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European Network

ILIADe 553:2022 | CLEN Method

Recording of Nuclear Magnetic Resonance Spectra of Organic Substances such as New Psychoactive Substances, or Designer Drugs

Version 27 January 2022

The table shows the most important changes that has been done compared to the latest former version.	
Date of the latest former version:	2018
Section	**Changes**
All sections	Layout harmonization according to new template and minor linguistic changes
Figure 1	Figure replaced with a better definition new one.
Annex 1	New annex with a brief description of main useful NMR experiments

Recording of Nuclear Magnetic Resonance Spectra of Organic Substances such as New Psychoactive Substances, or Designer Drugs

1. Scope and field of application

1.1 Introduction and field of application

The identification of the chemical structure of an unknown substance is required for purposes of control by enforcement authorities whether for verification of correct classification by Customs, detection of an illicit substance, or reporting of a new designer drug to the competent national and international authorities.

Nuclear Magnetic Resonance (NMR) is a powerful and flexible spectroscopic technique that provides key information facilitating the determination of the chemical structure of organic substances.
It can be used for pure organic substances or in certain cases for a mixture of several substances.

1.2 Scope

This method is suitable for recording ^{1}H and ^{13}C NMR spectra after preparation of the unknown substance in an appropriate solvent. This document presents the minimum requirements for ^{1}H NMR and ^{13}C NMR.

Depending on the spectroscopic instrumentation available, additional NMR experiments can be performed on the same preparation providing complementary information for elucidation of the chemical structure. These may include two dimensional NMR and spectra of other nuclei (^{15}N, ^{19}F, ^{31}P,…). It should be also mentioned that the presence of chloride or bromide anions can be detected in samples diluted in D_2O and can therefore be used to confirm the salt form of certain substances.
NMR spectroscopy offers a broad range of possible experiments, that cannot be presented in the current document. For information regarding their use, their set-up, and the hardware required for the instruments, the analyst should consult specialised scientific literature, NMR text books, and the instructions provided by the manufacturer of the spectrometer.
A short introduction of the main experiments of two-dimensional nuclear magnetic resonance spectroscopy of interest for the purpose of elucidation of the chemical structure of small molecules is provided in the annex.
It is beyond the scope of this document to present in depth details about the principles exploited for the set up of the sequences of these NMR experiments. For this the analyst should refer to the literature available on these arguments. On the other hand, a short mention of the physical phenomenons exploited in these sequences and the kind of information obtained through them can help the analyst to decide which experiments can be most helpful for the case under study and for further interpretation of the obtained NMR data.

2. Principle

The unknown organic substance is dissolved in an appropriate deuterated solvent for performing liquid state NMR spectroscopic experiments. The interpretation of results by a skilled and well trained scientist could lead to an hypothesis of the unknown molecule structure.

3. Reagents and materials

3.1 Deuterated Dimethyl sulfoxide (DMSO-d_6) for NMR analysis, CAS Number 2206-27-1, EC Number 218-617-0.

3.2 Any other analytical grade and deuterated solvents (e.g. $CDCl_3$, D_2O, CD_3OD) required to dissolve and analyse the chemical compound in question.

The solubility of the substance under study is a priori unknown. Due to its ability to dissolve a wide range of compounds and of the simplicity of its own NMR spectrum, deuterated dimethyl sulfoxide (DMSO-d_6) is routinely used as a first choice solvent for recording the NMR spectra of such unknown substances. Alternative solvents can be chosen considering

- *low solubility in DMSO or*
- *overlap of DMSO NMR signals with those of the substance under study*
- *low amount of material available*
- *extraction from herbal material or other bulk product (i.e. food product)*

Deuterated chloroform, methanol or water are the most commonly used other NMR solvents.
In case of low amount of sample, it can be preferable to first use a solvent such as deuterated chloroform or methanol which can then be left evaporated to recover the sample giving then the possibility to run successive experiments using DMSO-d6.

