Analytical Problem-Solving Procedures for Undergraduates by ^{1}H NMR

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ABSTRACT

^{1}H NMR Spectroscopy is widely used technique, but until recently was of limited practical importance in pharmaceutical and chemical education. Teaching ^{1}H NMR spectroscopy remains a challenge in all the chemistry labs, as the number of facts obtained from each experiment is easily overwhelming for the students. We developed four different experimental settings for the undergraduates which connect interdisciplinary problem-solving approaches with the hands-on experience in NMR. The set of the experiments consists of amino acids identification, log*P* value determination, quantitative determination of the marketed over the counter drugs, and p*K*a value determination. We could show that our approach to teach NMR has significantly improved the understanding of the technique among our students.

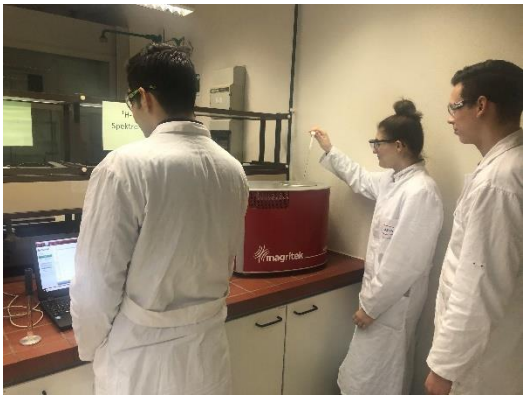

Photo: A. Zivkovic

Keywords: ^{1}H NMR, hands on experience, analytical chemistry, problem solving approach, qualitative analysis, quantitative analysis

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Introduction

Nuclear Magnetic Resonance spectroscopy (NMR) is a fundamental identification and structural determination technique in any chemical lab. This technique became more important with its introduction in the European Pharmacopoeia (*Ph. Eur)*. In 2008 about 900 cases of adverse events associated with the use of heparin were reported in Germany, resulting in the urgent need for increased analytical/quality control of complex compounds (Beyer et al., 2010). As a necessity, the first NMR-based identification control *via* ^{1}H NMR measurement has been introduced into *Ph. Eur*. (Beyer et al., 2010). This highlights the need for teaching NMR techniques in pharmacy and related sciences. Due to the high costs of NMR instrumentations, teaching NMR in the majority of academic practical courses in analytics has for a long time been restricted on the theoretical evaluation of NMR spectra, which have been recorded by technicians or other scientists, but not by the students themselves. Although high-field NMR spectrometers up to 1000 MHz or higher are extremely expensive to purchase as well as to run, the practical student courses can nowadays take advantage of the availability of low-field benchtop spectrometers (Anon n.d.).This technique development made experiments in NMR for students possible having a great variety of analytical aspects (Edgar et al., 2019; Kennedy et al., 2019; Kent and Bell 2019; Swartz et al., 2018; Yearty et al., 2017; Yennie et al., 2017). Recently, we developed and performed four different experiments using ^{1}H NMR benchtop instruments in the practical instrumental analytics course for undergraduate pharmacy students. The experiments are part of an interactive scientific puzzle where each element supports theunderstanding of a set of information obtained from ^{1}H NMR measurements. Identification of natural amino acids consists of using increment calculations, multiplicity predictions and chemical shifts in order to identify an unknown amino acid (Zivkovicet al., 2017). Another experiment consists of log*P* determination, where signal integration is used for the calculation of lipophilicity (Soulsby and Chica, 2017; Zivkovic et al., 2018). Furthermore, for the purpose of understanding the relative signal integration, the experiment where the quantitative analysis of multicomponent mixtures of over-the-counter (OTC) pain killer drugs as an example for nonsteroidal anti-inflammatory drugs (NSAIDs) is determined (Zivkovicet al., 2017). In order to deepen the understanding of the connection betweenacidity as altered protonation level and chemical shift, we developed an experiment where the p*Ka* value of the known drugs is determined (Zivkovicet al., 2017). Pedagogical goals of the battery experiments are to learn problem-based interpretation of ^{1}H NMR data (in one or two dimensions (COSY)), to process their data, understand the connection between chemical character and chemical shift, understand integration, use of D_2O exchange as well as in which way one can use the ^{1}H NMR for quantitative evaluation. Interdisciplinary, problem-orientedgroup learning should also increase motivation for learning.

