Modern Chemistry
2022; 10(1): 5-11
http://www.sciencepublishinggroup.com/j/mc
doi: 10.11648/j.mc.20221001.12
ISSN: 2329-1818 (Print); ISSN: 2329-180X (Online)

Analysis of the Alcohol Amine Mixtures by 1D Selective COSY

Zhang He[1], Huang Guilan[2, *], Yang Hongyu[1]

[1]Department of Underwater Weaponry and Chemical Defense, Dalian Naval Academy, Dalian, China

[2]State Key Laboratory of NBC Protection for Civilian, Institute of Chemical Defense, AMS, PLS, Beijing, China

Email address:

zhanghe92@126.com (Zhang He), hglhdm@163.com (Huang Guilan), wander0504@163.com (Yang Hongyu)

[*]Corresponding author

To cite this article:

Zhang He, Huang Guilan, Yang Hongyu. Analysis of the Alcohol Amine Mixtures by 1D Selective COSY. *Modern Chemistry*. Vol. 10, No. 1, 2022, pp. 5-11. doi: 10.11648/j.mc.20221001.12

Received: January 22, 2022; **Accepted**: March 18, 2022; **Published**: March 23, 2022

Abstract: Proton nuclear magnetic resonance (^{1}H-NMR) is one of the most commonly used techniques for structure identification in the field of chemical warfare convention (CWC) because of its strong ability in aiding structure assignment of chemicals. However, due to the lack of separation function coupled with chromatograph, the greatest disadvantage of ^{1}H-NMR in the analysis of environmental and biological samples is that the resonances can be easily covered or interfered. One-dimensional NMR selective excitation technology can effectively remove background interference and reveal the hidden spectral peak information. In this paper, three schedule chemicals of Chemical Weapons Convention (CWC), triethanolamine, N-methyldiethanolamine and 2-diisopropylaminoethanol, were selected as model chemicals. Homonuclear 1D COSY NMR selective excitation analysis method was established with these chemicals to solve the problem of resonances covered in ^{1}H-NMR. Key experiment parameters of 1D COSY were optimized. The optimized experimental conditions of 1D COSY are as follows: pulse sequence is selcogp, shaped pulse is G3, relaxation delay of d1 is 4 s, the excitation width of δ_H 0.91 (a), 2.51 (g), 2.62 (e) and 3.44 (d) is 39.16, 35.34, 20.00 and 26.03 Hz respectively. The established 1D COSY method was used to analyze 1~10 μg/mL alcohol amine mixtures with 2 mg/mL quinuclidinol as the background interference and the LOD was 5 μg/mL. This method is simple, fast and can wipe off the interference effectively in the ^{1}H NMR spectrum without proceeding complex sample pretreatment. 1D selective COSY reflects the more accurate position of hydrogen atom, and can be used as a reliable auxiliary technology for structural identification.

Keywords: Precursors of Chemical Weapon, Selective Excitation, 1D COSY, Mixture, Alcohol Amine

1. Introduction

In the field of Chemical Weapons Convention analysis, four one-dimensional NMR methods are commonly used for the analysis of small molecular compounds, namely ^{1}H-NMR, ^{31}P-NMR, ^{13}C-NMR and ^{19}F-NMR. Among them, ^{1}H-NMR sensitivity is the highest [1]. For 400M superconducting NMR spectrometer (9.4T field strength), if ^{1}H-NMR sensitivity is 1, then the sensitivity of ^{19}F spectrum, ^{31}P spectrum and ^{13}C spectrum are 0.8, 0.07 and 0.0001 respectively. Moreover, the ^{1}H-NMR provides the most abundant structural information. The hydrocarbon group information of compounds can be identified by the chemical shift, coupling constant, splitting condition, integral area ratio and other information of hydrogen atoms. ^{1}H-NMR is the preferred NMR method in the analysis of organic compounds, especially trace compounds [2-4], because most compounds contain hydrogen atoms and ^{1}H-NMR has the characteristics of high sensitivity and can show large amount of information.

The limitation of ^{1}H-NMR is that it requires high sample purity. Otherwise the target peaks can be easily covered by background interference. In the actual analysis of biological samples and environmental samples, most of the samples are doped in a complex matrix. The hydrogen spectrum peak signal of organic substances in the matrix is strong, which often overlaps with the hydrogen spectrum signal of trace target substances, resulting in that the hydrogen spectrum of target substances fail to meet the requirements of structural identification.

Generally, there are three ways to analyze the mixture in complex matrix by NMR technology. 1) Sample pretreatment: including filtration, centrifugation, extraction, concentration, solvent replacement, etc. 2) Change the method of nuclide detection: for the compounds whose target molecules do not contain fluorine and phosphorus atoms, first derive the compounds, and then detect the compounds by ^{31}P-NMR and 19F-NMR. 3) Correlation spectrum method: reveal the information of masked peaks through two-dimensional spectrum, such as COSY, ^{1}H-^{31}P HSQC and other technologies.

