

Electronic Supplementary Information

Rigorous pH measurement in liquid chromatography

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Contents

Comparison with earlier published abs ^w pH values of mobile phases.....	1
Figures.....	3
References.....	7

Comparison with earlier published $_{\text{abs}}^{\text{w}}$ pH values of mobile phases

The agreement between the $_{\text{abs}}^{\text{w}}$ pH values measured with 0.05 M Et₄NClO₄ in MeCN in the salt bridge^{1,2} and the new values measured in this work with ionic liquid [N₂₂₂₅]⁺[NTf₂]⁻ salt bridge (ILSB) is not good (Table 3 main text). Still, there is a linear correlation (Figure S1 A). The outliers are mobile phases with NH₃ in the aqueous phase. 1 mM NH₃ solution is not stable long term and is therefore difficult to measure. The differences with previously measured values are mostly less than ±0.5 units (Figure S1 B). The consistency standard deviation has improved from 0.14 for 83 measurements¹ to 0.09 for 300 measurements in this work.

The apparent reason is that the LJP estimation used in earlier works^{1,2} includes assumptions and simplifications that apparently are only partly valid, leading to biased results. Such assumptions and simplifications are not necessary for the currently used ILSB approach. Moreover, the standard uncertainty of the ILSB approach of 0.11 pH units mentioned in the text corresponds to LJP between neat solvents. It is highly likely to be lower when mixed-aqueous solutions with different amounts of water are compared, as the water content in both solvents makes them much more similar to each other. Therefore, the current values obtained with ILSB can be considered more accurate.

