MHRA
Regulating Medicines and Medical Devices

NMR in the European and US Pharmacopoeias

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Chapter 30 in *NMR in Pharmaceutical Sciences*. Edited by Jeremy R. Everett, John C. Lindon, Ian D. Wilson, and Robin K. Harris

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The purpose of a pharmacopoeia

- Publicly available set of quality standards for medicinal products
- Compliance is mandatory where a pharmacopoeia is referred to in drug regulations
- Monographs for individual active substances, excipients or for formulated preparations
- Supported by general notices and methods
- Product or article must comply throughout its period of use or lifetime – *if tested*

First Italian Pharmacopoeia: Ricettario Fiorentino

21 January 1499

- Giovanni Cipriani, Georgofili World, February 13 2015 and March 16 2015

European Pharmacopoeia

- The Council of Europe's Convention on the Elaboration of a European Pharmacopoeia (1964) and amendment protocol of 1994
- 37 member states (including all those in the European Union)
- European Union Directives on human and veterinary medicines specify use of pharmacopoeias (Ph Eur or national)

United States Pharmacopeia

- United States Pharmacopeia Convention publishes USP–NF
- combines two compendia, the United States Pharmacopeia (USP) and the National Formulary (NF)
- Recognized under the Federal Food, Drug, and Cosmetic Act.
- USP: monographs for drug substances, dosage forms and compounded preparations,
- NF: excipient monographs

NMR in Ph Eur

- 2.2.33 – Nuclear Magnetic Resonance Spectrometry
  - General requirements
  - Nuclei (mainly ^{1}H and ^{13}C)
  - Quantitative and qualitative techniques
  - Fourier Transform NMR
  - Parameters to be controlled
  - Solid-state spectroscopy eg for polymorphism
- 2.2.64 - Peptide Identification by Nuclear Magnetic Resonance Spectrometry
- Technical guides

NMR in USP

- General Chapter <761> Nuclear Magnetic Resonance Spectroscopy: qualification (installation, operational and performance), validation or verification
- General information chapter <1761> Applications of Nuclear Magnetic Resonance Spectroscopy: NMR applications
- General information chapter <1238> ‘Vaccines for Human Use – Bacterial Vaccines’: determination of depolymerized polysaccharides in protein purification section using O-acetyl groups

History of NMR in pharmacopoeias

- Mid 1970s in British Pharmacopoeia (BP) identification and quantification of the components of gentamicin (NMR spectroscopy as a general method in an Appendix)
- Quantitative determination of moisture content in Cloprostenol and Fluprostenol in the BP (Vet) 1977
- Identification tests for some corticosteroid sodium phosphates and aminoglycosidic antibiotics in the BP 1980
- Now superseded by Ph. Eur. monographs

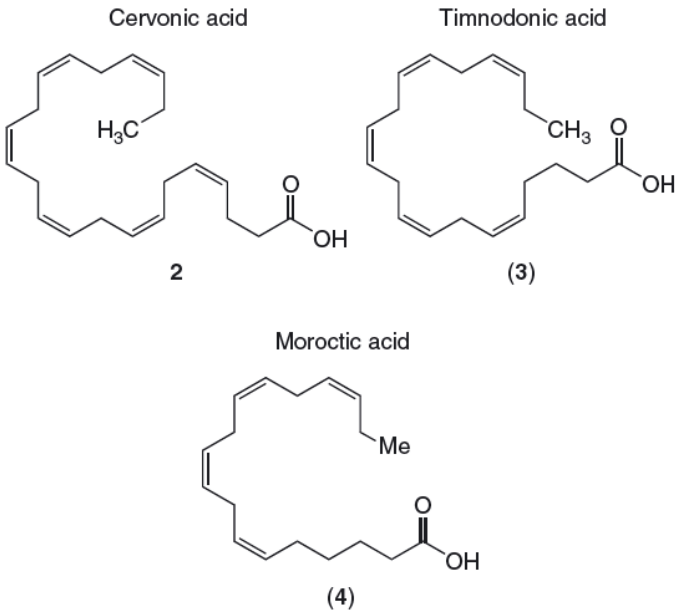
History in Ph Eur

- Appendix on NMR Spectroscopy was first published in the second edition in 1980
- Early 1980s monograph for Gentamicin Sulfate, later replaced by liquid chromatography (LC) test

Use of NMR in monographs

- Identification using ^{1}H and ^{13}C NMR spectroscopy
- Assay for content or composition, frequently for oligo- or polymeric materials, using ^{1}H and ^{13}C NMR spectroscopy
- Related substances, including isomers, and other
- impurities using ^{1}H NMR spectroscopy
- Specific tests, usually to determine composition of oligo- or polymeric substances
- Relaxivity for paramagnetic lanthanide diagnostic agents

Identification and Assay by ^{13}C NMR Spectroscopy: Farmed Salmon Oil and Farmed Cod Liver Oil (Ph. Eur.)