Aforementioned three methods can solve a part of practical problems [5-7], but the first two methods involve sample preparation. This not only requires more time, but may also cause damage to or even lose the original sample [8, 9]. Taking the 32nd OPCW proficiency test as an example [10], two amino alcohols were added to the organic liquid sample: dipropyl aminoethanol and diisopropyl aminoethanol. They are similar in structure and difficult to separate, which eventually leads to the inability to provide effective NMR spectra. So the indirect identification method of providing phosphorus spectra after phosphorylation derivatization has to be adopted. For the analysis of these mixtures with background interference, the two-dimensional NMR analysis method can reveal the spectral peak information of the target compound hidden in the background interference through the correlation points, but the relevant information of the interference compound and the target compound will appear on the two-dimensional spectrum at the same time, The target compound signal of low concentration will be inhibited by the background interference compound signal of high concentration, and the relevant information cannot be displayed. In addition, it also has the disadvantages of long data acquisition time. Also, spectral resolution is not as high as the conventional one-dimensional spectrum, and cannot reflect the splitting information of spectral peaks. In recent years, the gradually developed one-dimensional selective excitation technology of NMR has caught more attention for its obvious advantages in removing background interference, shortening data acquisition time, improving spectral resolution and simplifying spectral analysis [11-13]. For some special cases in small molecule analysis, for example, some peaks of compounds are covered up and there are bare hydrogen atom peaks, one-dimensional selective excitation technology of NMR can play a very effective role. Because of these advantages, one-dimensional selective excitation NMR has been widely used in the fields of metabolites, food analysis, chemical reaction intermediates and other fields [14-16]. But reports on its uses in the analysis of compounds related to chemical weapons are rare.

This paper determined the 1D selective COSY method to qualitatively analyze three typical alcohol amines by optimizing several critical experimental parameters and the amino alcohol mixture of N-methyldiethanolamine, triethanolamine and 2-(diisopropylamino) ethanol was analyzed qualitatively.

2. Experiment

2.1. Instrument and Sampling Requirements

Bruker AVANCE III HD 600 MHz NMR instrument (Bruker Corporation, Germany), Multi-core cryogenic probe with 5 mm pulsed gradient field broadband, workstation software of Topspin 3.5pl7. All tests are conducted in 5 mm NMR tubes. Sampling requirements: ^{1}H NMR operated at 600.13 MHz; tests performed at 25°C; Spectral width set as 12019.230 Hz; MestReNova software used for Spectral processing. Selectively excited pulse sequence selcogp, G3 shaped pulse.

2.2. Reagent

Heavy water (CIL Corporation, Deuterium Oxide (D, 99.9%)), Deuterated sodium hydroxide (Superhall Limited, Deuterium Oxide (D, 99.9%)), N-methyldiethanolamine (Shanghai Aladdin Bio-Chem Technology Co., LTD, purity 99%), triethanolamine (Shanghai Aladdin Bio-Chem Technology Co., LTD, purity 99%), 2-(diisopropylamino)ethanol (Energy Chemical CO., Ltd., purity 98%), Quinazole (Energy Chemical CO., Ltd., purity 98%).

