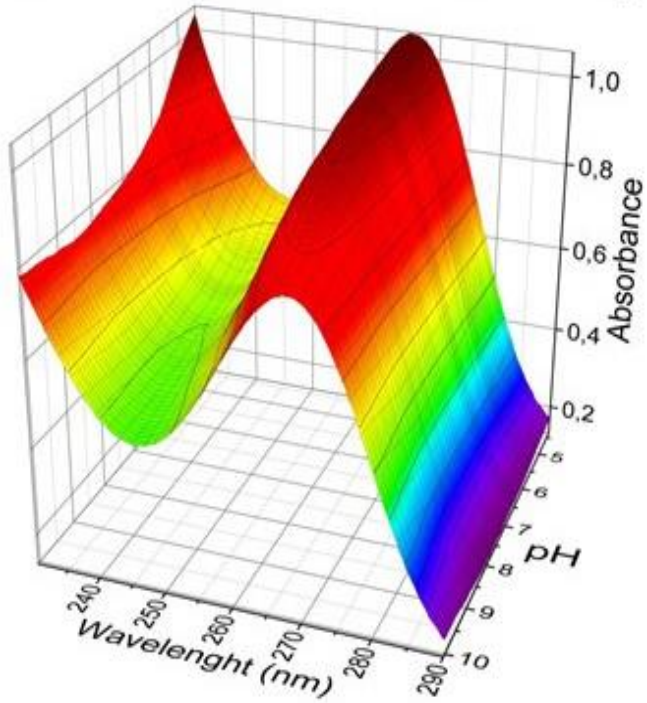
The protonation scheme of INN.HCl was suggested.

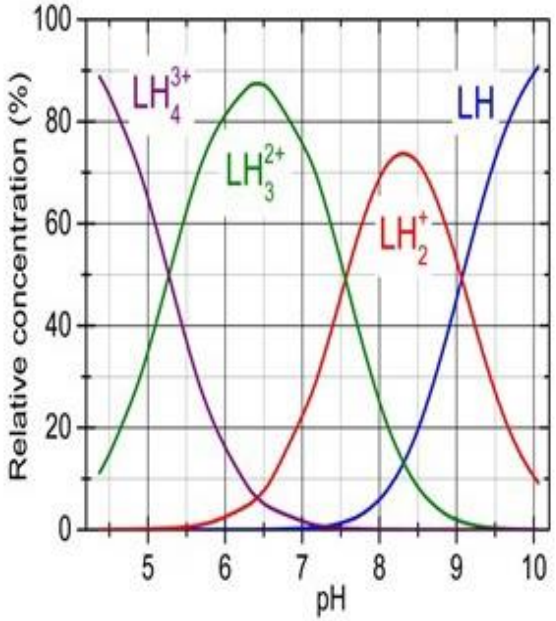
Protonation of Intepirdine for the Treatment of Alzheimer´s disease