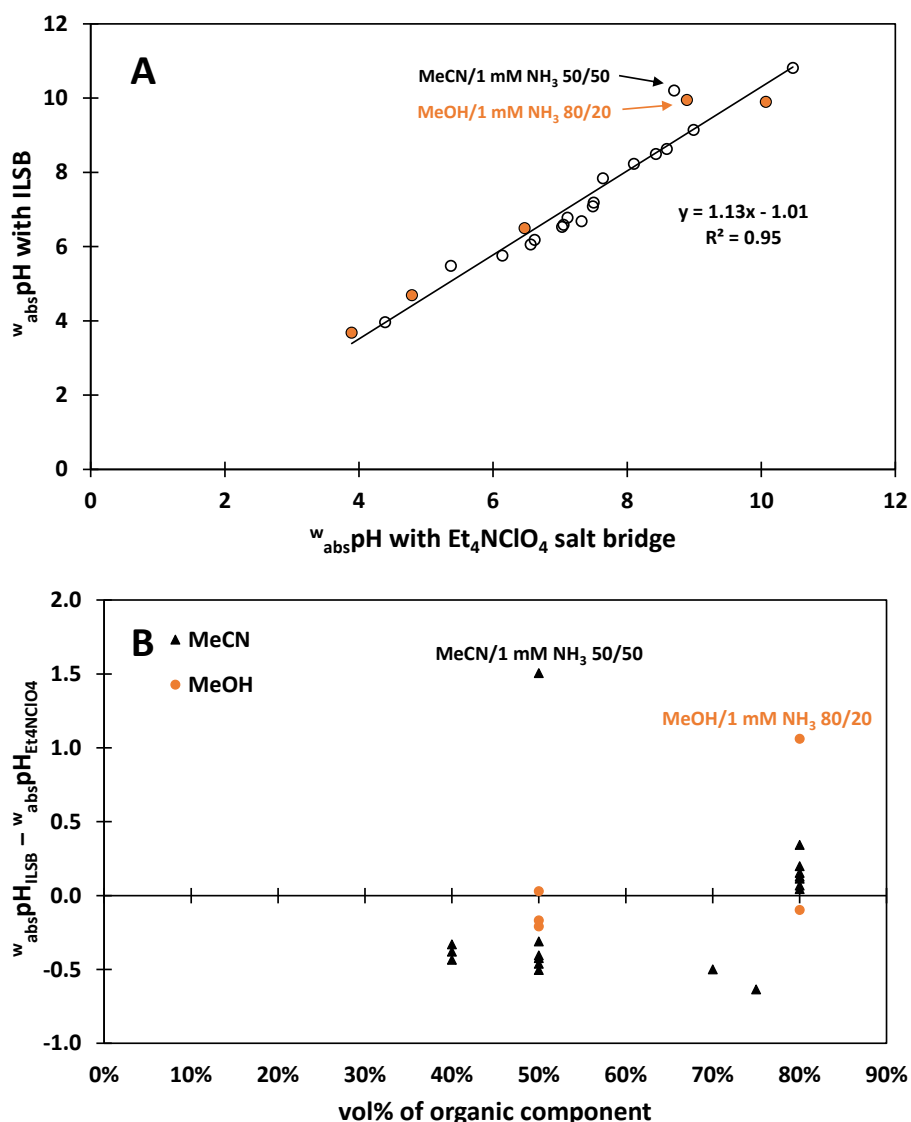


Figure S1. A: Correlation with earlier published $w_{\text{abs}}\text{pH}$ values in references 1 and 2. Empty rings are mobile phases with acetonitrile, and rings with orange filling are mobile phases with methanol. B: The dependence of the differences between $w_{\text{abs}}\text{pH}$ measured in this work and previously published values on the volume percent of the organic phase.

We remeasured³ some of the $w_{\text{abs}}\text{pH}$ values of fluoroalcohol-based mobile phases that had been previously estimated.⁴ The two $w_{\text{abs}}\text{pH}$ values used as reference points in reference⁴ to convert from $s_{\text{w}}\text{pH}$ to $w_{\text{abs}}\text{pH}$ were back then only measured with three relative measurements ($\Delta_{\text{abs}}\text{pH}$) and therefore are less trustworthy than the anchoring approach used in this work. In addition, the relation between $s_{\text{w}}\text{pH}$ and $w_{\text{abs}}\text{pH}$ values is not as constant as we thought (main text, Figure 2 A). Previous $w_{\text{abs}}\text{pH}$ values were underestimated and, in extreme cases, by more than two units. The other slight differences compared to the recently measured values³ are due to the new least squares minimisation of the “ladder” involving a large number of mobile phases.

Figures

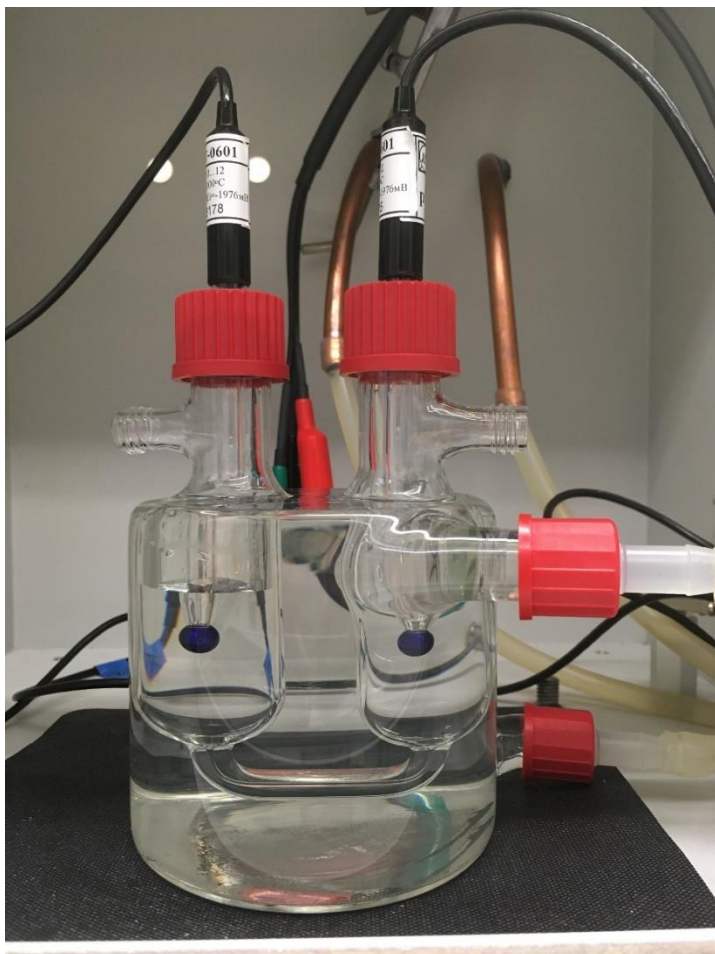


Figure S2. The reference method with cell I.