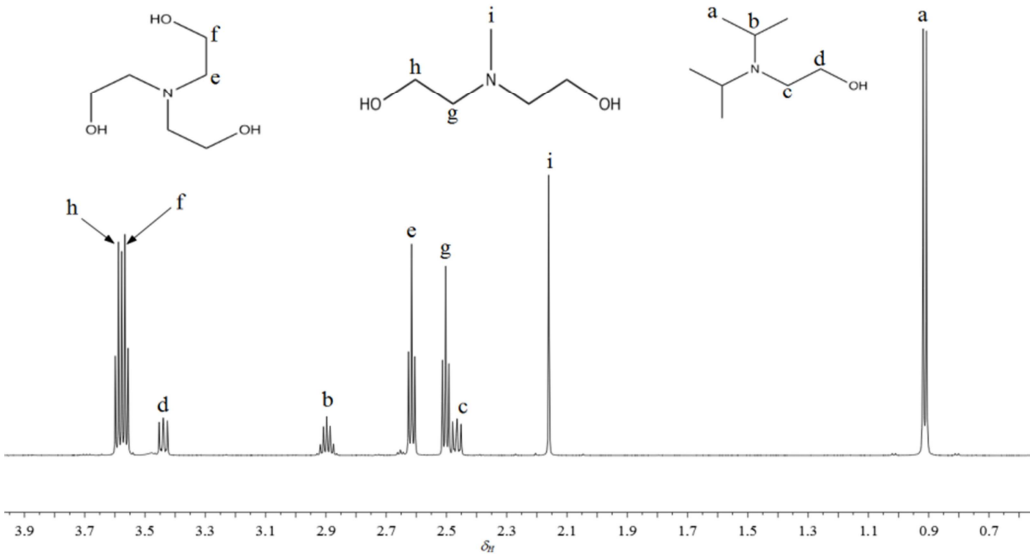

Figure 1. *^{1}H Spectra of Amino Alcohol Mixture.*

3. Result and Discussion

3.1. Optimization of the 1D COSY Parameters

Sample: Amino alcohol mixture dissolved in heavy water (concentration over 1 mg/mL) with deuterated sodium hydroxide modifying pH=14. The regular ^{1}H spectra are shown in Figure 1.

Based on theoretical analysis and information from the 2D COSY spectra, it can be confirmed that 3J hydrogen atom coupling systems existed in the three amino alcohol molecules. They are (a, b), (c, d), (g, h), (e, f). Among them, a, b, c, d, e, f, g, h respectively refers to the hydrogen atoms marked in Figure 1. Ways to optimize parameters of 1D COSY experiments are determined to be follows: 1) 1D COSY spectra should only reflect the information of hydrogen atoms having 3J coupling relationships with the excited hydrogen atoms; 2) 1D COSY should be with normal peak phase and peak splitting; 3) 1D COSY spectra should have the best signal-to-noise ratio of spectral peaks.

3.2. Optimization of Related Parameters

3.2.1. Choice of Pulse Sequence

According to the instrument configuration, selcogp and selco are chosen as the tested pulse sequences. 1D COSY experiments are respectively conducted with pulse sequences of selcogp and selco to the samples of alcohol amine mixture, i.e. N-methyldiethanolamine, triethanolamine and 2-(diisopropylamino) ethanol, with heavy water as solutions. The main difference between selcogp and selco is whether having gradient fields. Because of this, slightly different experimental parameters are required for the pulse sequences. The pulse sequences are shown in Figure 2 with d14, sp2 and p12 respectively referring to mixing time, shaped pulse and the time when shaped pulse is applied.