^{13}C NMR procedure for the determination of positional distribution of (β(2)-acyl) of fatty acids and identification of the drug substance

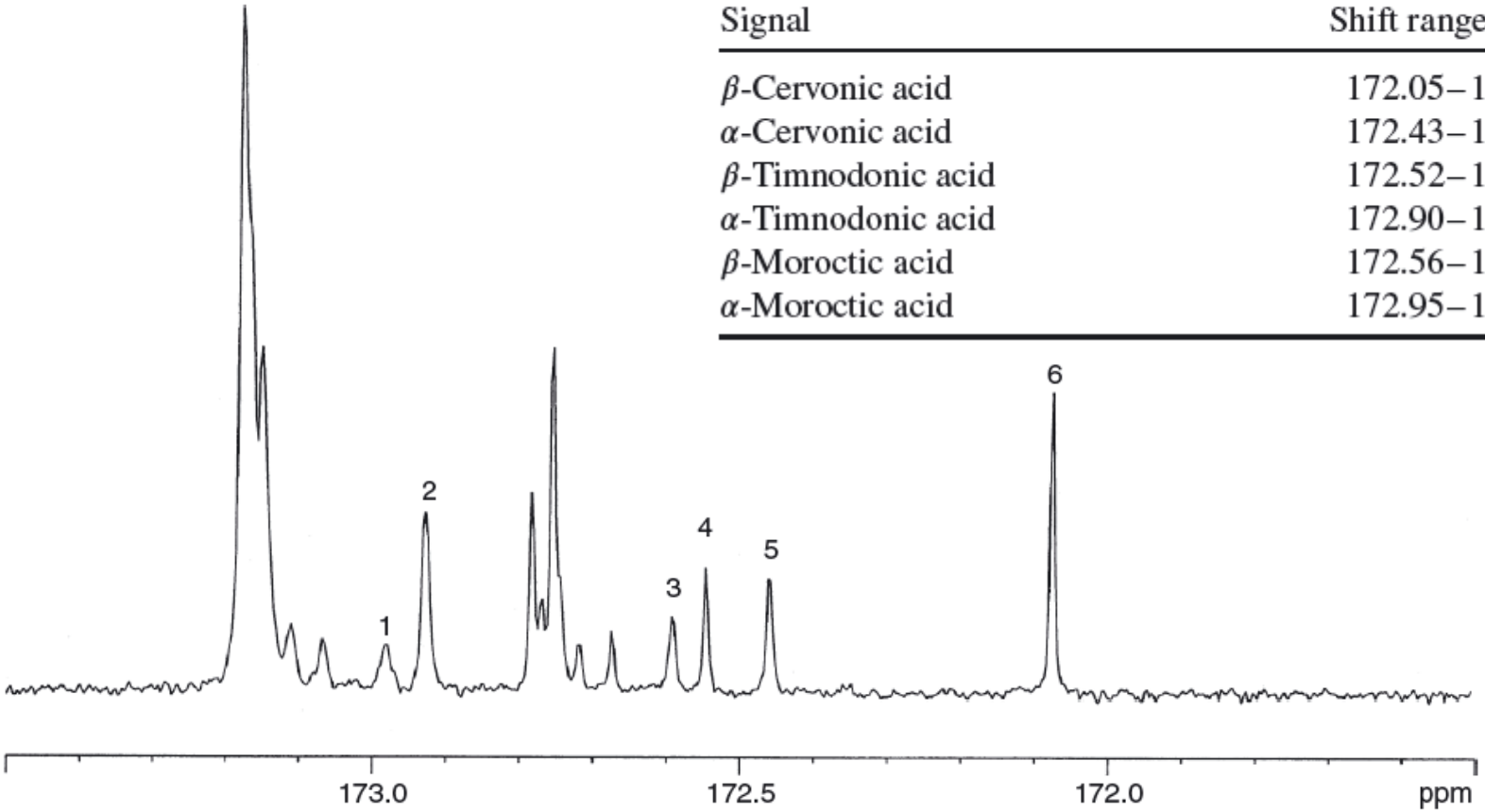
Table 30.3. (β(2)-acyl) positional distribution in farmed salmon oil and farmed cod liver oil