Experimental

All of the measurements have been performed on the Magritek Spinsolve Benchtop (42.5 MHz) (Aachen, Germany) with the following resolution parameters: 50% linewidth <0.7 Hz (16 ppb), 0.55% line width<20 Hz. The measurements have been done in standard 5 mm NMR tubes in either D_2O for amino acids identification and p*Ka* determination, H_2O for log*P* value determination or DMSO-d_6 for qualitative and quantitative analysis of the OTC mixtures (and subsequent addition of D_2O).

The procedures are simple and can be performed and reproduced by inexperienced lab students. Undergraduate students have performed all parts of the experiment: dissolution, measurement, processing of the data (using MNova software) and identification.

As first milestone in a battery of experiments, the students have identified an unknown amino acid. We developed(Zivkovicet al., 2017) an easy algorithm thatstudents can follow (Figure 1).

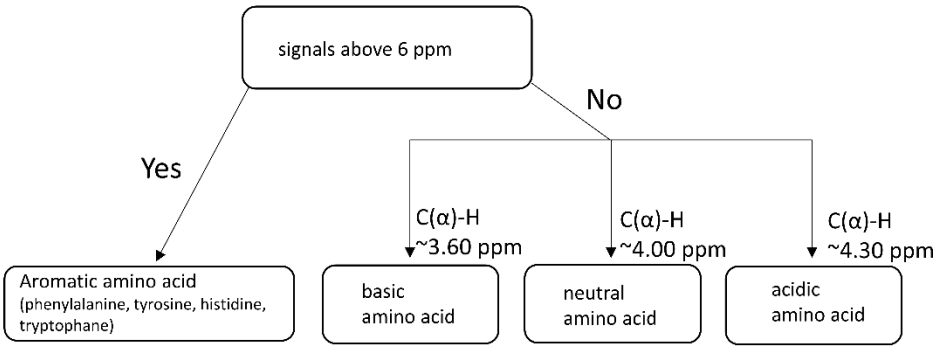
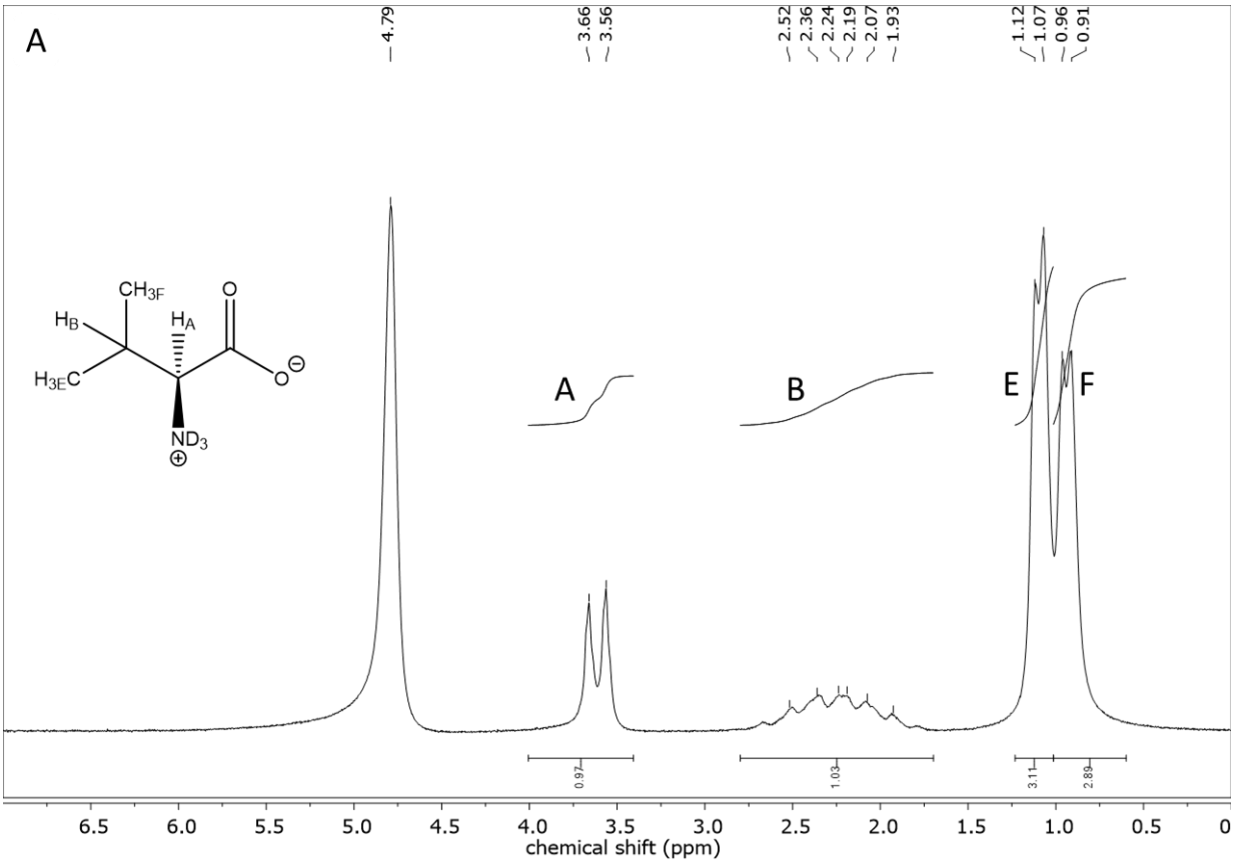