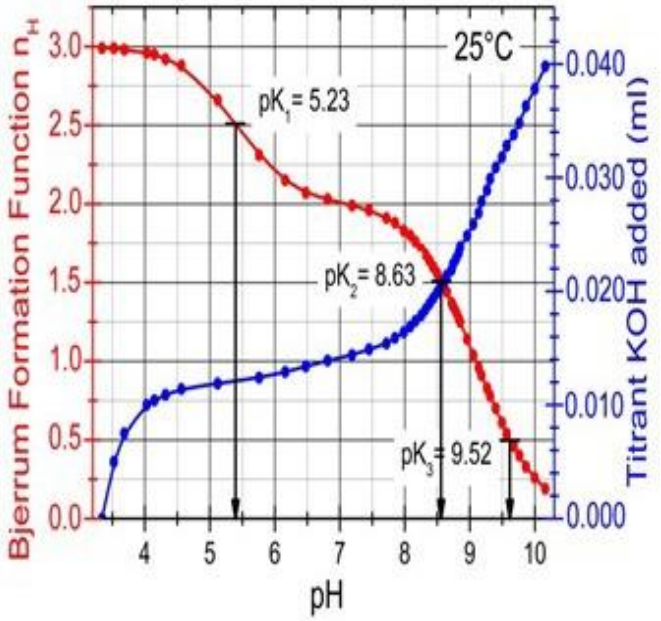




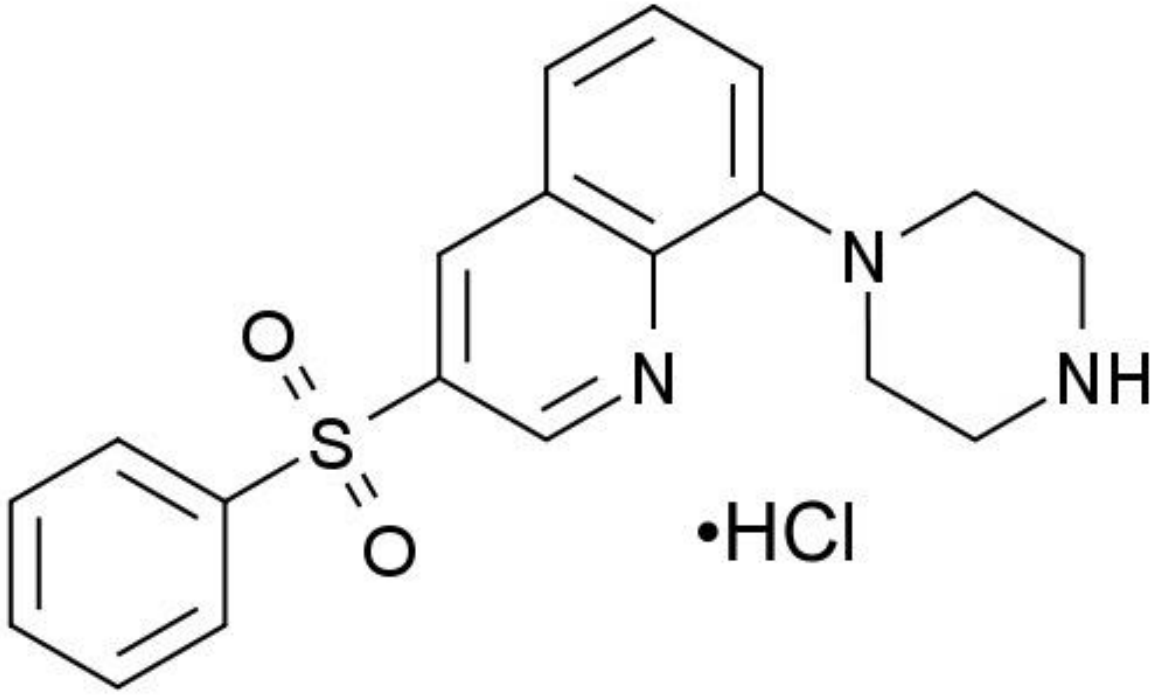








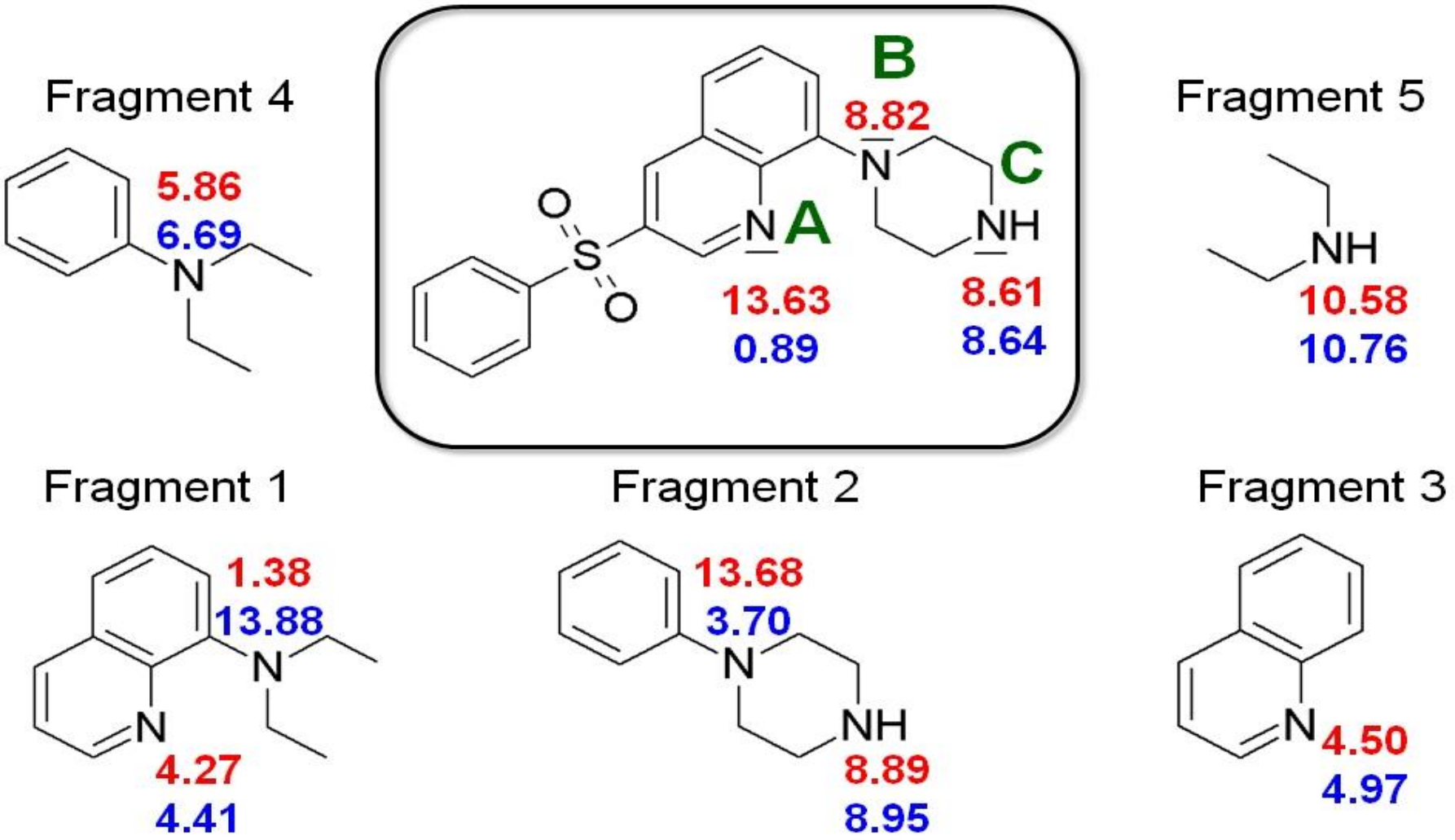
Structural formula of Intepirdine hydrochloride, INN.HCl



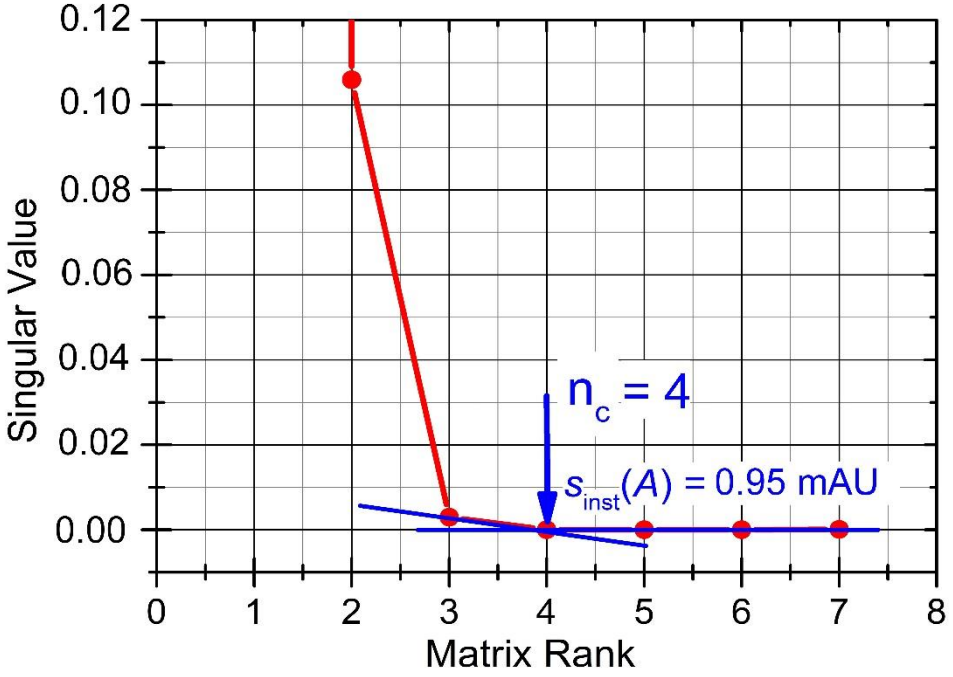
Intepirdine (INN) is a novel 5-HT_6 receptor antagonist in development for the treatment of patients with mild-moderate Alzheimer's disease.