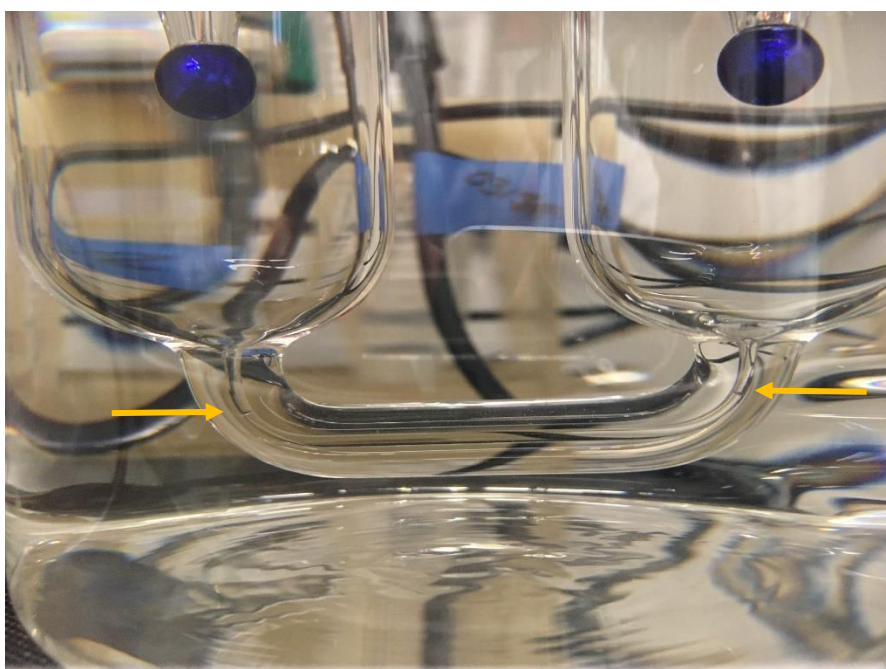


Figure S3. The reference method with cell I. A close-up of the salt bridge containing the ionic liquid $[\text{N}_{2225}]^+[\text{NTf}_2]^-$. Liquid junctions are shown with arrows.

