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Pharmaceutical Industry: Regulatory Control and Impact on NMR Spectroscopy.

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Note

The opinions expressed in this presentation are based on the speaker's experience as Pharmaceutical Assessor and do not necessarily reflect the views or policies of the MHRA.

Aims

- NMR spectroscopy in regulatory dossiers
- NMR for DS, DP and excipients
- Presentation of NMR data
- NMR deficiencies frequently encountered during assessment
- Concluding remarks

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NMR Spectroscopy in Regulatory Dossiers

NMR: general aspects.

• NMR is beneficial for identification, purity for the drug substance (DS), the drug product (DP) and excipients.

• In either post-manufacture testing or real-time release testing (RTRT), ^{1}H, ^{13}C, or multinuclear NMR spectroscopy (e.g.^{19}F, ^{31}P, ^{77}Se, etc.) may be used as appropriate.

• RTRT can be applied to discrete unit operations and to chemical reactions or separations (e.g. of diastereoisomers) during DS synthesis.

NMR: general aspects.

- The ability of NMR to provide greater structural specificity is well-recognized.

- NMR is used in identity tests for more complex molecules such as peptides and proteins as well as heparins.

- NMR is also used to confirm that the drug substance (DS) is present in the correct polymorphic form. This has impact on bio-availability when assessing BE studies!

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NMR for DS, DP and excipients

NMR for the Drug Substance (DS)

Manufacture:

• The Control of the DS manufacturing process requires the application of specifications to starting materials, intermediates, solvents and reagents as well as the final DS.

• Any of these may include NMR tests, usually to provide identification of the molecule in question.

NMR for the Drug Substance (DS)

Characterization:

• NMR beneficial for characterization of the DS, to include evidence of structure, discussion of potential isomerism, polymorphism, and physicochemical characterization.

• A wide range of ^{1}H, ^{13}C, and multinuclear NMR techniques have been presented in dossiers for MAAs to support the structures proposed for DS, both chemical and biological.

NMR for the Drug Substance (DS)

Characterization (cont'd):

- ^{1}H–^{1}H correlation (COSY), ^{1}H–^{13}C proton–carbon heteronuclear correlation, to identify short and long-range couplings, and Nuclear Overhauser Enhancements experiments (NOESY) to identify intramolecular interactions, protein structures, etc.

- NMR useful in establishing the stereochemistry of the DS, examples being the use of NOESY to identify *cis*–*trans* isomerism or to distinguish between *endo* and *exo* isomers of bicyclic molecules.

NMR for the Drug Substance (DS)

Characterization (cont'd):

- Single-crystal X-ray diffraction is the best method of establishing the absolute configuration of molecules containing chiral centers but NMR methods such as those employing chiral shift reagents may also be of value in establishing the presence or absence of optical isomers.

- NMR pivotal in establishing the identity and correct conformation of macromolecules (essential for activity!). This type of data is now being seen routinely in regulatory dossiers.

NMR for the Drug Substance (DS)

Specifications:

• NMR spectroscopy is used at times as an identity test in DS specification.

• Identification of Organic Impurities of the DS normally includes NMR methods in conjunction with other spectroscopic techniques and confirmation by *ad hoc*/independent synthesis.

NMR for the Drug Substance (DS)

Stability:

• The NMR spectroscopy plays an important role in the identification of by-products arising from degradation. Degraded samples may also be used to test the specificity of the analytical methods applied to the DS.

• Solid-state NMR seen during the first stability study to identify polymorphs in the DS. However, such test might not be eventually necessary as routine test in the DS specification, if justified by evidence and experience.

NMR for Excipients

• Some of the information outlined earlier for DS may also be needed to characterize novel excipients (i.e. those never included in a DP before).

• Such excipients though, may have been used previously in the cosmetic or food industry and therefore will already have substantial characterization data packages and established safety data.

• NMR used particularly to control the composition of polymeric excipients used in the dosage form, the proportion of monomeric components, the proportion of functional groups, or their positional distribution.

NMR for the Drug Product (DP)

• As for DS, stressed stability studies are used to establish likely degradation in the DP, and NMR spectroscopy is used in the characterization of any compounds that can be isolated.

• The causes of degradation may be either the usual chemical breakdown mechanisms or specific interactions with excipients (e.g. formation of Maillard by-products).

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Presentation of NMR data in dossiers

Example: ^{1}H-NMR for a NCE as bromide salt.