Figure 1. Algorithm for amino acidsidentification

To illustrate our measurements, we demonstratein Figure 2 the ^{1}HNMR spectrum of L-valine (20 mgmL^{-1} in D_2O), with ^{1}H-^{1}H COSY measurement.


A
4.79
3.66
3.56
2.52
2.36
2.24
2.19
2.07
1.93
1.12
1.07
0.96
0.91
CH3F
HB
HA
H3EC
O
O⊖
ND3
⊕
A
B
E
F
0.97
1.03
3.11
2.89
6.5
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chemical shift (ppm)

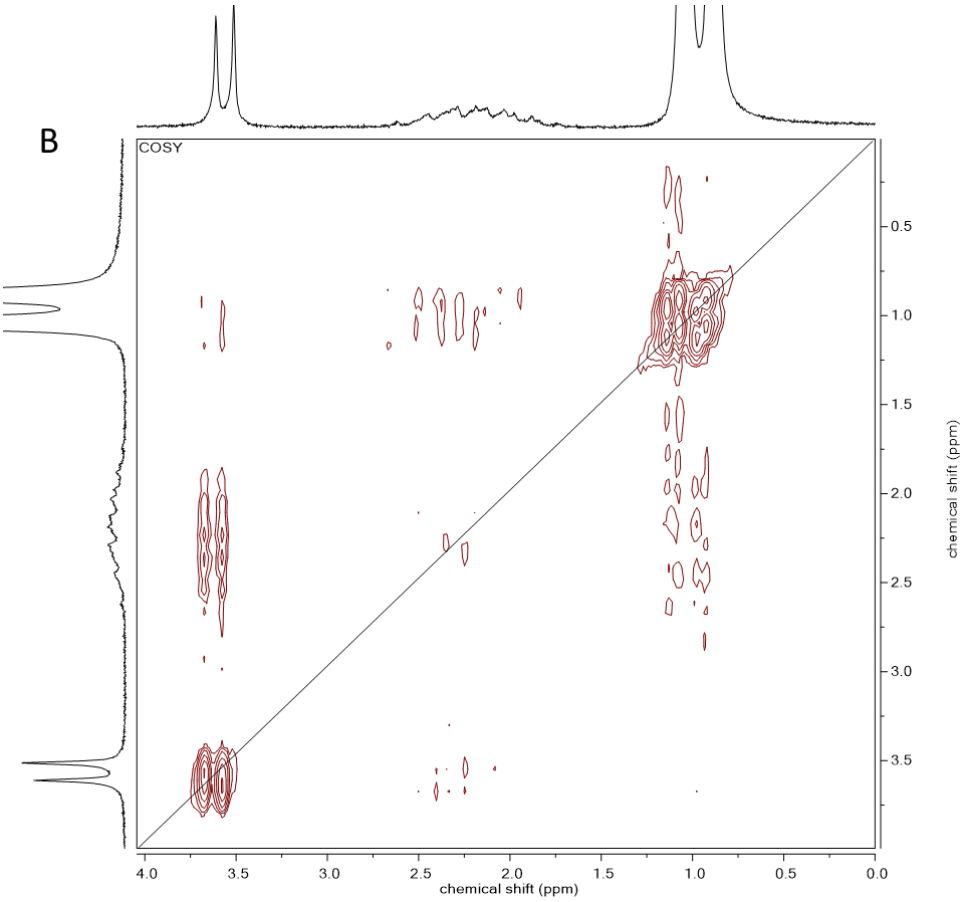

Figure 2. ^{1}HNMR (A) and ^{1}H-^{1}H COSY (B) measurement of L-valine in D_2O (~20 mgmL^{-1}).