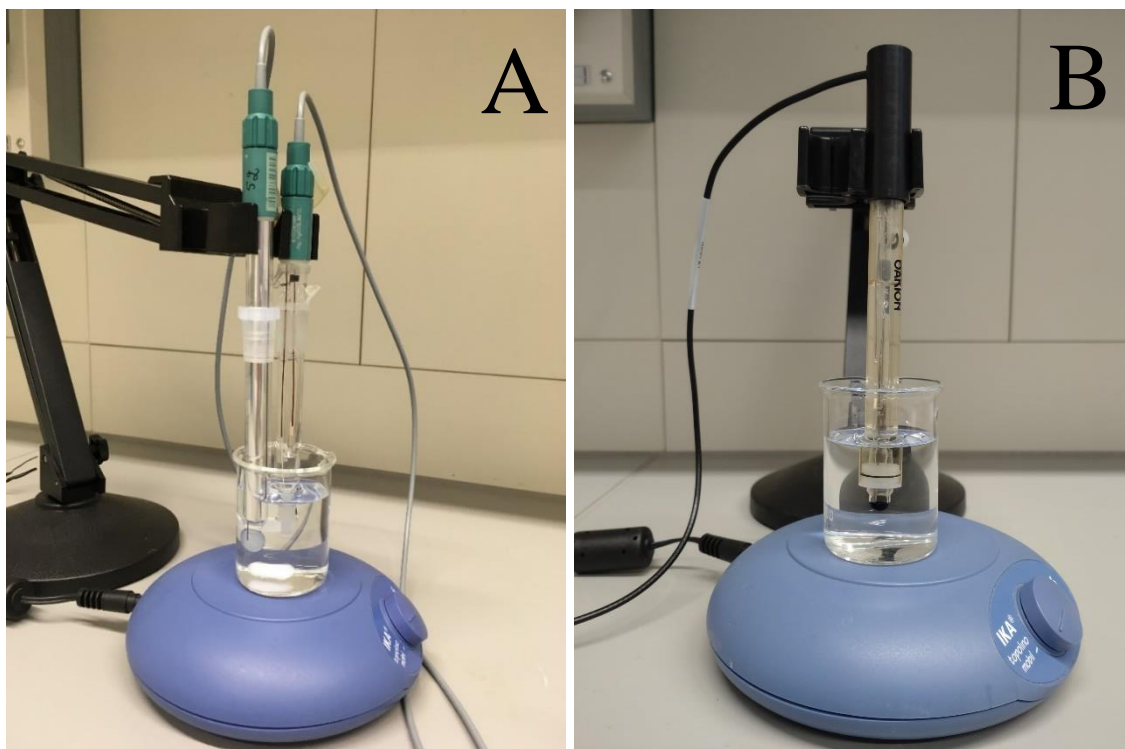


Figure S4. A: The routine method with cell II.1. B: The routine method with cell II.2.



Figure S5. Left: IL – 80 % MeOH junction is not visible. Right: droplets and unclear junction region at IL – 80 % MeCN.