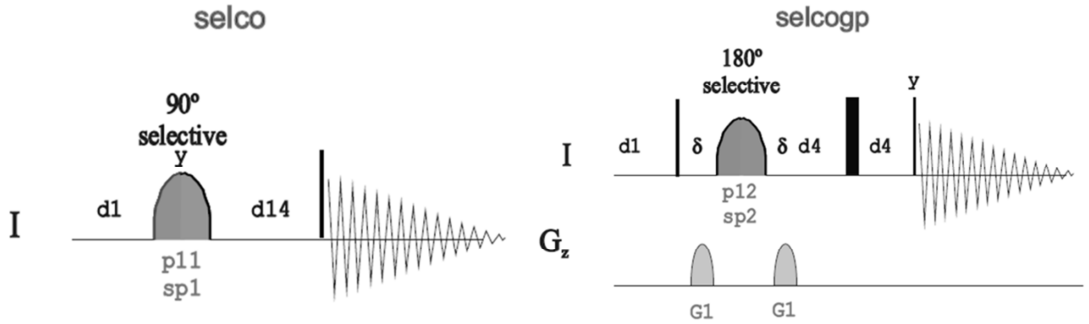

Figure 2. *Schematic diagram of 1D COSY pulse sequence.*

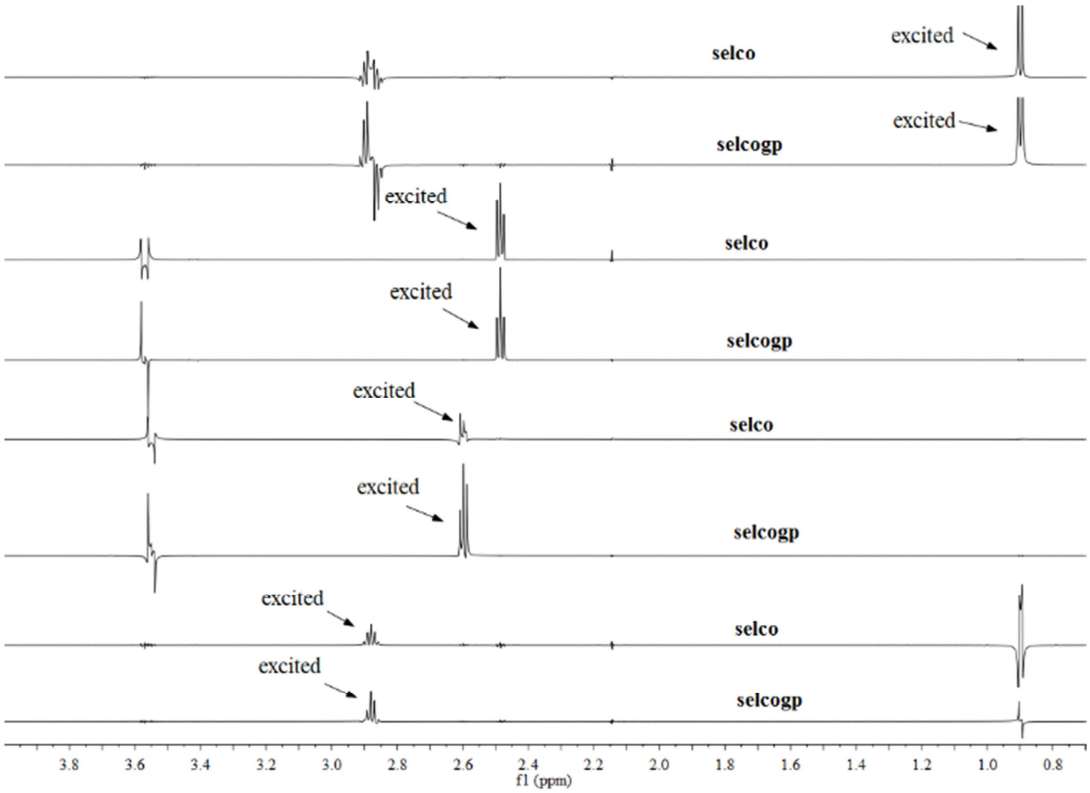

Figure 3. *1D COSY spectra of amino alcohol mixtures under different pulse sequences.*

In the experiments, δ_H 0.89, 2.46, 2.57, 2.85 are excited, 32 scans are operated and the bias is 0 Hz. According to signal-to-noise ratios, peak splitting, whether there are phase distortions and whether only the hydrogen atoms having 2J and 3J coupling relationships with the excited hydrogen atoms are reflected, the optimized pulse sequence is picked out. The result shows that selcogp and selco are both able to remove the unrelated peaks and only show the hydrogen atoms having 3J coupling relationships with the excited peaks. The spectra are show in Figure 3. With pulse sequence selco, peak phase distortions are more obvious and signal-to-noise is not as good either. For optimization of other parameters, 1D COSY experiments are mainly conducted with pulse sequence selcogp.

3.2.2. Optimization of Shaped Pulse

According to the instrument configuration, ten shaped pulses are selected as the test objects. They are Reburp, Q3, G3, Borbup, Q3-surbop, Q5, Seduce3, Seduce1, Square, Sinc. Experiments are conducted to find the best one.

Samples of amino alcohol mixtures with the concentration of 1 mg/mL are used for the 1D COSY experiments. Chemical shifts of δ_H 0.91 (a), 2.52 (g), 2.57 (e) and 3.44 (d), which correspond to the different 3J coupling systems in the three alcohol amine compounds, are excited.

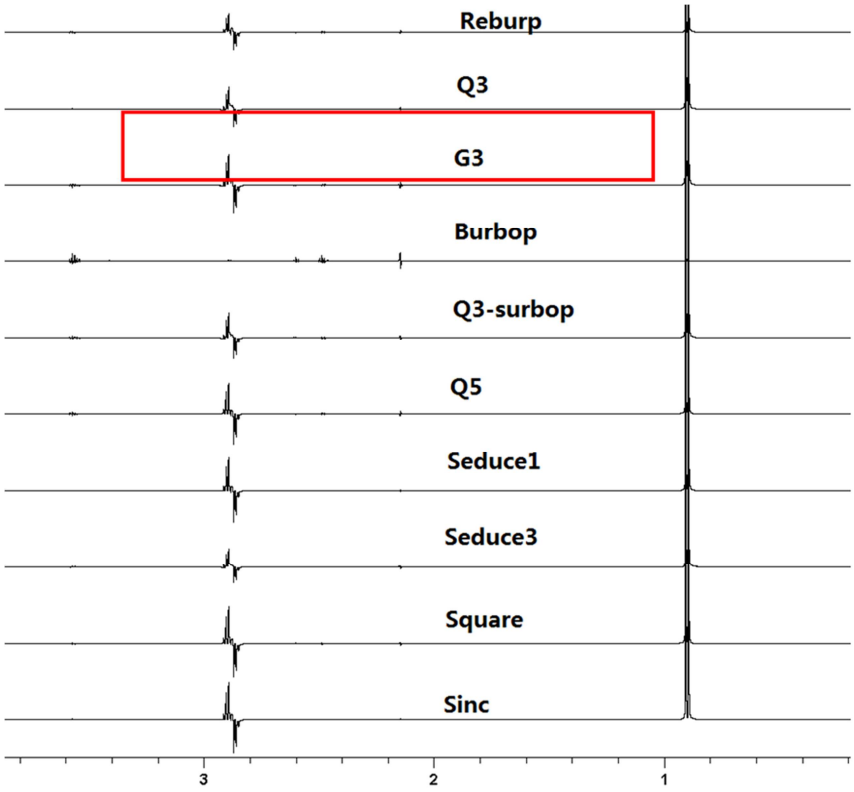

Figure 4. *1D COSY spectra of amino alcohol mixtures under different pulse sequences, excitation position δ_H 0.91.*