In the second experiment, the students determined the log*P* value of one of the common solvents (acetone, methanol, ethanol, dimethylformamide,*etc.*). The log*P* parameter together with the p*K*a value is of high importance in the design and synthesis ofpharmaceuticallyactive compounds, especially for lipophilicity, solubility, protein binding, and permeability (Manallack, 2007; Manallack et al., 2014). To keep an experiment simple for the beginners, ithas been done with frequently used solventsin water and water/octanol mixture. In the first measurement,^{1}H NMR of water and the solvent mixture has been measured. Then the integration has been done to the water peak integral as a reference, which is always set at 1.000.00. After thismeasurement, 1-octanol has been added to the mixture, shaken and after separation of the phases directly transferred into the NMR tube, the second measurement has been done. When water is integrated on the same value (1.000.00), the integral reduction of the solvent (from which log*P* is determined) fits to the concentration that is now present in 1-octanol.The experiment is replicated thrice. The mean value and the standard deviation are calculated. The measurement of ethanol in water and water/octanol mixture is shownin Figure 3. The log*P* value was calculated as follows (A_w (solvent integral in water) and A_{ow} (solvent integral in water/octanol) are taken from the NMR in Figure 3):

$$logP = log\left(\frac{A_w - A_{ow}}{A_{ow}}\right)$$

$$\log P = log\left(\frac{203.18 - 136.50}{136.50}\right) = -0.31$$

The log*P* value obtained corresponds with literature data for ethanol log*P* values (Erhart et al., 2015; Wasserkort and Koller, 1997).