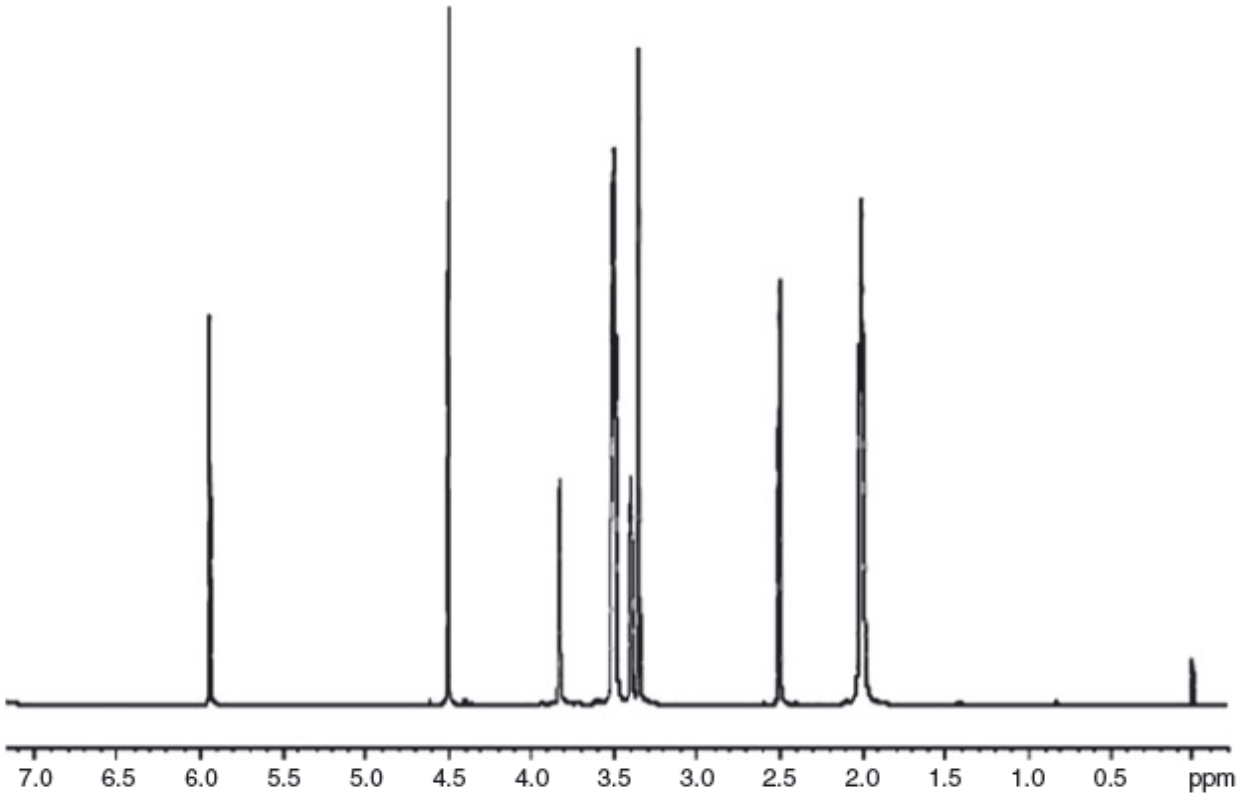

Figure 1. Example of ^{1}H NMR spectrum (partial) of a drug substance (as bromide salt) in DMSO-d_6. Solvent DMSO-d_6 and water resonances appear at 2.50 and 3.35 ppm, respectively. Propan-1-ol impurity resonances observed at 0.83, 1.41, and 4.37 ppm

Example: ^{13}C-NMR for a NCE as bromide salt.

