MQUANTIFICATION OF IMPURITIES
Where the impurity limit is specified in the individual monograph, a reference solution corresponding to that level of impurity should be prepared by diluting the test solution. For example, where the limit is 5 per cent, a reference solution would be a 1:20 dilution of the test solution. No impurity (any band other than the main band) in the electropherogram obtained with the test solution may be more intense than the main band obtained with the reference solution.

Under validated conditions impurities may be quantified by normalisation to the main band using an integrating densitometer. In this case, the responses must be validated for linearity.

2.2.32. LOSS ON DRYING
Loss on drying is the mass of loss expressed as per cent m/m.

Method. Place the prescribed quantity of the substance to be examined in a weighing bottle previously dried under the conditions prescribed for the substance to be examined. Dry the substance to constant mass or for the prescribed time by one of the following procedures. Where the drying temperature is indicated by a single value rather than a range, drying is carried out at the prescribed temperature ± 2 °C;

a) "in a desiccator": the drying is carried out over diphosphorus pentoxide R at atmospheric pressure and at room temperature;

b) "in vacuo": the drying is carried out over diphosphorus pentoxide R, at a pressure of 1.5 kPa to 2.5 kPa at room temperature;

c) "in vacuo within a specified temperature range": the drying is carried out over diphosphorus pentoxide R, at a pressure of 1.5 kPa to 2.5 kPa within the temperature range prescribed in the monograph;

d) "in an oven within a specified temperature range": the drying is carried out in an oven within the temperature range prescribed in the monograph;

e) "under high vacuum": the drying is carried out over diphosphorus pentoxide R at a pressure not exceeding 0.1 kPa, at the temperature prescribed in the monograph.
2.2.33. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

INTRODUCTION

Nuclear magnetic resonance (NMR) spectrometry is an analytical method in particular suitable for the elucidation of the chemical structure of organic molecules by means of interpretation of their NMR spectra, arising from, for example, $^1$H or the X-nuclei $^{13}$C, $^{19}$F, $^{15}$N, $^{31}$P. The spectra can be used for qualitative and quantitative purposes.

Under suitable experimental conditions, the integrated NMR intensities of the signals are directly proportional to the number of nuclear spins of the molecular group responsible for the signal. These integrals can be used for quantitative analysis.

GENERAL PRINCIPLES

Placing an ensemble of nuclei with angular momentum and a magnetic moment in a static magnetic field ($B_0$) causes the nuclei to arrange themselves in different, quantum-mechanically controlled orientations in relation to the axis of the magnetic field. These orientations are different in energy. An oscillating high-frequency magnetic field ($B_1$), perpendicular to $B_0$, will cause transitions between these orientations with net energy absorption. According to the resonance condition $\omega_0 = \gamma B_0$ ($\gamma$ = gyromagnetic ratio, $\omega_0$ = Larmor frequency), either the $B_1$ magnetic field or the frequency ($\omega_0$) of the $B_1$ field may be varied to achieve a spectrum (continuous wave (CW) method). Nowadays the $B_1$ irradiation is achieved by the use of a radiofrequency (RF) pulse (Fourier transform (FT) method). The coherent radiation emitted during the return to the initial state is observed in the form of a decay curve, called the free induction decay (FID). Subsequent Fourier transformation gives the spectrum in the frequency domain, providing information on the molecular structure. Additional radiofrequency fields may be applied during acquisition of the FID signal to suppress scalar (through-bond) interactions between nuclei (called ‘decoupling’). One- and multi-dimensional techniques can be applied for qualitative and quantitative purposes, on samples in either the liquid or the solid state.