Figure 4 shows the excitation result on δ_H 0.91 with different shaped pulses. The results of excited position on δ_H 2.52 (g), 2.57 (e) and 3.44 (d) are similar to Figure 4. Shaped pulse which correctly reflects the 3J coupling relationships in hydrogen atom systems is marked with red rectangle. Result shows that excited at different positions, the shaped pulse of G3 is all able to reflect the right 3J coupling relationships in hydrogen atom systems. Meanwhile, it also has a rather ideal signal-to-noise ration. Therefore, in the following experiments, G3 are used as the shaped pulse.

3.2.3. Optimization of the Excitation Width

Different from 1D NOESY, 1D ROESY and 1D TOCSY experiments, 1D COSY experiments do not involve the optimization of mixing time. 1D COSY experiments are more related to excitation width and relaxation delay D1. Excitation of δ_H 0.91 (a), 2.52 (g), 2.57 (e) and 3.44 (d), different experiments are conducted according to the excitation width. The result on δ_H 2.57 (e) is shown in Figure 5. Based on peak splitting, peak shape and whether there are interference peaks around the target peak, different excitation widths are selected. The best excitation width, in red rectangular, can only reflect hydrogen atom systems with 3J coupling relationships, and also with more ideal signal-to-noise ration and relatively less phase distortion.

3.2.4. Optimization of Relaxation Delay D1

To improve the signal-to-noise ration of 1D COSY spectra, D1 (Relaxation Delay D1) is optimized. D1 are respectively set at 1, 2, 3 and 4 s and δ_H 0.91 is excited. The result shows in Figure 6. When D1 is 4 s, we get the most ideal signal-to-noise ration.

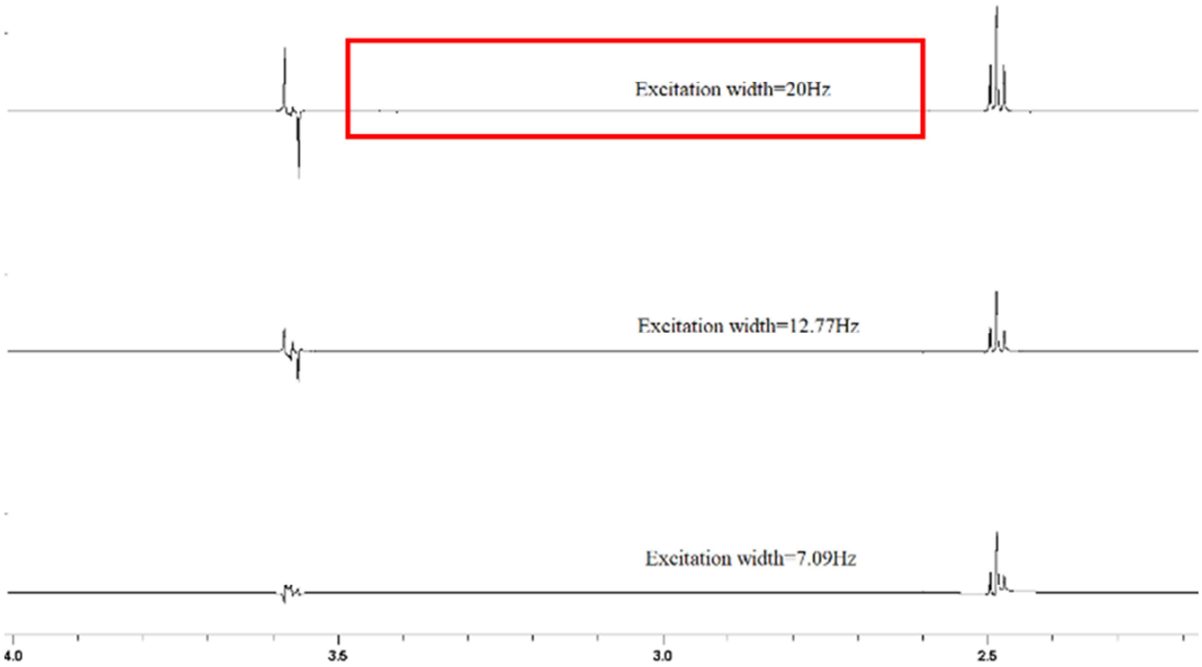

Figure 5. *1D COSY spectra of amino alcohol mixtures with different excitation widths, excitation position δ_H 2.57.*