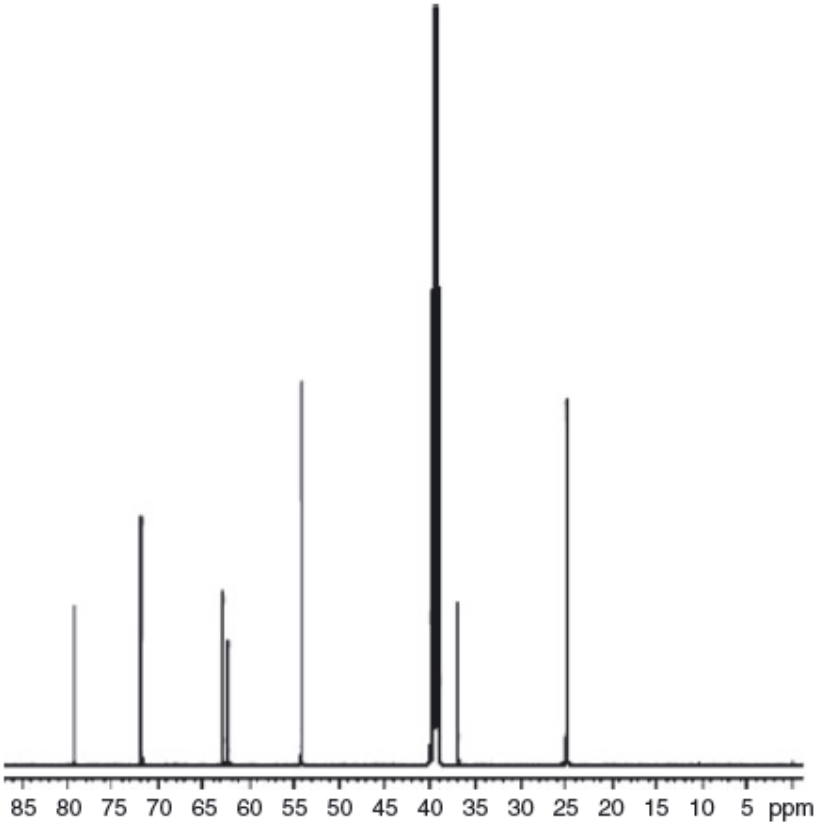

Figure 2. Example of ^{13}C NMR spectrum (partial) of a drug substance (as bromide salt) in DMSO-d_6. Solvent DMSO-d_6 resonance appears at 39.4 ppm

Example (cont'd): Chemical shifts.

Table 3. Interpretation of example ^{1}H NMR spectrum

Chemical shift (δ)[a]	Multiplicity	No. of H atoms	Assignment
2.01	t	6	1, 2, 3
3.40	m	2	4
3.50	t	6	5, 6, 7
3.83	m	2	8, 9
4.51	s	2	10, 11
5.94	s	1	12

[a]ppm referenced to tetramethylsilane at 0 ppm.
s = singlet, d = doublet, t = triplet, and m = multiplet.

Table 4. Interpretation of example ^{13}C NMR spectrum

Chemical shift (δ)[a]	Assignment
24.9	1, 2, 3
37.0	4
54.2	5, 6, 7
62.4	8
62.8	9
71.9	10
79.3	11

[a]ppm referenced to tetramethylsilane at 0 ppm.

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NMR deficiencies frequently encountered

Deficiencies

• *Illegible and unassigned spectra*: Care should be taken to ensure that reproductions of spectra are completely legible (a common problem in regulatory dossiers), and full assignments should be given where possible.

• *Vicinal coupling constants*: Incorrect assignment of *J* values for vicinal protons present on *cis/trans* geometrical isomers (i.e. the highest value of the *J* assigned to the *cis* isomer instead of the *trans* isomer).

Deficiencies (cont'd)

• *Deuterated solvents not corresponding*: The deuterated solvent used to run the proton and carbon NMR is $CDCl_3$. However, the table of chemical shifts' values state d_6-DMSO.

• *Proton integrations*: Inaccurate description of the overall number of hydrogens present in the DS. Mismatch between spectra and table with protons' integration.

Deficiencies (cont'd)

• *Tautomers*: Drug substances containing primary amides functionalities may give rise to tautomers (i.e. keto or enol forms). In this scenario, the applicant is asked to run dynamic NMR experiments (proton and carbon) with a view to confirming the exact structure of the drug substance.

Deficiencies (cont'd)

• *Ipso carbons on aromatic rings*: ^{13}C NMR spectra of drug substances containing aromatic rings do not clearly show peaks belonging to *ipso* carbons. The applicant is requested to run the NMR spectrum either with a more concentrated sample of drug substance or to run the NMR for a longer period (e.g. overnight).

• *Lack of multinuclear NMR*: For drug substances containing fluorine, phosphorous, carbon, and hydrogen atoms, only NMR spectra for the latter two are provided. In this context, the applicant is asked to provide ^{19}F and ^{31}P NMR spectra also.

Deficiencies (cont'd)

• *Lack of 2D NMR spectra*: Spectra not provided for more complex drug substances (e.g., containing bicyclic structure with *endo* end *exo* protons). Only one-dimensional ^{1}H and ^{13}C carbon NMR spectra are provided, which are not considered sufficient to characterize the molecule satisfactorily.

Deficiencies (cont'd)

• *Carbon multiplicity*: A DEPT-135 spectrum is useful to complement a standard ^{13}C NMR spectrum as it allows distinction between CH, CH_3 (positive signals), and CH_2 carbons (negative signals). However, this information is often missing in the initially submitted dossier and might be sometimes required from Applicants.