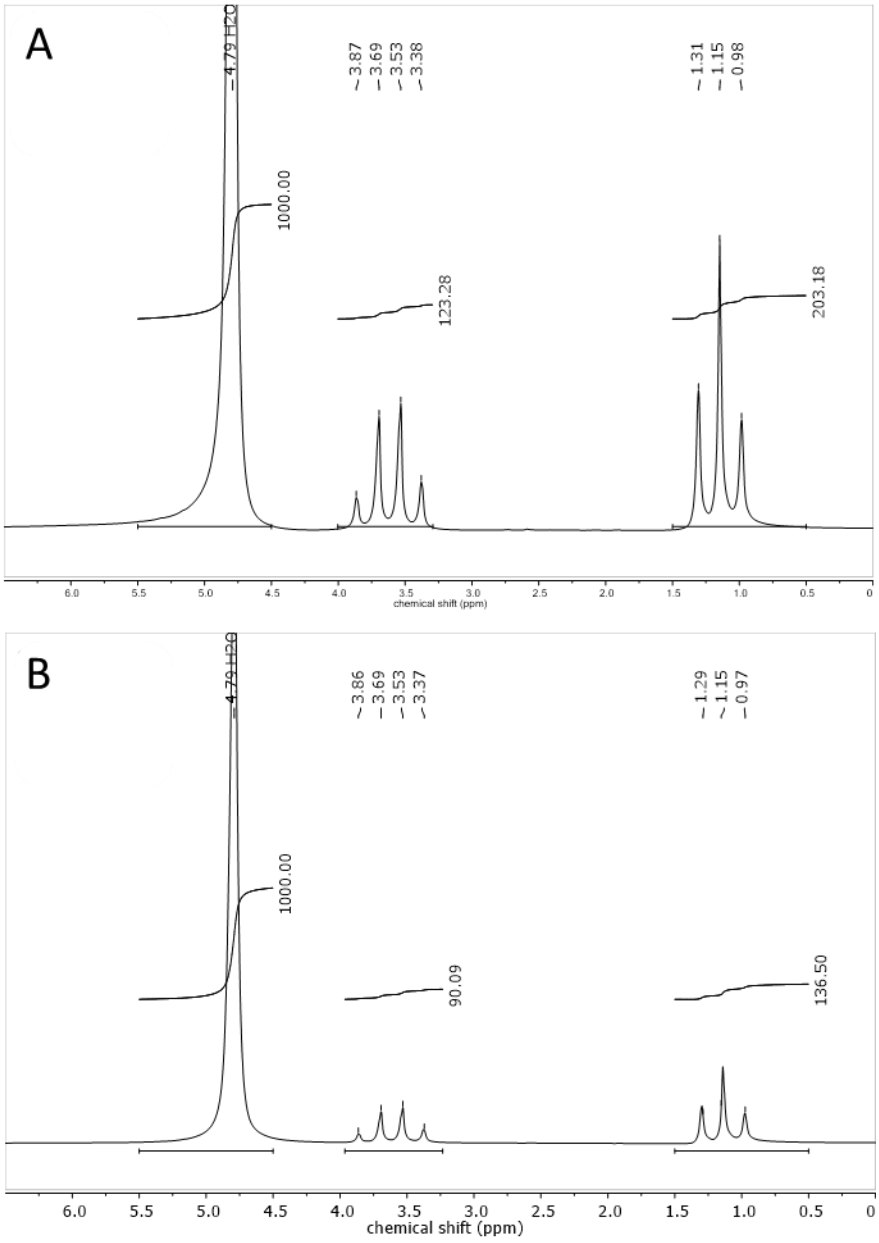

Figure 3.[1]HNMR measurement of ethanol in water (A) and water/octanol (B)

A slightly moreadvanced experiment (performed as the third in this series) is the qualitative and quantitative determination of one compound and two-component mixtures using common OTC drugs as shown in Figure4.

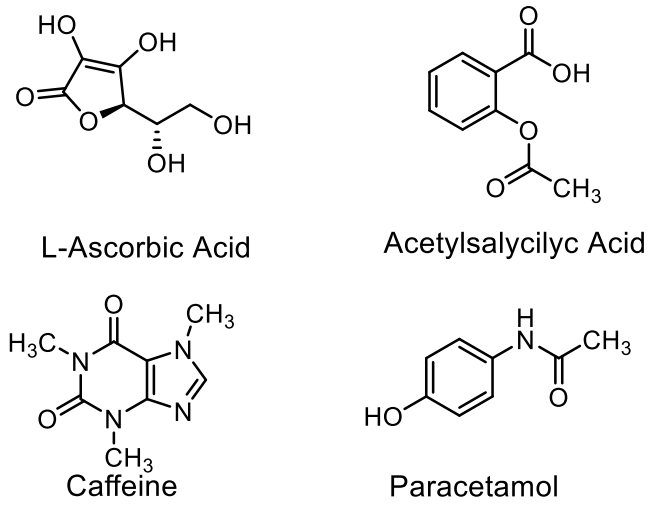

Figure 4. The structures of the common OTCs in quantitative determination

In this analysis step, it is important tofirst identify the substances in the mixture and assign all the signals (in both NMRs, with and without D_2O) to the correspondingprotons in the structures. After this, the students need to choose one signal from each OTC drug that is going to be used for relative quantitative determination. The chosen NMR signal cannot be exchangeable with D_2O;it has to be isolated and clearly has to belong to only one of the substances. If there are more than one possibility, the signal belonging to the largest number of protons should be chosen. The signal/noise ratio would have the smallest influence on the result, so these selection criteria are the most efficient. In this challenging experiment for NMR beginners, each group has to find the problem solving way on their own, the best for their measurements (Zivkovic et al., 2017). One measurement of a compound mixture with the corresponding calculations from the quantification is shown in Figure 5 and Table 1.