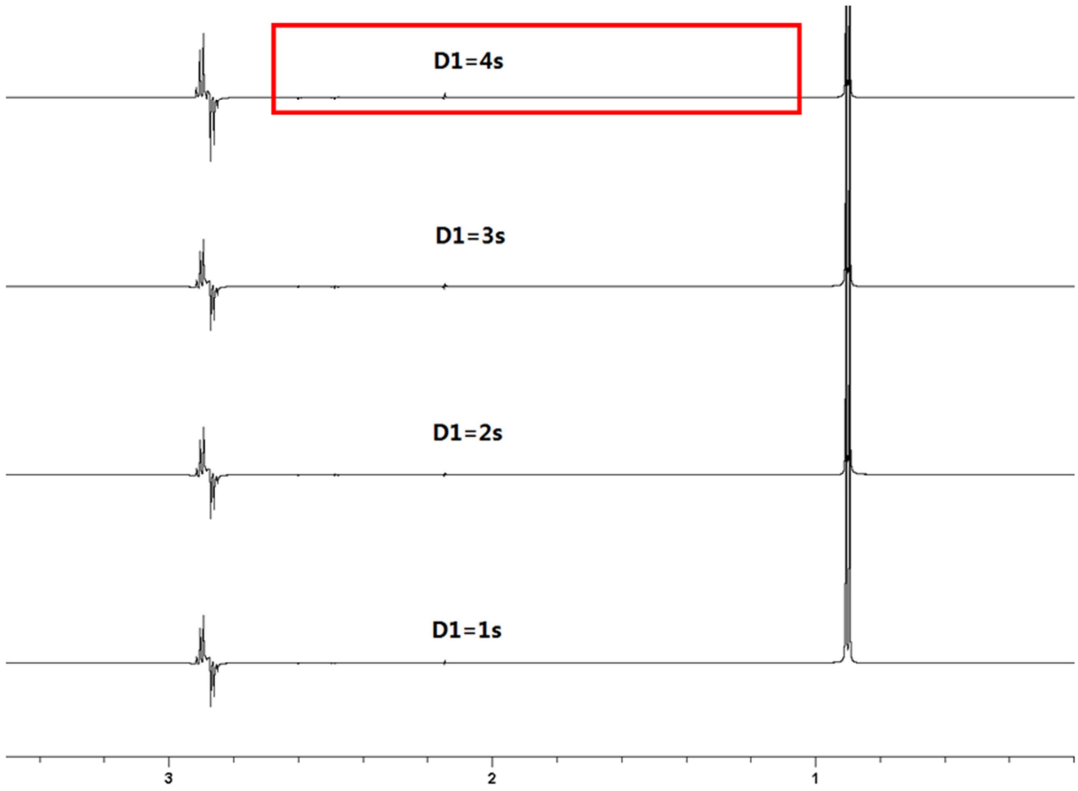

Figure 6. *1D COSY spectra of amino alcohol mixtures with different D1, excitation position δ_H 0.91.*

3.3. Application of 1D COSY to Simulation Samples

In the simulation samples, concentration of N-methyldiethanolamine, triethanolamine, and 2-(diisopropylamino) ethanol are respectively 20 μg/mL and 200 μg/mL quinuclidinol is used as the background interference. First, ^{1}H NMR experiments are conduced to the simulation samples and the spectra are shown in Figure 7. Peaks between δ_H 2.4-2.7 of the three alcohol amine compounds are totally covered because of quinuclidinol. Between δ_H 3.5-3.6, hydrogen peaks of N-methyldiethanolamine and triethanolamine overlap and unknown impurity peaks present around as well.