Important structural information is derived from the following spectroscopic features:

<table>
<thead>
<tr>
<th>resonance frequency</th>
<th>kind of nuclei observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of resonance signals (singlets, multiplets)</td>
<td>number of chemically distinctly groups of nuclei</td>
</tr>
<tr>
<td>chemical shift $\delta$ (ppm)</td>
<td>chemical nature and environment of the structural group observed</td>
</tr>
<tr>
<td>intensity of resonance signals</td>
<td>relative number of resonant nuclei per chemically distinct group</td>
</tr>
<tr>
<td>multiplicity of coupling pattern</td>
<td>number of nuclei that are scalar coupled to the observed nucleus</td>
</tr>
<tr>
<td>coupling constant $J$ (Hz)</td>
<td>number of bonds in the coupling pathway, and its geometry</td>
</tr>
</tbody>
</table>

Correlations of different spectral parameters (e.g. chemical shift and coupling constant, or chemical shifts of different nuclei within one molecular system) can be performed by homo- and hetero-nuclear two- and higher-dimensional methods. Information about the relaxation times $T_1$ and $T_2$, nuclear Overhauser effects (NOEs) and the kinetics of time-dependent processes are also accessible from appropriate experiments.

APPARATUS

A high-resolution NMR spectrometer consists of at least the following parts:

- a magnet to deliver the constant magnetic field $B_0$;
- a temperature-controlled probe to contain the sample, to deliver the radiofrequency pulse and to detect radiation emitted by the sample;
- an electronic console to generate high-power radiofrequency pulses and to collect and digitise the FID signal; this unit also maintains the stability of the instrument electronics;
- a data acquisition and processing unit (computer);
- a system for pulsed field gradient NMR.

The high magnetic field is generated by a superconducting coil in a Dewar flask filled with liquid helium. The probe typically contains the sample in a 5 mm-outer-diameter sample tube or in a flow cell, and is connected to the electronics cabinet by RF cables carrying lock, $^1$H, and X-nucleus frequencies. Additional devices for tuning and matching the electronic circuits are essential, and sample temperature control is often used.

The NMR spectrometer should be demonstrated to be operating correctly. Appropriate tests to demonstrate this are, typically, measurement of linewidths at half height for defined peaks under defined acquisition conditions, signal-to-noise ratios ($S/N$) for standard mixtures, pulse power (measured as a 90o pulse width), and pulse reproducibility. All instrument manufacturers publish specifications and measurement protocols for these parameters for specific instrument/probe combinations, and compliance with these specifications should be demonstrated.

FOURIER TRANSFORM NMR (FT-NMR)

Contemporary spectrometers generally operate according to the Fourier transform (FT) principle: after exciting the sample with a radiofrequency pulse of appropriate frequency ($\nu$), amplitude ($B_1$) and duration ($\tau_p$) and a succeeding short dead time ($\tau_d$) (to enable the electronics to recover), the amplified analogue FID signal is sampled during the acquisition time ($\tau_{ac}$) and digitised with an analogue-to-digital converter (ADC), and the results are stored in the spectrometer memory. The receiver output is amplified prior to digitisation to maximise sensitivity without saturating the ADC. In case of observation of X-nuclei, the standard experiment includes, if necessary, broadband $^1$H decoupling, i.e. irradiation of all the protons during the experiment. To increase the $S/N$, multiple FID signals may be accumulated coherently and summed. Fourier transformation of this time-domain data gives the frequency-domain spectrum.

PARAMETERS

The following acquisition parameters influence the result of an FT experiment, and should be adjusted and controlled.

Pulse width ($\tau_p$). The excitation pulse is directed along the x-axis of the so-called rotating frame, its duration (or ‘width’, $\tau_p$) determines the flip angle ($\theta$) and thus the intensity ($I$) of the resonance signal:

\[
\theta = \gamma \cdot \tau_p \cdot B_1 \cdot \tau_p
\]

The observed magnetisation $M_y$ is maximum at $\theta = 90^\circ$. The pulse duration should be short to guarantee that all signals in the spectral width (SW) are excited to a similar degree. The magnetisation decays due to relaxation processes.

Dead time ($\tau_d$). The dead time is the time between the end of the pulse and start of the acquisition, it is necessary for technical reasons and care should be taken as it may influence signal intensities and peak phase. Rapidly decaying signals (giving rise to broad spectral lines) are reduced in intensity by more than slowly decaying signals (which give rise to narrow spectral lines).

Acquisition time ($\tau_{ac}$). The acquisition time ($\tau_{ac}$) is related to the spectral width (i.e. the whole observed region) and the number of digital data points (DP) collected during signal acquisition.