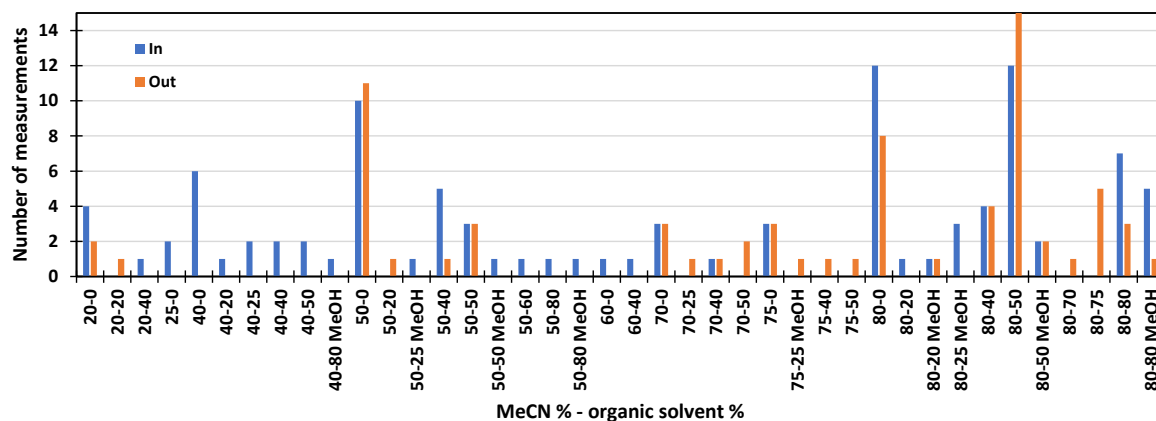
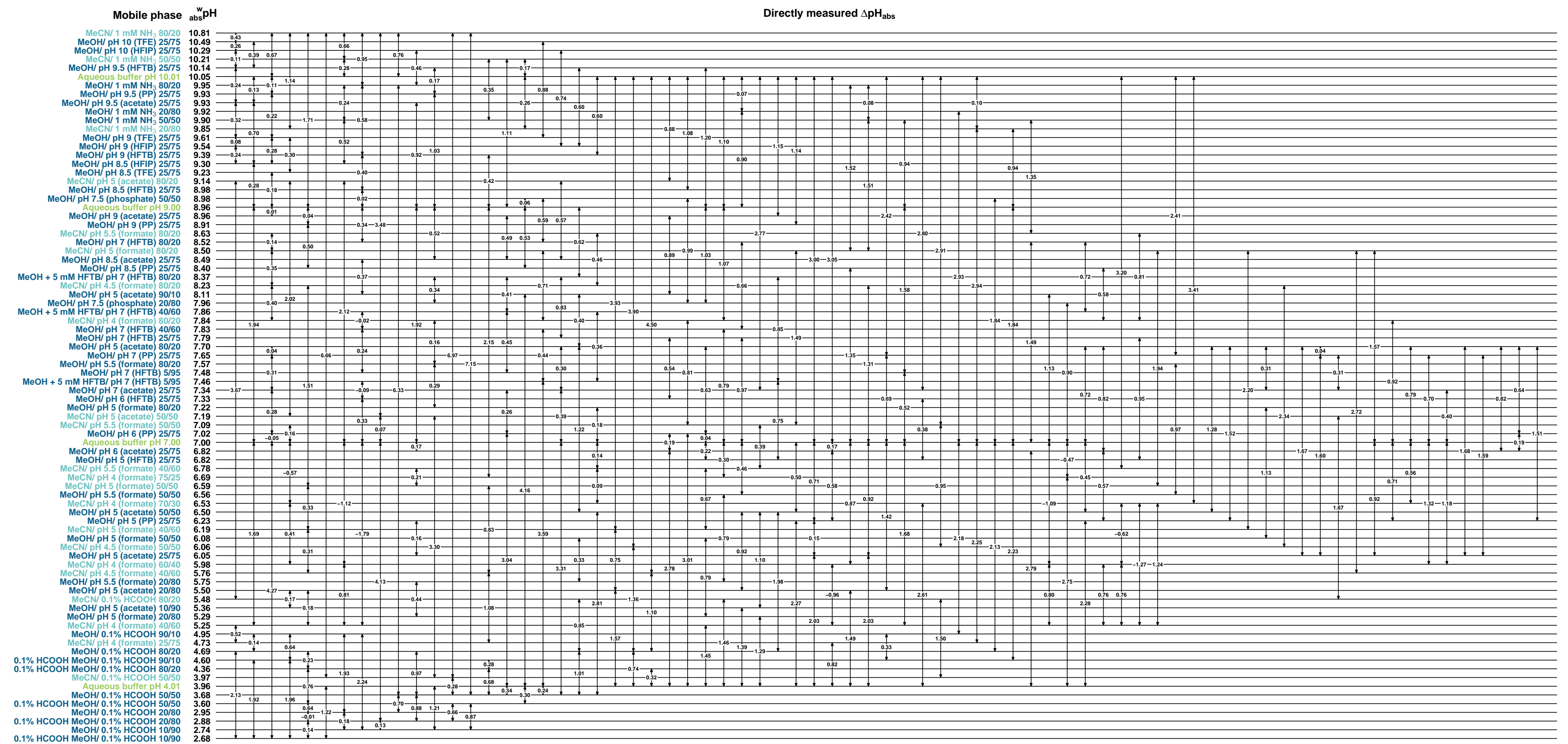


Figure S6. Number of measurements done with different acetonitrile (MeCN) solvent pairs. In and out indicate whether the measurement was used in the least squares minimization. MeCN % - other solvent % explanation: the first number is the volume percent of MeCN, and the second is the volume percent of the other solvent used. 0 means water, MeOH is methanol, and others are MeCN solutions.



References

- 1 A. Suu, L. Jalukse, J. Liigand, A. Kruve, D. Himmel, I. Krossing, M. Rosés and I. Leito, *Anal Chem*, 2015, **87**, 2623–2630.
- 2 P. Liigand, A. Heering, K. Kaupmees, I. Leito, M. Girod, R. Antoine and A. Kruve, *J Am Soc Mass Spectrom*, 2017, **28**, 2124–2131.
- 3 K. Lossmann, R. Hecht, J. Saame, A. Heering, I. Leito and K. Kipper, *J Chromatogr A*, 2022, **1666**, 462850.
- 4 R. Veigure, K. Lossmann, M. Hecht, E. Parman, R. Born, I. Leito, K. Herodes and K. Kipper, *J Chromatogr A*, 2020, **1613**, 460667.