3.3 Phenethylamine; CAS Number 64-04-0, EC Number 200-574-4.

3.4 NMR tubes 5mm id diameter and caps.

3.5 Pipette tips and vials for preparation of chemicals.

4. Apparatus

4.1. Balance with 0.1 mg accuracy or better.

4.2. Vortex mixer or ultrasonic bath

4.3. Common laboratory equipment such as micropipettes.

4.4. Nuclear Magnetic Resonance spectrometer equipped with at least:

- 5mm probe and hardware and software allowing the recording of ^{1}H spectra and of ^{13}C spectra decoupled from ^{1}H (^{13}C {^{1}H}).
- electronic channel for the field-frequency stabilization (lock) using the deuterium signal of the solvent.
- temperature control unit for maintaining the sample at ± 0.1 K at the temperature set for the NMR experiments.
- spinners and the gauge

Unless otherwise specified, an apparatus with a field strength giving an operating frequency for proton NMR of at least ν_o = 300 MHz (corresponding to a magnetic field Bo = 7.05 T) or higher frequencies are generally convenient for obtaining spectra with adequate resolution and signal to noise ratio fit for the purpose presented in the above introduction and scope. The NMR instrument can possibly be equipped with an automatic sample changer device.

5. Procedure

5.1 Apparatus set up

The correct set up of the instrument includes the calibration of the pulses and power used in the classical sequences of NMR experiments (i.e. parameters used to acquire ^{1}H, ^{13}C spectra and classical two dimensional NMR spectra). The resolution is adjusted through the shimming of the magnetic field. The performance of the spectrometer is then checked on test compounds and/or ad-hoc NMR tubes (e.g. by recording the spectra of '1% $CHCl_3$ in acetone-d_6' or of a control NMR sealed tube of ethylbenzene generally delivered with the instrument). This is achieved following the procedure defined by the manufacturer to check the performance of the instrument against its technical specifications. When needed, the operator can control the performance of the instrument with the same procedure. Performance checks are executed at least annually and at restarts/modification of the equipment.

5.2 Sample preparation

Normal laboratory conditions are sufficient for the preparation and analysis of the compounds. In order to reduce any risk of exposure, usual precautions for manipulation of chemicals should be observed. Moreover it is recommended to manipulate limited amounts of material sufficient for the preparation of the solutions.

On powders/crystals the first solvent used is routinely DMSO-d_6 (3.1) and , if some insoluble matter remains, then other different solvents may be used. On herbal product the first choice as a solvent is deuterated methanol.

Note: the operator should take care of the high hygroscopicity of DMSO to avoid possible contamination with moisture during the preparation.

5.2.1 Weigh ca. 10 mg of the sample in a vial.

5.2.2 Add 600 µL of DMSO-d6 (3.1).

5.2.3 Eventually use a vortex mixer or ultrasonic bath to ensure complete dissolution.

5.2.4 Carefully pipet and transfer the solution into a 5mm NMR tube.

5.2.5 Place the tube containing the sample to analyse in a spinner, ensuring that the height is correctly adjusted using the gauge provided. Wipe both NMR tube and spinner with a lint-free tissue to remove all smudges or dirt.

5.2.6 The tube is introduced directly in the probe (inside the magnet) or eventually placed at the appropriate position of the sample changer if the instrument is equipped with such a device.

5.3. NMR set up

The modern NMR instruments allow the execution of the following operations in automation mode. Alternatively this can also be done "manually" by experienced NMR operators.

5.3.1 Once positioned in the probe, the field/frequency of the instrument has to be 'locked' on the deuterium signal of the solvent.

5.3.2 Then the tuning and matching of the probe for the frequencies used for the experiments have to be performed.

5.3.3 Lastly shimming is performed to obtain a homogenous magnetic field and good resolution of the NMR spectra.

5.3.4 Before starting the experiment, the Receiver Gain parameter is adjusted.

5.4. NMR experiments

Select the appropriate experiments (i.e. for launching the sequences of acquisition for ^{1}H spectra, then ^{13}C $\{^{1}H\}$ spectra and eventually additional NMR experiments for recording two-dimensional NMR spectra or experiments on other nuclei). Create the parameter file. Examples of most used parameter sets are shown in Table 1 .