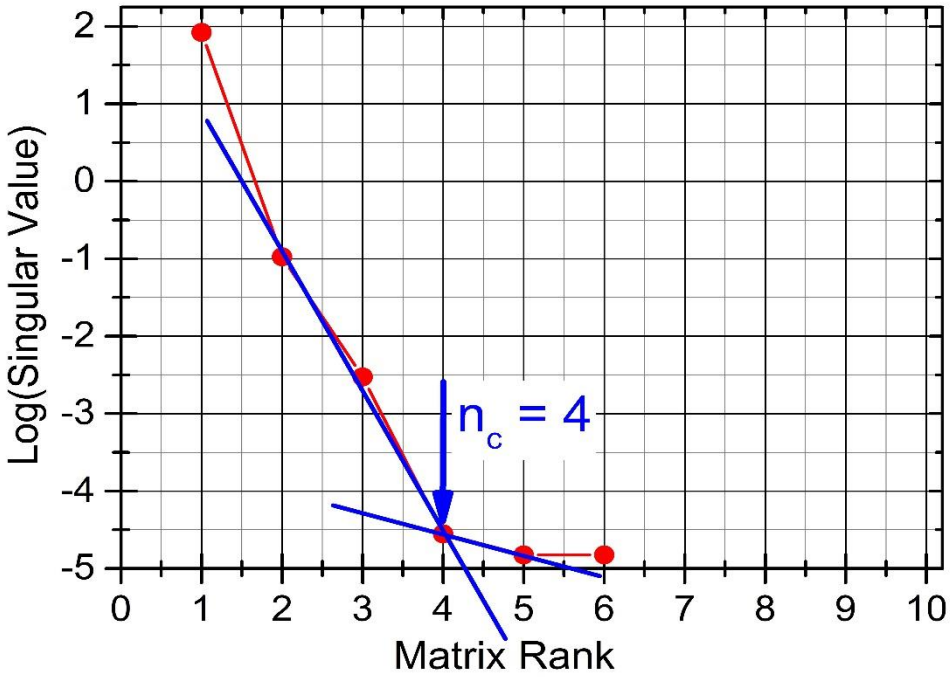
As a 5-HT_6 receptor antagonist, intepirdine works in part by relieving interneuron-mediated inhibition and promoting the release of acetylcholine and other neurotransmitters in the brain.

Predicted pK_{pred} of Intepirdine with MARVIN and ACD



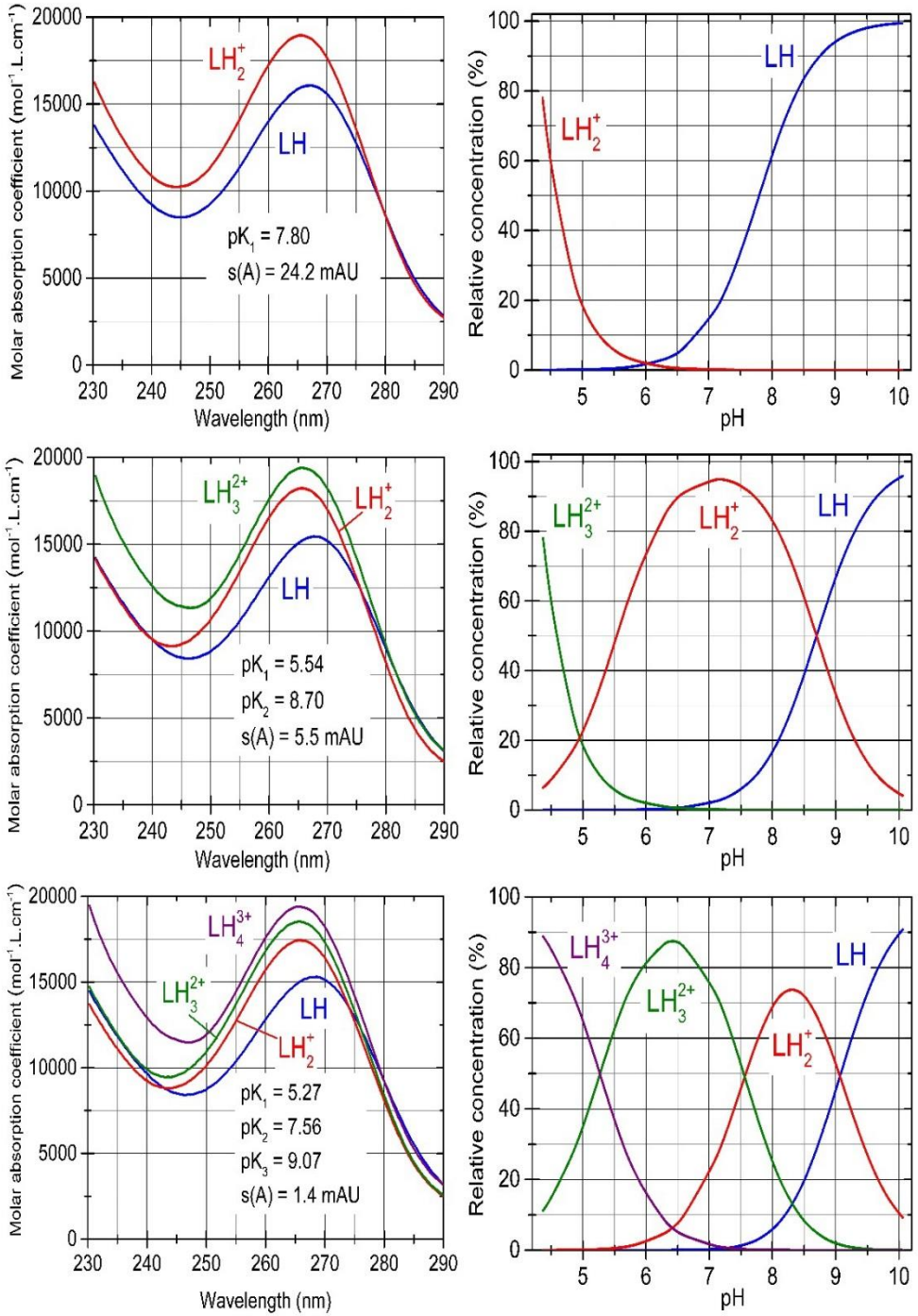
Molecular structure of Intepirdine INN (inset) with highlighted basic centres A, B and C and predicted pK_a values using MARVIN/ACD prediction programs. Structure of auxiliary fragments 1-5 and their predicted pK_a.