	(β(2)-acyl) positional distribution (%)	
	Farmed Salmon Oil	Farmed Cod Liver Oil
Cervonic acid	60–70	71–81
Timnodonic acid	25–35	32–40
Moroctic acid	40–55	28–38

^{13}C NMR reference spectrum for farmed salmon oil for identification

Table 30.4. ^{13}C NMR chemical shifts for fish oil omega-3 fatty acids

Signal	Shift range (ppm)
β-Cervonic acid	172.05–172.09
α-Cervonic acid	172.43–172.47
β-Timnodonic acid	172.52–172.56
α-Timnodonic acid	172.90–172.94
β-Moroctic acid	172.56–172.60
α-Moroctic acid	172.95–172.99



Assay

$$\frac{\beta}{\alpha + \beta} \times 100$$

α = peak area of α-carbonyl
β = peak area of β-carbonyl

Table 30.3. (β(2)-acyl) positional distribution in farmed salmon oil and farmed cod liver oil

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Assay for Monomeric Composition by ^{1}H NMR Spectroscopy: Poloxamer USP and Poloxamers Ph. Eur.

Poloxamer

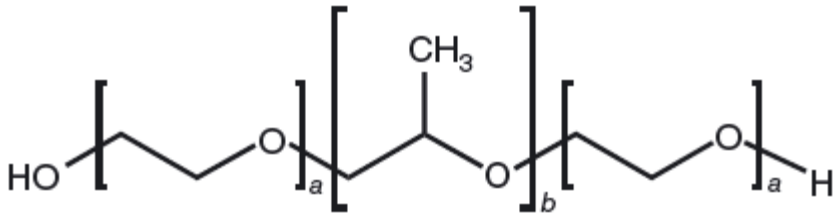


Table 30.5. Poloxamer characteristics (Ph. Eur.)

Poloxamer type	Ethylene oxide units (a)	Propylene oxide units (b)	Content of oxyethylene (%)	Average relative molecular mass
124	10–15	18–23	44.8–48.6	2090–2360
188	75–85	25–30	79.9–83.7	7680–9510
237	60–68	35–40	70.5–74.3	6840–8830
338	137–146	42–47	81.4–84.9	12 700–17 400
407	95–105	54–60	71.5–74.9	9840–14 600

Relative/normalization method USP/Ph Eur

$$\alpha = (A_2/A_1) - 1$$

$$\text{Result} = 3300 \times \alpha/(33 \times \alpha + 58)$$

*A*1= average area of the oxypropylene methyl group doublet at about 1.08 ppm compared to the reference standard

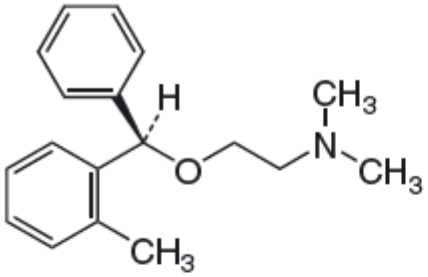
*A*2 = average area of composite band in the range 3.2–3.8 ppm due to CH_2O of both the oxyethylene and oxypropylene units and the CHO groups of the oxypropylene units

Isomeric-related Substances by ^{1}H NMR spectroscopy: Orphenadrine Citrate USP

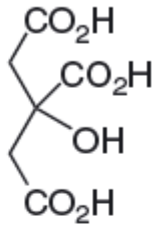
Relative method of quantitation to limit *meta*- and *para*-isomers to 3.0% using methine peak.