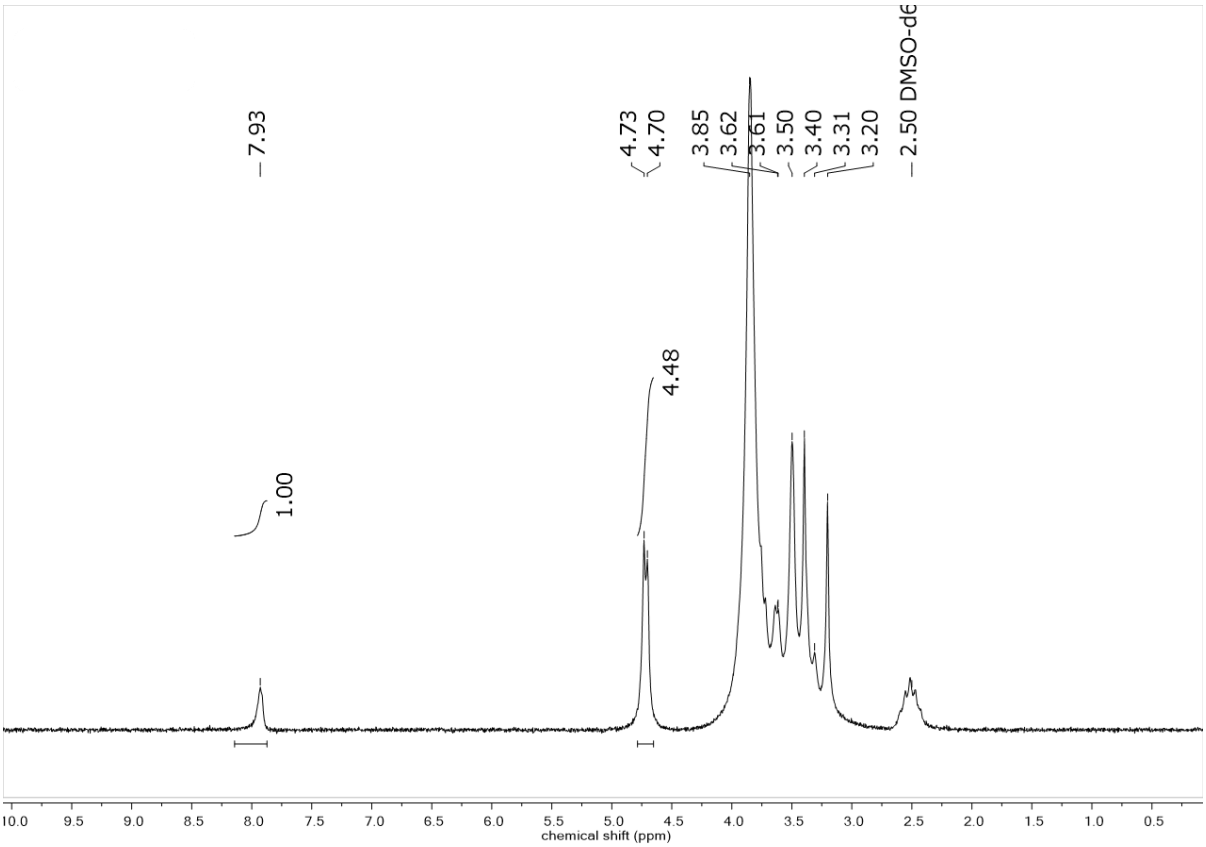

Figure 5. ^{1}H NMR measurement of the mixture consisting of caffeine (20%) and L-ascorbic acid (80%) in DMSO-d_6 (~20 mgmL^{-1}) after addition of few drops of D_2O (exchangeable protons)

The aromatic proton in caffeine (at 7.93 ppm) and the doublet signal of one proton at 4.72 ppm is used for the quantification in this mixture.

Table 1. Quantification from the ^{1}H NMR measurement of the mixture in Figure 5

Compound	*L-ascorbic acid*	*Caffeine*
Molecular Weight (Mr)	176.1	194.2
Integral Height (I)	4.48	1.00
Number of Protons (P) that belongs to Integral (I)	1	1
Ratio I/P	4.48	1.00
Mass Ratio (Mr•I/P)	789.0	194.2
Mass Ratio in %	80.2	19.8
Actual Composition (mg)	80	20

In the last experiment in ^{1}H NMR spectroscopy in this series, the students have determined the p*K*a value of one of the following drugs: nicotinamide, isoniazid and pyridoxine hydrochloride. Therefore, the change in the chemical shift in one of the aromatic protons was documented upon p*H* change (achieved with the addition of either acid or base). Results were represented as the function of the chemical shift (ppm) of p*D* value (Figure 6) (De Almeida Drumond Dos Santos et al., 2010; Gift et al., 2012; Mumcu and Küçükbay, 2015; Zivkovicet al., 2017). The titration curve was evaluated in the form of a classical titration curve with MS-Excel or related programs. The obtained results for all the probes agree with literature values for the corresponding drugs (Becker et al., 2007; De Almeida Drumond Dos Santos et al., 2010; Perrin, 1969)