Number of light-absorbing species in mixture

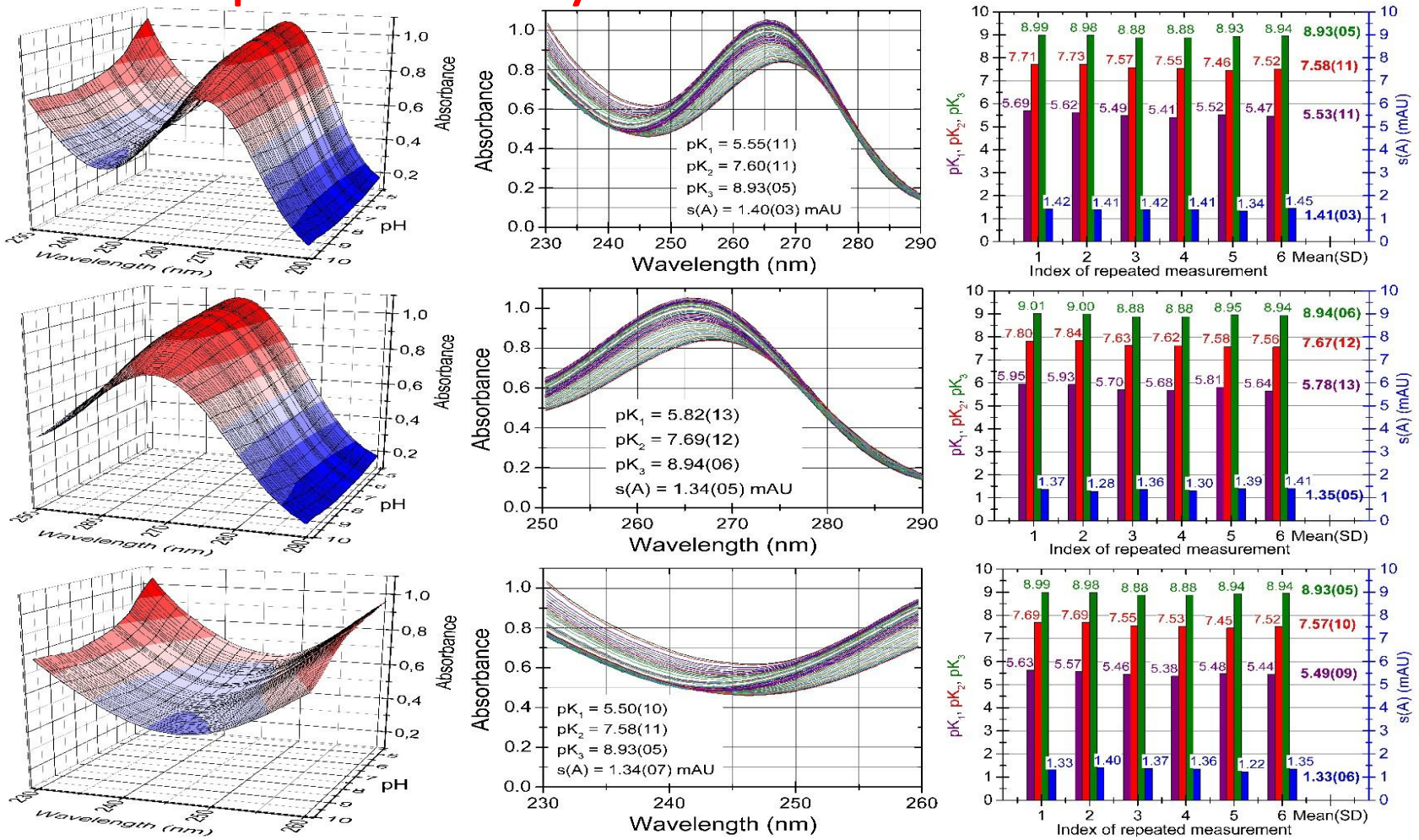
The Cattel's scree plot $s_k(SV) = f(k)$ for the rank of the absorbance matrix $k^* = 4$ in normal scale and in logarithmic scale which leads to four light-absorbing species in the equilibrium mixture, $n_c = 4$.



Search for the protonation model building and testing

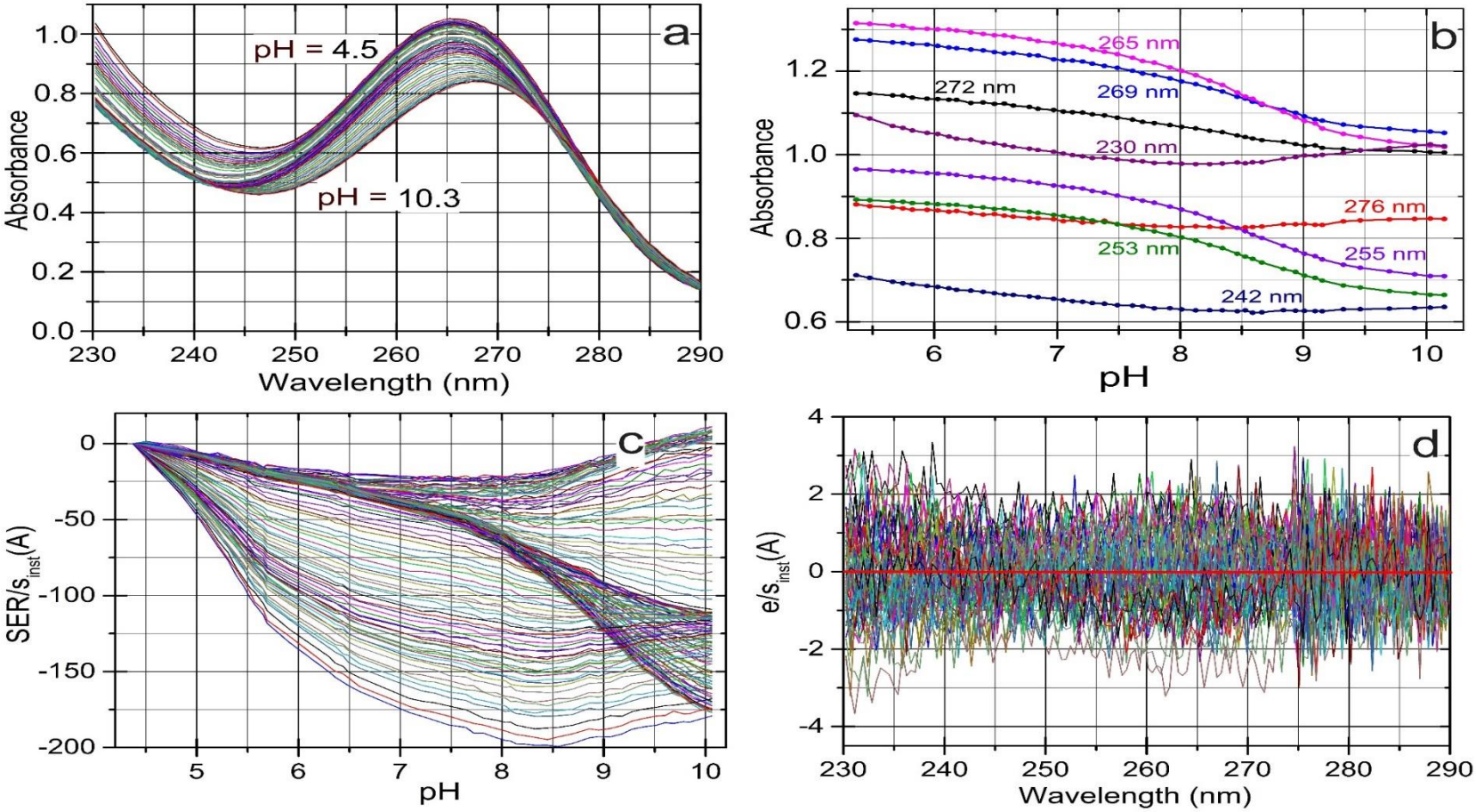
The SQUAD84 working environment searching the best protonation model of Intepirdine hydrochloride for one (**Upper**), two (**Middle**) and three (**Lower**) dissociation constants pK_{a1}, pK_{a2}, pK_{a3}

