Reference NMR Spectrum Based on ^{1}H Spin Parameters and Application to Reagents in the Japanese Pharmacopoeia 〈*G5-9-190*〉

(^{1}H スピン情報に基づいた参照 NMR スペクトルと日本薬局方試薬への応用〈*G5-9-190*〉)

For quantitative or qualitative tests of drug products, "JP reference standards" that have appropriate quality for the intended purpose have been established. For crude drugs and related drugs, various physicochemical tests targeting marker compounds contained in crude drugs and related drugs have been established and it is desirable to establish JP reference standards of marker compounds for these tests. However, as described in Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy and its Application to Reagents in Japanese Pharmacopoeia <*G5-5-170*>, marker compounds of crude drugs and related drugs are derived from natural resources, and there are various difficulties in establishing JP reference standards.

In many cases, the Japanese Pharmacopoeia sets the specifications of a marker compound available as a reagent at that time in Reagents, Test Solutions <*9.41*> of the Japanese Pharmacopoeia, and the reagent is defined as the reference material for analysis. Since the reagent may contain related substances of the marker compound, purity tests are established in the specifications of the reagent to detect impurities in addition to the identification test. Although chromatographic methods are specified as the purity test in many cases, it is sometimes difficult to separate impurities like diastereomers from marker compounds. On the other hand, Nuclear Magnetic Resonance Spectroscopy <*2.21*> is a useful spectroscopic technique in such discrimination. When ^{1}H NMR measurement is performed in a superconducting magnetic field, as generally performed at present, even in the case where an impurity having a very similar structure such as a diastereomer is contained, a signal derived from the ^{1}H nucleus of the impurity is observed as a clearly different signal in the measured spectrum, mainly in a part with different structures between the marker compound and the impurity contained in the reagent. Therefore, if ^{1}H NMR is specified in identification tests, the presence of impurities in the reagent can be easily confirmed from the obtained spectrum.

Originally, ^{1}H spin parameters such as chemical shifts and spin-spin coupling constants observed from a ^{1}H NMR spectrum are specific to a compound and independent of strength of the magnetic field of an NMR apparatus. In addition, accurate ^{1}H spin parameters of a marker compound contained in the reagent can be obtained based on the ^{1}H NMR spectrum of the reagent with extremely high purity because software products for iterative calculation to obtain accurate ^{1}H spin parameters from measured ^{1}H NMR spectra can be utilized[1)]. Moreover, there are software products that can generate the spectrum from the ^{1}H spin parameters corresponding to the magnetic field strength of each measurement[1)], so that the reference NMR spectrum corresponding to any magnetic field strength for identification tests can be easily generated. Therefore, if accurate ^{1}H spin parameters are specified in the Japanese Pharmacopoeia, identification tests can be performed by direct comparison of spectra in the same manner as the infrared reference spectrum. In addition, detection of the distortion and unnecessary signals caused by the existence of structurally similar impurities by comparison of spectra can be used as a purity test. Details of the ^{1}H spin parameters and precautions to be taken in the identification test using the reference NMR spectrum are described below.

1. ^{1}H spin parameters

^{1}H spin parameters are generally obtained by measuring the position and multiplicity (peak pattern) of a signal obtained directly from a spectrum, and these can be specified as a chemical shift and a spin-spin coupling constant, respectively. It is desirable to know the ^{1}H spin parameters for all protons that are contained in the substance. However, even in the case of a substance with very high purity, the signals may overlap in a limited observation range, resulting in a very complex peak pattern. Therefore, these signals are often described as multiplet signals, and an accurate reference NMR spectrum cannot be generated because a spectral analysis by observation does not provide accurate ^{1}H spin parameters.

In order to obtain accurate ^{1}H spin parameters from a spectrum that consists of such a complex peak pattern, it is effective to use an iterative calculation method. Software products that generate spectra based on ^{1}H spin parameters have already been available, and software products equipped with an additional function for iterative calculation have become easy to use. In this method, total-line-shape fitting is performed each time until iterative calculation converges to obtain the ^{1}H spin parameters (chemical shift and spin-spin coupling constant) from the observed spectrum of the substance. When iterative calculation converges and the calculated spectrum agrees with the observed spectrum, the ^{1}H spin parameters obtained in that time can be regarded as being extremely close to the exact solution. By specifying this ^{1}H spin parameters in the Japanese Pharmacopoeia, the reference NMR spectrum can be used for the identification test.