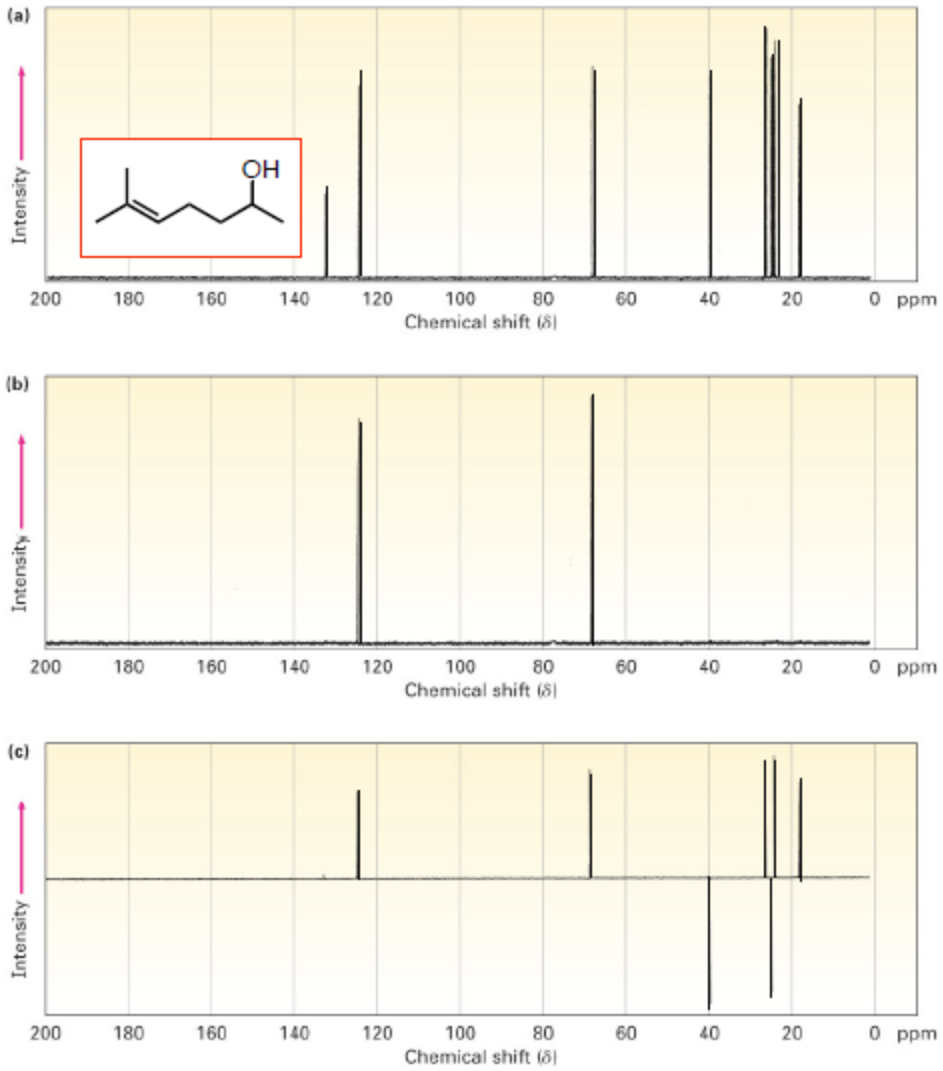
Example: ^{13}C NMR DEPT-90 & DEPT-135 Spectra

^{13}C NMR spectrum: shows all carbon types

DEPT-90 spectrum: shows only signals for the CH carbons

(not commonly used)

DEPT-135 spectrum: shows positive signals for CH and CH_3 carbons; shows negative signals for CH_2 carbons



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Concluding remarks

In summary

- Role of NMR in regulatory dossiers is also key.
- NMR deficiencies found during assessment can be addressed.
- Regulatory dossiers will increasingly include examples of DS & DP that have relied on NMR techniques for part of their R&D or control during routine manufacture for the marketplace.
- Advances in NMR technology and techniques mean that NMR has become more accessible and the method of choice in certain circumstances, particularly for the control of more complex molecules, including natural products and biologicals.

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References

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This article reviews the international requirements for the registration of pharmaceuticals, focusing on the information needed to define the pharmaceutical quality of drug substances and drug products. It highlights the role of NMR spectroscopy in regulatory dossiers that support applications to market medicines. The power of NMR spectroscopy in structure elucidation is well recognized but the specialized equipment required has prohibited its widespread use in routine control of drug substance or drug products through their specifications. However, advances in technology and techniques mean that NMR spectroscopy has become more accessible and the method of choice in certain circumstances, particularly for the control of more complex molecules, including natural products and biologicals. NMR spectroscopy may also be used more extensively during the research and development process, making optimal use of the latest techniques.

Keywords: regulatory dossier, NMR spectroscopy, International Conference for Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH), common technical document

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Thank you