Reproducibility of dissociation constants

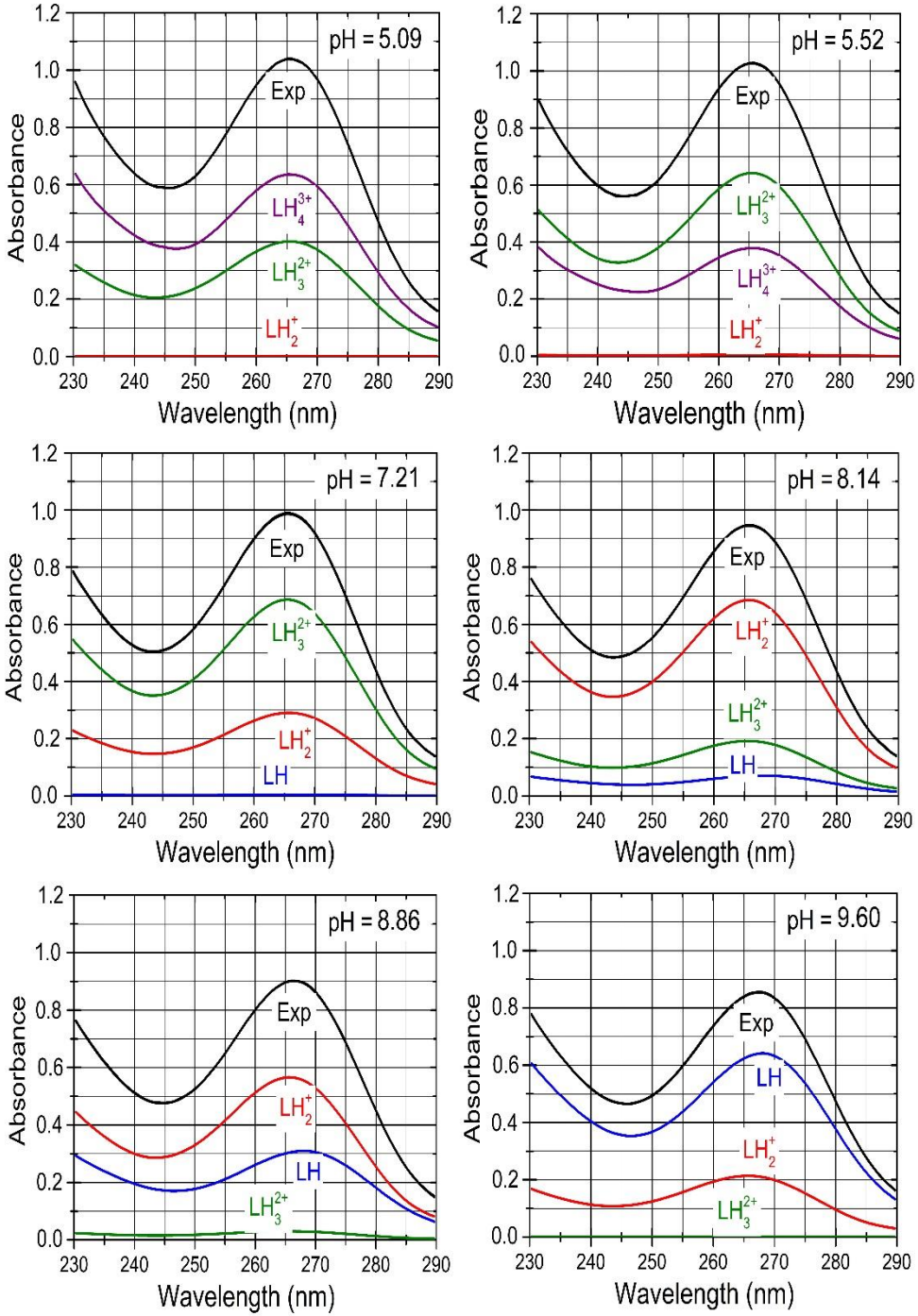


The plot of 2D and 3D-absorbance-response-matrix for Intepirdine and reproducibility of the estimated dissociation constants from three absorption bands. pK_{a1}, pK_{a2}, and pK_{a3} with their standard deviation in the last two digits. The goodness-of-fit is expressed on the right axis as the standard deviation of absorbance after regression, $s(A)$ [mAU].