*now superseded by gas chromatography cf Ph Eur

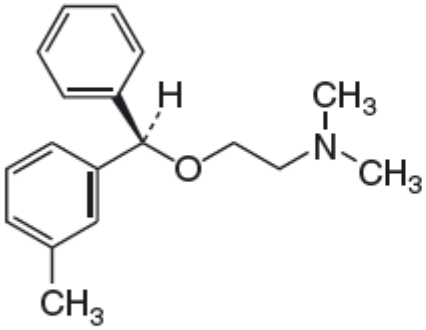
Orphenadrine and its impurities A and B



and enantiomer

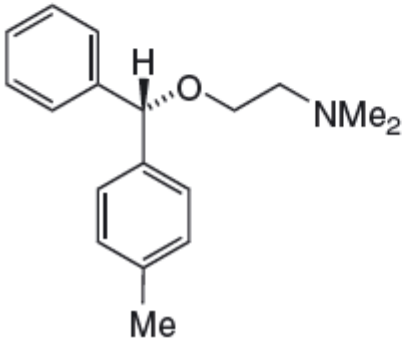


(7)



and enantiomer

(8)



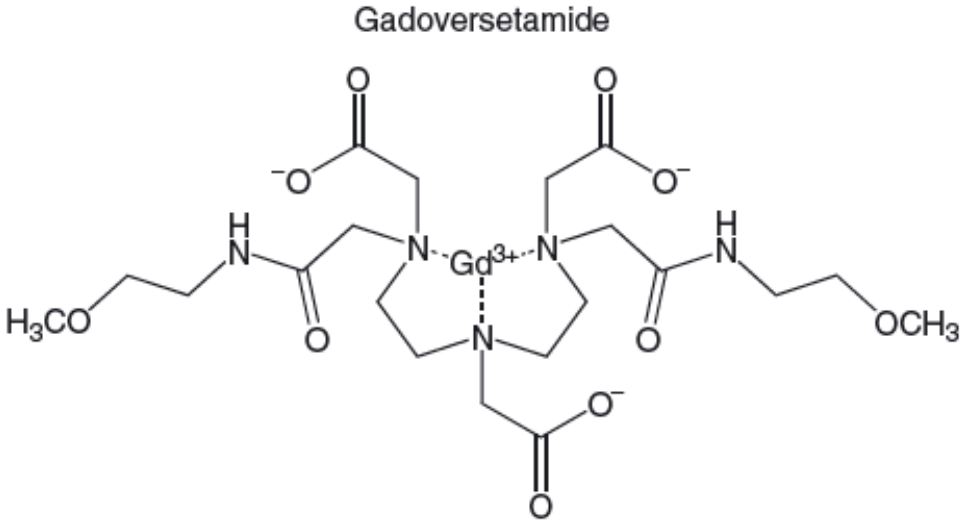
and enantiomer

(9)

Relaxivity: Gadoversetamide Injection USP

- Relaxivity is the magnitude of a substance's capacity to enhance the relaxation rate of a nucleus, expressed in units of s−1 mM−1.
- Relaxivity of a substance is determined experimentally by measuring the spin-lattice relaxation time (*T*1) of a test substance and plotting 1/*T*1 against the concentration in units of mM
- The slope of the curve is the numerical relaxivity

Relaxivity: Gadoversetamide Injection USP



- Test solutions are prepared by diluting 5.0 ml of the injection in water to give 0.504, 1.008, 2.016, and 3.024 mM, respectively
- The slope of the plotted line, the relaxivity, should be between 4.0 and 5.0 s–1 mM–1.

Some other examples

- Identification and Assay by ^{13}C NMR Spectroscopy: Heparin Sodium (USP and Ph. Eur.)
- Assay for Drug Substance Content by ^{1}H NMR Spectroscopy: Amyl Nitrite and Amyl Nitrite Inhalant USP
- Assay for Control of Functional Group Substitution by ^{1}H NMR Spectroscopy: Hydroxypropyl Starch Ph. Eur. And USP
- Test for Composition by ^{13}C NMR Spectroscopy: Lauromacrogol 400 Ph. Eur.
- Specific Test for Amino Acid Content by ^{13}C NMR Spectroscopy: Goserelin Acetate USP
- Impurities by ^{1}H NMR Spectroscopy: Medronic Acid for Radiopharmaceutical Preparations Ph. Eur.

Concluding remarks

- Pharmacopoeias provide quality standards and methods for a wide range of users and have to be generally accessible
- Not always the case with NMR techniques - sophisticated and relatively expensive equipment
- Only included in monographs when other methods not feasible or when an NMR technique has something unique to offer
- Unique capability for structural discrimination is exploited in monographs, particularly for polymeric compounds of natural or biological origin
- Characterisation of reference standards

Ricettario Fiorentino

- *“D’ingegno et di corpo destro, di buoni costumi, non avaro e fedele”*

- Giovanni Cipriani, Georgofili World, February 13 2015 and March 16 2015

- Grazie
- Qualsiasi domanda?