Table 1 Example of the most used parameter sets for Bruker NMR

NMR experiment	Pulse program	Parameter set
^{1}H	zg, zg30	PROTON
^{13}C	jmod	C13APT
	zgpg30	C13CPD
	deptsp135	C13DEPT135
COSY	cosygpppqf	COSYGPSW
HSQC	hsqcetgpsisp2.2	HSQCETGPSISP_ADIA
HMBC	hmbcgplpndqf	HMBCGP

5.5 Examination of the spectra

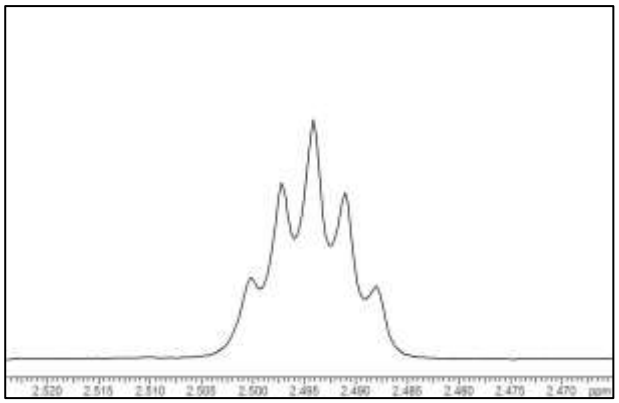
Since the chemical structure and/or composition of the material under analysis is a-priori unknown, it is not possible to set-up specific criteria to evaluate the quality of the acquired spectra.
Nevertheless keeping in mind the general objective of the current method as presented in introduction and scope, one can establish a few general criteria to guide the chemist for :

i) further exploitation of the NMR spectra obtained to derive sound information or hypothesis about the chemical structure and/or composition of the substance under investigation or
ii) propose to perform further tests either in NMR or with other analytical techniques. This may be needed to check some hypotheses or to obtain spectra with better resolution, signal to noise ratio or to observe a signal of the compound overlapping with that of the solvent of that experiment.

The spectroscopist can therefore consider the following criteria to judge the resolution of the ^{1}H spectra:

- When DMSO-d_6 is used as solvent, the typical quintet corresponding to the signal of the residual amount of C_2D_5HOS (chemical shift 2.50 ppm) should be well resolved: a symmetric signal of five peaks well visible with intensities roughly corresponding to the theoretical distribution 1,4,6,4,1 expected for a NMR quintet signal should be observed (see example in figure 1):

Figure 1, ^{1}H NMR DMSO-d_6 signal



- When the solvent used is deuterated chloroform, the line shape of the single peak corresponding to the signal of the residual amount $CHCl_3$ (chemical shift 7.26 ppm) can be compared to that obtained for the checking of the performance of the instrument with '1% $CHCl_3$ in acetone-d_6' (5.1).
The resolution is more critical for ^1H spectra which, with the conditions of concentration used in the present method, can be acquired with a sufficient signal to noise ratio for all signals within a few minutes. The operator can therefore try to improve this by once again "shimming" the instrument if insufficient resolution is observed on the first experiment. Eventually a test in another solvent, at another temperature, or higher dilution can be carried out if the resolution remains unsatisfactory.
Once the resolution of the ^1H spectra is acceptable, the field homogeneity is also sufficient for the $^{13}C\{^1H\}$ spectra. In that case the more critical parameter is rather the signal to noise ratio (S/N) which should be sufficient to clearly distinguish from the background noise all the peaks observed in the spectra. In case of doubt for certain weak signals, the operator can simply repeat the acquisition with a higher number of scans (NS) keeping in mind that the increase of (S/N) is proportional to the square root of (NS).
It should also be remembered that, in certain cases, the product under examination may be a mixture of several organic compounds and/or eventually contain mineral components. This may affect both the resolution and the signal to noise ratio especially of the ^1H spectra.
Typical features expected for the general criteria described above could be previously established from the spectra obtained for a known "control" compound measured using similar conditions of concentration, solvent, and pulse sequences. Phenethylamine (3.3) could be suggested for carrying out such tests. It is similar to amphetamine (but freely available in chemical catalogues), with a set of signals covering both aliphatic and aromatic regions and the multiplicity and relative intensities of its signals are well known.

6. Calculation

Not relevant

7. Results

Depending on the further use of the NMR data, the results can consist of:

7.1 Electronic NMR files (FID or transformed spectra) for possible further processing and storage in electronic libraries.

7.2 Table of NMR chemical shifts (δ in ppm), coupling constants (J in Hz), multiplicity (singlet, doublet, …, multiplet) and relative integrals of the signals observed in the 1H spectrum and chemical shifts observed for carbon in the 13C spectrum recorded with proton decoupling.