2. Identification using reference NMR spectrum

The NMR spectrum of the sample is obtained by measuring ^{1}H NMR under the specified conditions. When accurate ^{1}H spin parameters are available for the substance to be examined, a reference NMR spectrum in the same magnetic field as the sample measured above can be generated via the software. When the chemical shift, multiplicity, and relative intensity of each signal in the NMR spectrum of the sample

are congruent to those in the reference NMR spectrum, the sample is judged to meet the identification test. Following points should be considered for confirming the congruence of the NMR spectrum of the sample and the reference NMR spectrum.

2.1. Line width of a signal

Fig. 1 shows partial reference NMR spectra of hesperidin at the ^{1}H resonance frequency of 60, 400, 600 and 800 MHz. It can be seen that the peak patterns of the spectra are different depending on strength of the magnetic field. Although these peak patterns are specific to hesperidin in each magnetic field strength, it should be noted that the peak pattern changes in the observation region where the peaks are close to each other due to the change in the line width of the signal. In generating the reference NMR spectra shown in Fig. 1, the line width of the signal has been standardized to 1 Hz.

In the spectrum of a sample, the line width of its signals are changed by adjusting the shim and/or the resolution of the apparatus. Therefore, when confirming the congruence of the NMR spectrum of the sample and the reference NMR spectrum of hesperidin, it is desirable to match the line width of a signal of the reference NMR spectrum with that of the sample or to apply a broadening factor of about 2 Hz to both spectra.

2.2. Signals derived from deuterated solvents and water

In the NMR spectrum of a sample, signals derived from the deuterated solvent used to dissolve the sample and from water, as well as signals from a reference compound for chemical shifts when it is used, can be observed. Note that in the case where these signals overlap with those of the marker compound in the sample, this region should not be used to confirm the congruence of the spectrum to the reference NMR spectrum. Since signals derived from slight impurities in the deuterated solvent may be observed, it is desirable to measure a blank containing only the deuterated solvent in advance to confirm the range and intensity of the observed signals of the impurities. In addition, the signal derived from water tends to increase in the case of a highly hygroscopic deuterated solvent which has been stored for a while after the container is opened. Therefore, it is desirable to use freshly opened or ampule type deuterated solvents.

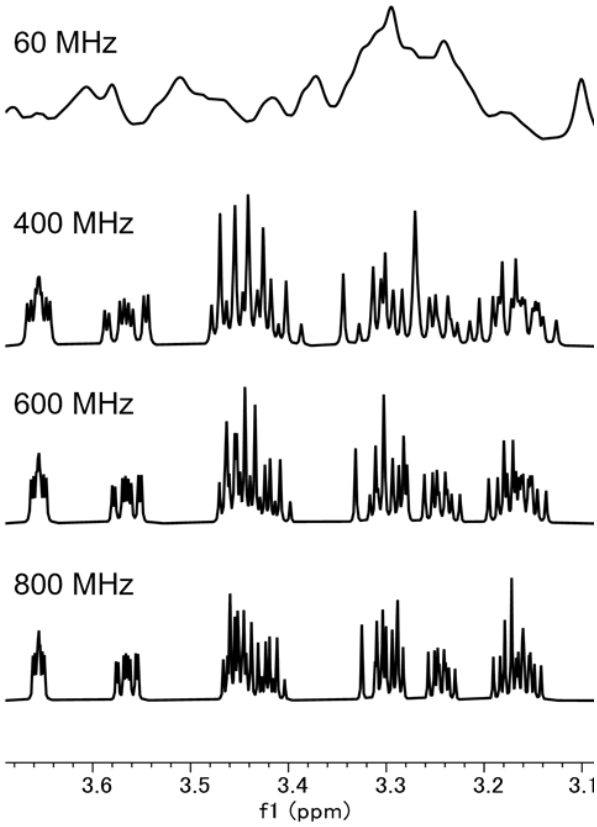

Fig. 1 Reference NMR spectra of hesperidin in deuterated dimethyl sulfoxide at ^{1}H resonance frequencies of 60, 400, 600 and 800 MHz

3. References

1) P.S. Achanta, *et al*., J. Pharm. Biomed. Anal., 192, 113601 (2021).