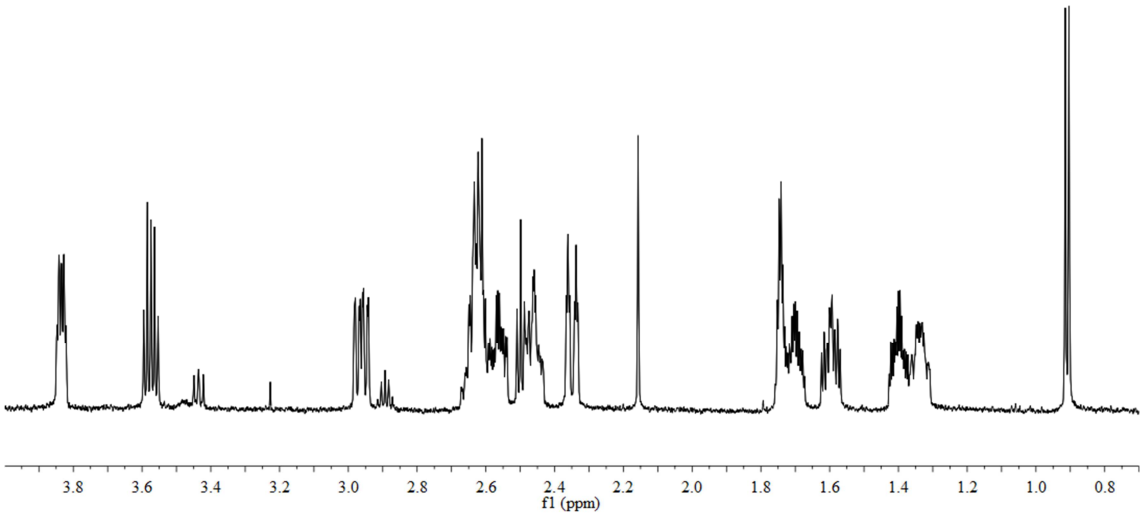

Figure 7. *Simulated sample ^1H spectra.*

Excited at δ_H 0.91 (a), 3.44 (d) and 3.57 (f, h), we get the 1D COSY spectra in Figure 8. This 1D COSY spectra removes interference from background and shows the hydrogen peaks which have 3J coupling relationships with the excited peaks. For example, exciting δ_H 0.91 (a), signals of hydrogen atom δ_H 2.91 (d) are shown in the 1D COSY spectra; exciting δ_H 3.44, signals of hydrogen atom δ_H 2.49 (c) are shown; exciting δ_H 3.57 (f, h), signals of hydrogen atoms δ_H 2.52 (g) and δ_H 2.62 (e) are shown. Due to the relatively small molecular weights and simple structures of the three amino alcohols, 1D COSY can locate all of their hydrogen atoms and make qualitative analysis of them possible. However, in practical application, able to reflect hydrogen atoms with 2J and 3J coupling systems, 1D COSY is often used as auxiliary tool for structural analysis.

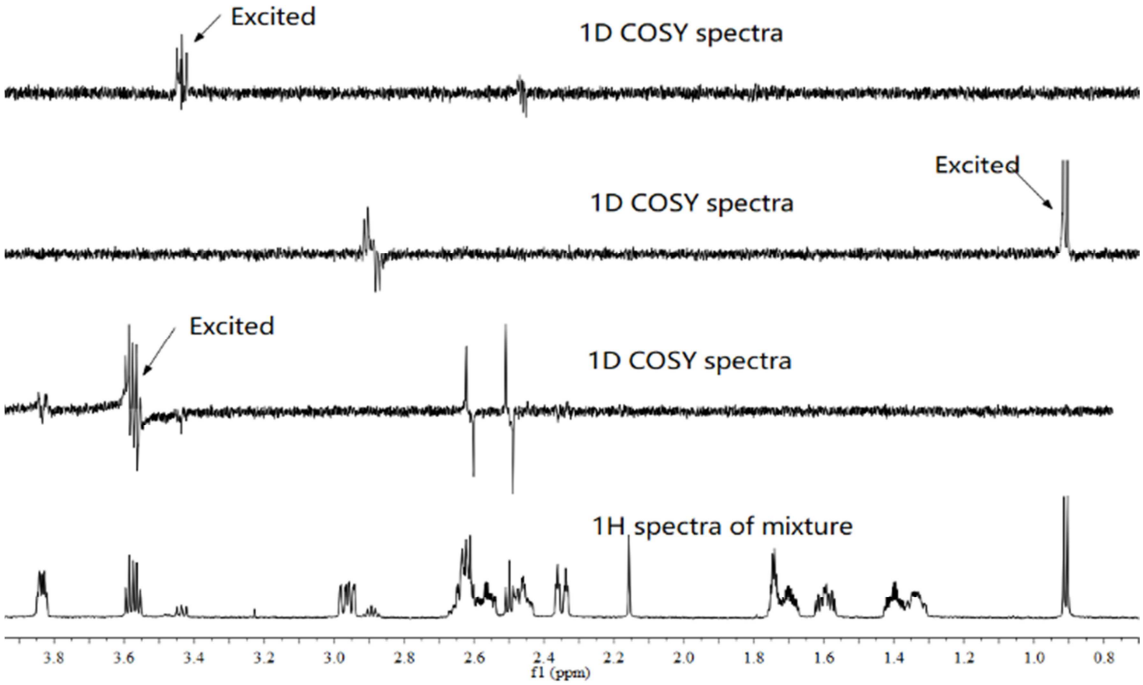

Figure 8. *Simulated sample 1D COSY spectra.*

4. The LODs of 1D COSY

1D COSY experiments conducted to mixtures with alcohol amine's concentration respectively at 1, 3, 5, 10 μg/mL and with 2 mg/mL quinuclidinol as background interference. Exciting δ_H 0.91, 2.52, 2.62 and 3.44, the result shows that the LODs (S/N>3) of 1D COSY for the alcohol amines with quinuclidinol at background is 5 μg/mL.