Effect of small absorbance changes

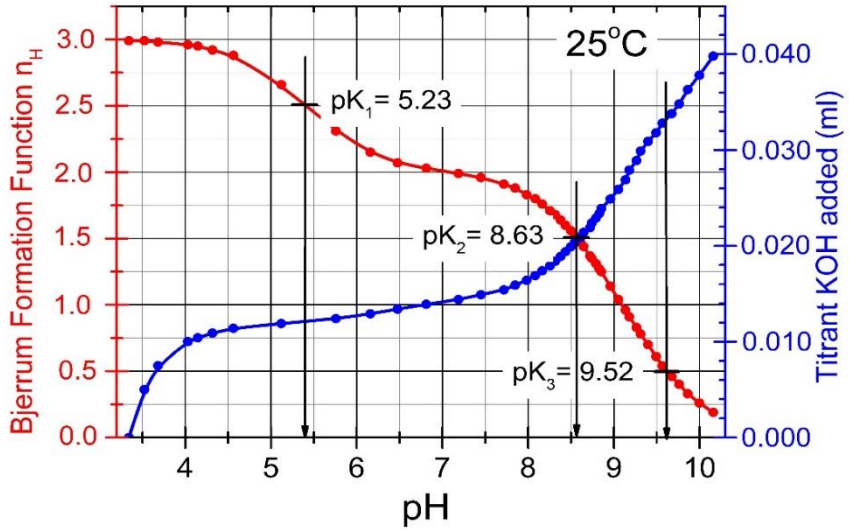


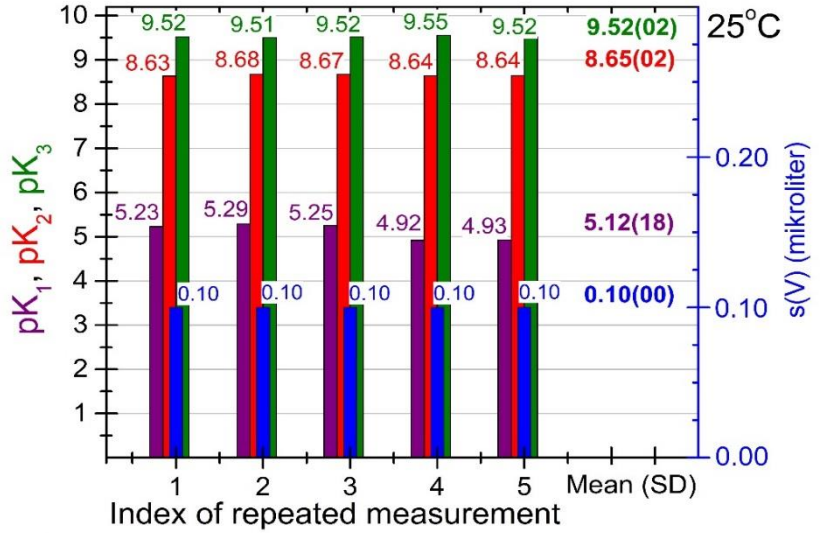
Plot of small absorbance changes in the Intepirdine 2D-spectra set within pH-titration and Absorbance-pH curves at wavelengths. Plot of small absorbance shift in spectrum within pH-titration $SER_{ij} = A_{ij} - A_{i,acid}$ divided with the $s_{inst}(A)$ leading to $SER/s_{inst}(A)$. Residuals $\boldsymbol{e}$ [mAU] are divided by $s_{inst}(A)$ leading to $e/s_{inst}(A)$ to test if the residuals $\boldsymbol{e}$ are of the same magnitude as the $s_{inst}(A)$.

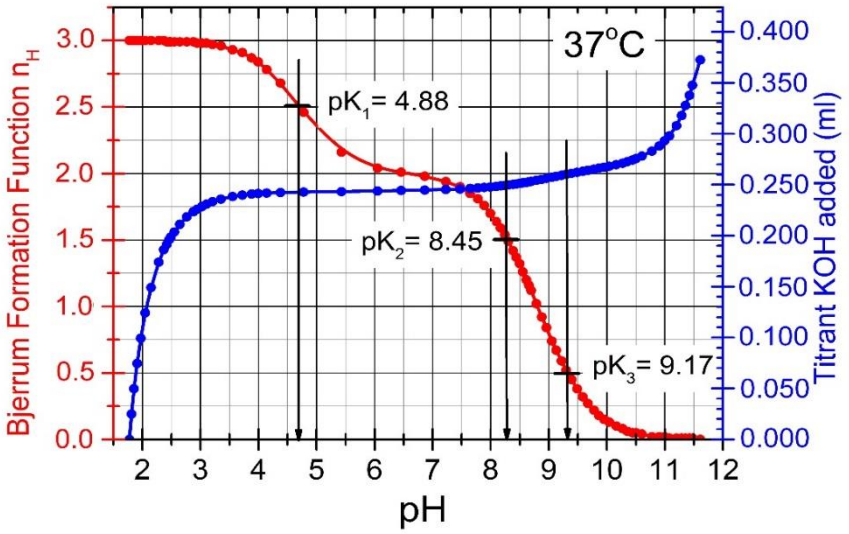


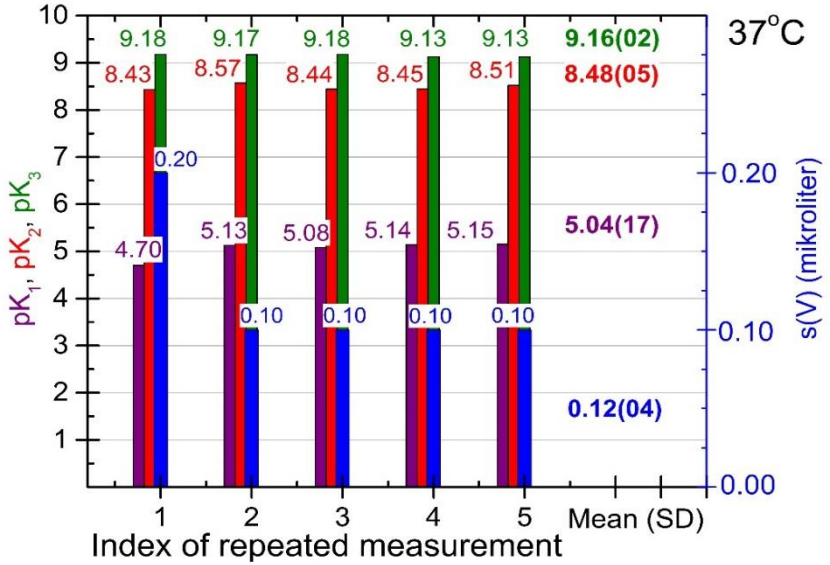
Spectra deconvolution

Deconvolution of the experimental spectrum of Intepirdine into spectra of the protonated species LH, LH_2^+, LH_3^{2+}, LH_4^{3+} in mixture for pH: 5.09, 5.52, 7.21, 8.14, 8.86, and 9.60 using SQUAD84.