7.3 Observations about purity (presence of other substances).

7.4 Hypotheses of possible chemical structure recorded in electronic format such as Molfiles and/or SMILES (simplified molecular-input line-entry system).

7.5 Generated identifier InChI (IUPAC International Chemical Identifier) and the derived InChIKey (InChITM) non-proprietary identifier for chemical substances (http://www.iupac.org/home/publications/e-resources/inchi.html)

8. Precision

Not relevant

Annex 1: NMR experiments

Homonuclear correlation spectroscopy (COSY)

The two-dimensional spectrum that results from the COSY experiment shows the frequencies for a single isotope, most commonly hydrogen (^{1}H) along both axes.
Diagonal peaks correspond to the peaks in a 1D-NMR experiment, while the cross peaks indicate couplings between pairs of nuclei (much as multiplet splitting indicates couplings in 1D-NMR) their presence indicates that two nuclei are coupled.

Total correlation spectroscopy (TOCSY)

The TOCSY experiment is similar to the COSY experiment, in that cross peaks of coupled protons are observed. However, cross peaks are observed not only for nuclei which are directly coupled, but also between nuclei which are connected by a chain of couplings. This makes it useful for identifying the larger interconnected networks of spin couplings.

Heteronuclear single-quantum correlation spectroscopy (HSQC) and heteronuclear multiple quantum coherence (HMQC)

HSQC detects correlations between nuclei of two different types which are separated by one bond. This method gives one peak per pair of coupled nuclei, whose two coordinates are the chemical shifts of the two coupled atoms.
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HSQC works by transferring magnetization from the proton nucleus to the Carbon nucleus.

Heteronuclear multiple-bond correlation spectroscopy (HMBC)

HMBC (Heteronuclear Multiple Bond Correlation) spectroscopy is suitable for determining long-range connectivities. Since it is a long-range chemical shift correlation experiment, HMBC provides the information about the chemical shift of atoms that are about 2-4 bonds away from the proton to which they correlate. Hence, in case of carbon correlation, also quaternary carbon atoms can be detected. HMBC is mainly used for determining long-range ^{1}H-^{13}C connectivities, but also other nuclei can be detected, as for example ^{15}N.

Diffusion-ordered spectroscopy (DOSY)

Diffusion-ordered spectroscopy (DOSY) seeks to separate the NMR signals of different species according to their diffusion coefficient. A series of spin echo spectra is measured with different pulsed field gradient strengths, and the signal decays are analysed to extract a set of diffusion coefficients with which to synthesize the diffusion domain of a DOSY spectrum.
DOSY can be used for identification of overlapping signals of single molecules present in mixtures.

Nuclear Overhauser effect spectroscopy (NOESY)

Whereas a COSY generally correlates protons via geminal or vicinal scalar spin couplings, a NOESY shows correlations of protons, that are spatially close, no matter whether they are as well close through bonds and therefore scalarly coupled or not. The interaction that is important for an NOE transfer is the so called dipolar coupling, which can only be directly observed in solid or partially oriented phases. However, the NOE (Nuclear Overhauser Effect) also leads to an intensity change of a signal, if the signal of a coupled proton is saturated. This effect is based on the relaxation behaviour and it can lead to a signal enhancement as well as to a signal reduction.

Attached proton test (APT)

The attached proton test (APT) is a 1D ^{13}C NMR experiment that is used as an aid to assignment by separating carbons unattached to protons and CH_2 signals from CH and CH_3 signals. The APT experiment yields methine (CH) and methyl (CH_3) signals positive and quaternary (C) and methylene (CH_2) signals negative. It is slightly less sensitive than DEPT (Distortionless Enhancement by Polarization Transfer) but a single experiment shows all carbon signals at once unlike DEPT that suppresses quaternary carbons and requires up to three different acquisitions to yield full results.

Distortionless enhancement by polarization transfer (DEPT)

This experiment allows to determine multiplicity of carbon atom substitution with hydrogens. For this purpose, three experiments need to be recorded, where pulse has flip angle of 45^o (**a**), 90^o (**b**), and 135^o (**c**).

Signs of signals in these experiments will reveal the substitution of carbon atoms:

- in **a** (DEPT-45) all signals will be positive
- in **b** (DEPT-90) only signals of CH groups will show
- in **c** (DEPT-135) signals from CH_2 will be negative, while CH and CH_3 - positive