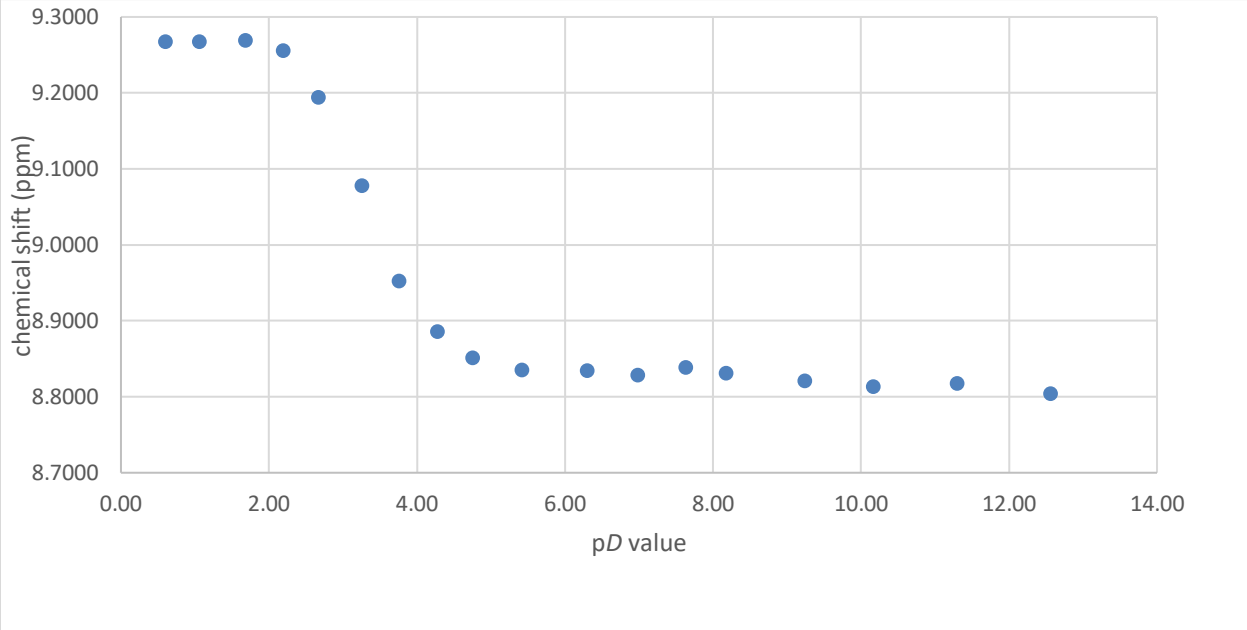

Figure 6. An example plot of the ^{1}H NMR chemical shift of aromatic proton of nicotinamide as a function of p*D*

From the titration curve in Figure 6, the calculated pKa value of 3.54 was in good accordance with the literature value of 3.35 (Perrin, 1969).

Results and Discussion

We have developed and implemented four experiments with different difficulty level in the undergraduate pharmacy student's lab that are easily transferable to related compound studies in other scientific fields. In the last five years more than 500 students performed the experiments. Each of the experiments was conducted up to today more than 400 times. The first three experiments, amino acid identification, log*P* determination and quantification of the mixtures,were performed in one 5 h laboratory course, and the pKa determination in the second 5 h course. Amino acid determination was incorrect on the first try in 13 determinations (3.1%). In the second try all of the students have correctly determined the natural amino acid. In the log*P* determination experiments, of all the determinations performed, 12.5%

had to be repeated, mostly due to the incorrect experimental performance. In the slightly more sophisticated experiment with OTC drugs, the correct mixture composition was given only once improperly. The students' quantification evaluation failed in 20.1% of cases. Here one should mention that not all of the mixtures show the same tendency for a failure, mostly depending on the signal overlap and student detection of exchangeable protons. In the second try, only 2.1% of the students failed, and on the third try, all of the determinations were done properly. The p*Ka* determination experiment requires precise and focused practical work.

Conclusion

We were able to develop and perform four experiments in order to deepen our students'understanding of ^{1}H NMR principles as well as potential applications. After the introduction of spectroscopy experience in practice, the average of 56% points in the NMR spectroscopy questioning (test/exam) increased to almost 81%. Pharmacy students in Germany take the state exam in instrumental analytics after the fourth semester, where approximately ten of the 45 questions are NMR related. Since the introduction of the experiment into our lab course, we observe that at the NMR state exam, our students achievestatistically significantly better results than the average (all pharmacy faculties in Germany).

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Conflict-of-Interest Statement

The authors declare no competing financial interests.

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