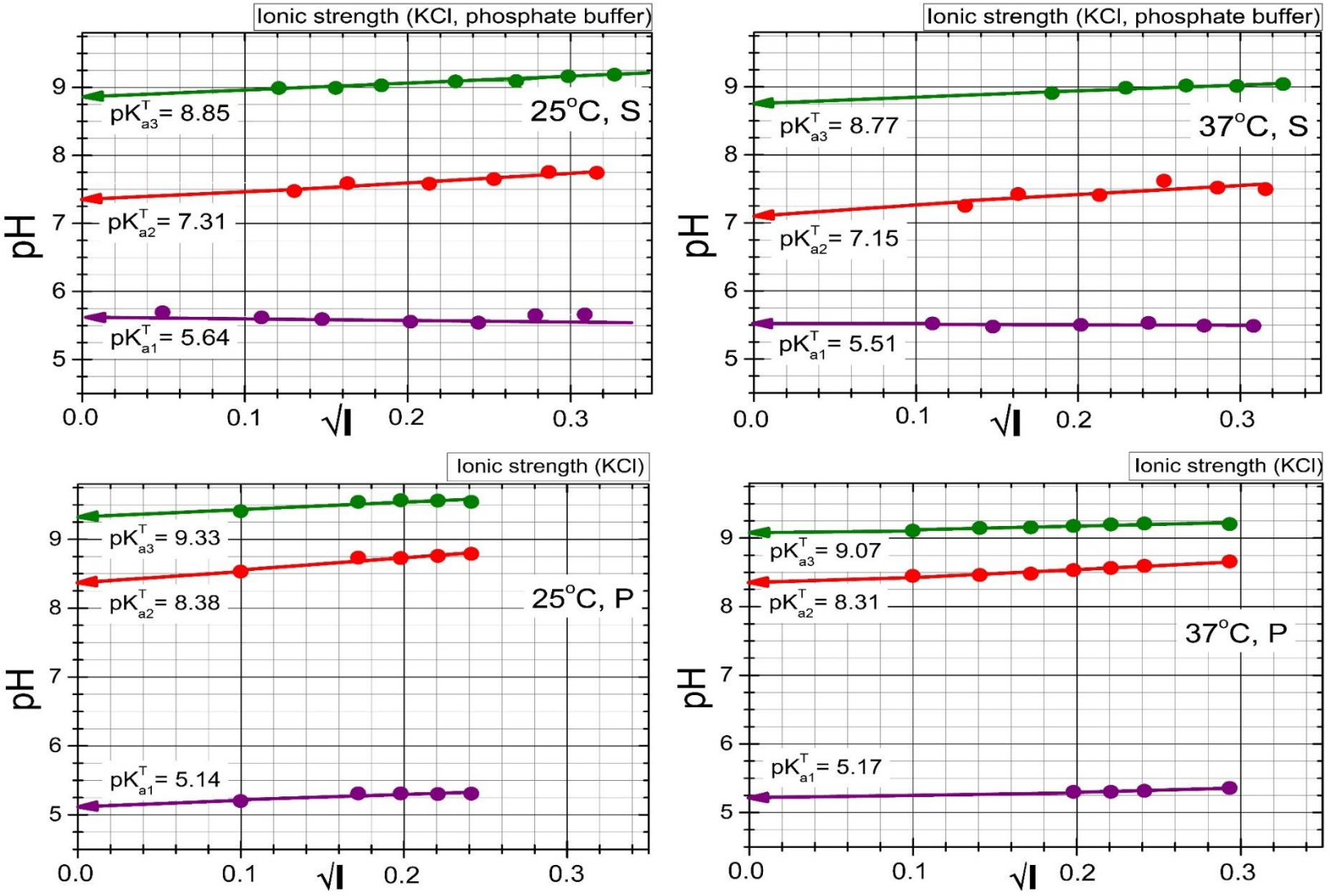






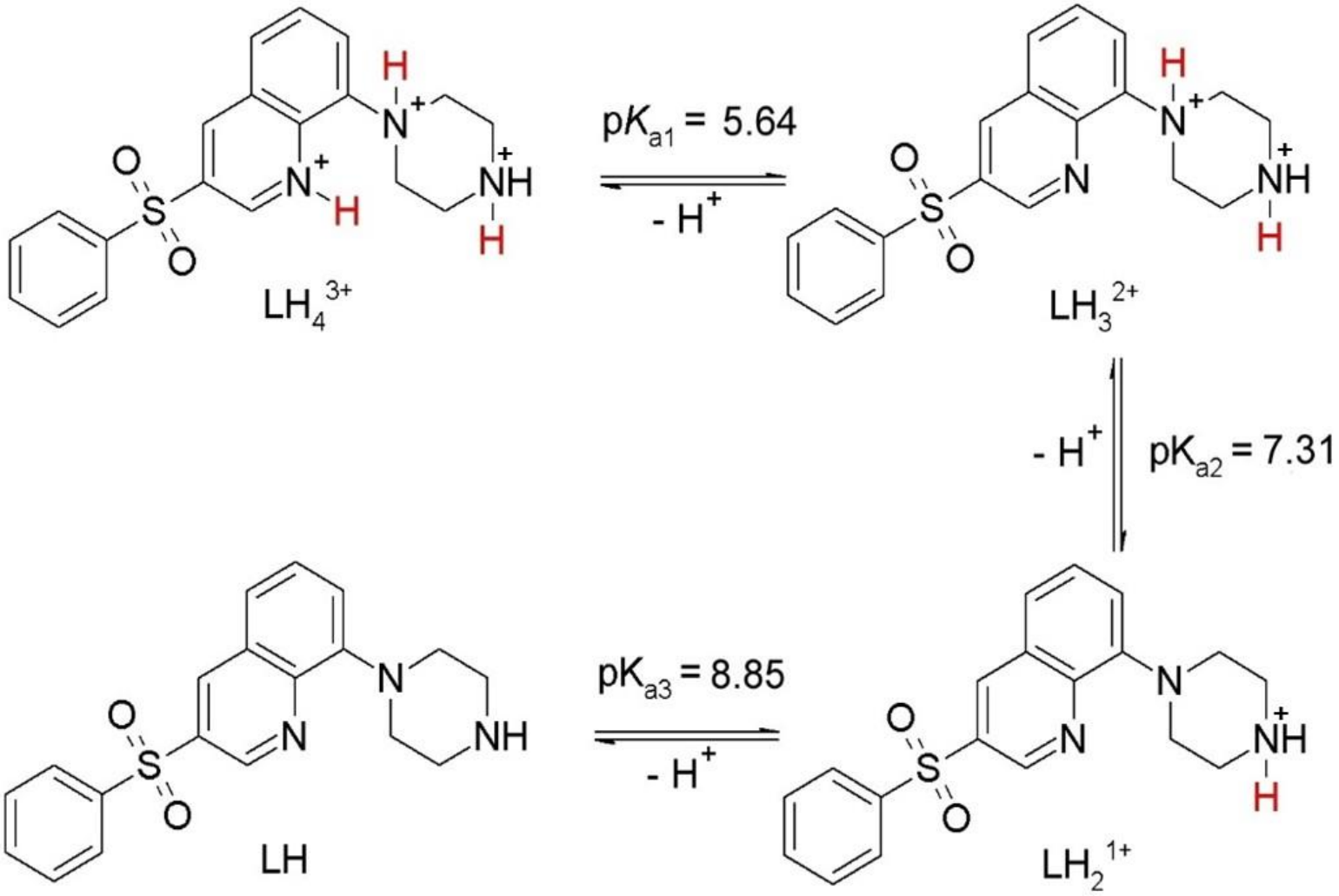


The search for the protonation model analysing the potentiometric titration curve of acidified Intepirdine and titrated with KOH and plotted with the Bjerrum protonation function indicating three pK_a values. Dissociation constants are estimated with ESAB at 25°C and 37°C .



Dependence of the mixed dissociation constants of Intepirdine on the square root of the ionic strength for three dissociation constants leading to the thermodynamic dissociation constant pK_a^T at 25°C and 37°C with UV-metric (**S**) and pH-metric technique (**P**).

Protonation scheme of Intepirdine



Conclusion

1. UV-metric and pH-metric determination of three dissociation constants of Intepirdine (Scheme 1).

2. The sparingly soluble molecule LH of Intepirdine capable of protonation to form three cations LH_2^+, LH_3^{2+} and LH_4^{3+} in pure water.

3. Three thermodynamic dissociation constants of Intepirdine hydrochloride can be reliably determined with SQUAD84 and REACTLAB reaching to similar values with both programs, pK^T_{a1} = 5.64, pK^T_{a2} = 7.31, pK^T_{a3} = 8.85 at 25°C and pK^T_{a1} = 5.51, pK^T_{a2} = 7.15, pK^T_{a3} = 8.77 at 37°C.

4. Three thermodynamic dissociation constants of Intepirdine hydrochloride were determined by the regression analysis of potentiometric titration curves using ESAB, pK^T_{a1} = 5.14, pK^T_{a2} = 8.38, pK^T_{a3} = 9.33 at 25°C and pK^T_{a1} = 5.17, pK^T_{a2} = 8.31, pK^T_{a3} = 9.07 at 37°C.

5. Prediction of the pK of Intepirdine hydrochloride using the MARVIN and ACD/Percepta programs specified protonation locations.

Recommended papers

Intepirdine:

1. Upton, N., Chuang, T.T., Hunter, A.J., Virley, D.J.: 5-HT6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. Neurotherapeutics **5**(3), 458-469 (2008)

2. Callaghan, C.K., Hok, V., Della-Chiesa, A., Virley, D.J., Upton, N., O'Mara, S.M.: Age-related declines in delayed non-match-to-sample performance (DNMS) are reversed by the novel 5HT6 receptor antagonist SB742457. Neuropharmacology **63**(5), 890-897 (2012)

3. Codony, X., Vela, J.M., Ramirez, M.J.: 5-HT6 receptor and cognition. Current Opinion in Pharmacology **11**(1), 94-100 (2011)

4. Lombardo, I., Ramaswamy, G., Friedhoff, L., Asare, E.: Intepirdine (RVT-101), a 5-HT6 Receptor Antagonist, as an Adjunct to Donepezil in Mild-to-Moderate Alzheimer's Disease: Efficacy on Activities of Daily Living Domains. Am J Geriat Psychiat **25**(3), S120-S121 (2017)