5. Conclusion

1D COSY, showing the other hydrogen atoms that have 2J and 3J coupling relationships with the excited hydrogen

atoms, can indicate hydrogen atom position information accurately. The LODs of this method for mixtures of amino alcohols are 5 μg/mL, which makes it with higher sensitivity compared to 1D NOESY and 1D ROESY. Because 1D COSY only reflect 2J and 3J coupling relationships, it normally not used alone to identify the compounds but often as assistive technology for structural identification. Due to the relatively small molecular weights and simple hydrogen atom coupling systems of the N-methyldiethanolamine, triethanolamine and 2-(diisopropylamino)ethanol, 1D COSY selective excitation techniques can locate signals of all hydrogen atoms inside the three amino alcohol molecules and identify them based on the standard ^{1}H spectra.

References

[1] Claridge T D W. High-resolution NMR techniques in organic chemistry [M]. Elsevier Books, 2001: 12-13.

[2] Htun K T, Pan J, Pasanta D, et al. Identification of Metabolic Phenotypes in Young Adults with Obesity by 1H NMR Metabolomics of Blood Serum [J]. Life, 2021, 11 (6): 574.

[3] Sulaiman F, Azam A A, Bustamam M, et al. Metabolite Profiles of Red and Yellow Watermelon (Citrullus lanatus) Cultivars Using a 1H-NMR Metabolomics Approach [J]. Molecules, 2020, 25 (14).

[4] Shumilina E, Andreasen C, Bitarafan Z, et al. Determination of Glyphosate in Dried Wheat by 1H-NMR Spectroscopy [J]. Molecules, 2020, 25 (7).

[5] Guo C, Fritz M P, Struppe J, et al. Fast 19 F Magic Angle Spinning NMR Crystallography for Structural Characterization of Fluorine-Containing Pharmaceutical Compounds [J]. Analytical Chemistry, 2021.

[6] Zhang L, Gellerstedt G. Quantitative 2D HSQC NMR determination of polymer structures by selecting suitable internal standard references [J]. Magnetic Resonance in Chemistry Mrc, 2011, 45 (1): 37-45.

[7] Changlun, Shao, Zhigang, et al. 1H and 13C NMR assignments for two anthraquinones and two xanthones from the mangrove fungus (ZSUH-36) [J]. Magnetic Resonance in Chemistry, 2007.

[8] Brennan, Lorraine. NMR-based metabolomics: from sample preparation to applications in nutrition research. [J]. Prog Nucl Magn Reson Spectrosc, 2014, 83: 42-49.

[9] Halabalaki, Bertrand, Stefanou, et al. Sample Preparation Issues in NMR-based Plant Metabolomics: Optimisation for Vitis Wood Samples [J]. PHYTOCHEM ANALYSIS, 2014.

[10] Kim H, Jung C, Lee Y. Analysis Study on 32nd OPCW Proficiency Test Sample with GC-TSQ CI [J]. Journal of the Korea Institute of Military Science and Technology, 2014, 17 (6): 828-835.

[11] Uhrinova S, Du X, An U, et al. Detection of long-range couplings in oligomers and evaluation of coupling constants in polymers. Application of the 1D COSY technique [J]. Magnetic Resonance in Chemistry, 1991, 29 (1): 144-147.

[12] Pham T N, Liptaj T, Barlow P N, et al. J-modulated 1D directly COSY for precise measurement of proton-proton residual dipolar coupling constants of oligosaccharides [J]. Magnetic Resonance in Chemistry, 2011, 40 (11).

[13] Stryjewski D, Oschkinat H, Leibfritz D. Detection of metabolites in body fluids and biological tissue by a 1D soft COSY technique [J]. Magnetic Resonance in medicine Official Journal of the Society of Magnetic Resonance in Medicine, 1990, 13 (1): 158.

[14] Dioukhane K, Aouine Y, Nakkabi A, et al. Characterization of the Structure of 2-(4-methyl-2 Phenyl-4, 5-dihydrooxazol-4-ylmethyl)-isoindole-1, 3-dione by 1D, COSY and HSQC 2D NMR Spectroscopy [J]. Asian Journal of Chemical Sciences, 2021: 30-37.

[15] Hu H, Bradley S A, Krishnamurthy K. Extending the limits of the selective 1D NOESY experiment with an improved selective TOCSY edited preparation function [J]. Journal of Magnetic Resonance, 2004, 171 (2): 201-206.

[16] Howe P. Removal of zero-quantum peaks from 1D selective TOCSY and NOESY spectra [J]. Journal of Magnetic Resonance, 2006, 179 (2): 217-222.