5. Ferrero, H., Solas, M., Francis, P.T., Ramirez, M.J.: Serotonin 5-HT6 Receptor Antagonists in Alzheimer's Disease: Therapeutic Rationale and Current Development Status. Cns Drugs **31**(1), 19-32 (2017)

6. de Bruin, N.M.W.J., van Loevezijn, A., Wicke, K.M., de Haan, M., Venhorst, J., Lange, J.H.M., de Groote, L., van der Neut, M.A.W., Prickaerts, J., Andriambeloson, E., Foley, A.G., van Drimmelen, M., van der Wetering, M., Kruse, C.G.: The selective 5-HT6 receptor antagonist SLV has putative cognitive- and social interaction enhancing properties in rodent models of cognitive impairment. Neurobiol Learn Mem **133**, 100-117 (2016)

7. Mason, V.L.: Alzheimer's Association International Conference on Alzheimer's Disease 2015 (Aaic 2015) (July 18-23, 2015-Washington, Dc, USA). Drug Today **51**(7), 447-452 (2015)

Methodology:

22. Meloun, M., Ferenčíková, Z., Javůrek, M.: Reliability of dissociation constants and resolution capability of SQUAD(84) and SPECFIT/32 in the regression of multiwavelength spectrophotometric pH-titration data. Spectrochim Acta A Mol Biomol Spectrosc **86**, 305-314 (2012)

23. Meloun, M., Nečasová, V., Javůrek, M., Pekárek, T.: The dissociation constants of the cytostatic bosutinib by nonlinear least-squares regression of multiwavelength spectrophotometric and potentiometric pH-titration data. Journal of Pharmaceutical and Biomedical Analysis **120**, 158-167 (2016)

24. Meloun, M., Bordovská, S., Syrový, T., Vrána, A.: Tutorial on a chemical model building by least-squares non-linear regression of multiwavelength spectrophotometric pH-titration data. Anal Chim Acta **580**(1), 107-121 (2006)

25. Meloun, M., Bordovská, S., Vrána, A.: The thermodynamic dissociation constants of the anticancer drugs camptothecine, 7-ethyl-10-hydroxycamptothecine, 10-hydroxycamptothecine and 7-ethylcamptothecine by the least-squares nonlinear regression of multiwavelength spectrophotometric pH-titration data. Anal Chim Acta **584**(2), 419-432 (2007)

26. Maeder, M., King, P.: Analysis of Chemical Processes, Determination of the Reaction Mechanism and Fitting of Equilibrium and/or Rate Constants. (2012)

28. Meloun, M., Syrový, T., Bordovská, S., Vrána, A.: Reliability and uncertainty in the estimation of pK (a) by least squares nonlinear regression analysis of multiwavelength spectrophotometric pH titration data. Anal Bioanal Chem **387**(3), 941-955 (2007)