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Quantitative NMR spectroscopy in pharmaceutical applications

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Nomenclature

ASIS	aromatic solvent induced shifts
CCQM	Comité Consultatif pour la Quantité de Matière
CSA	chiral solvating agents
CZE	capillary zone electrophoresis
COSY	correlation spectroscopy
DOSY	diffusion-ordered spectroscopy
ee	enantiomeric excess
ERETIC	electronic reference to access *in vivo* concentrations
FID	free induction decay
fwhh	full width at half signal height
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum correlation
HPLC	high performance liquid chromatography
ICH	International Conference on Harmonization
IS	internal standard
HR-MAS NMR	high-resolution magic angle spinning NMR
LOD	limit of detection
MS	mass spectrometry
MRI	magnetic resonance imaging
NOE	Nuclear Overhauser Enhancement (effect)
NOESY	two-dimensional NOE spectroscopy
OSCS	oversulfated chondroitin sulfate
PhEur	European Pharmacopoeia
Pre-SAT	presaturation
PULCON	pulse length based concentration determination
ROESY	rotating-frame NOE spectroscopy
TOCSY	total correlation spectroscopy
Sdv	standard deviation
S/N	signal-to-noise ratio
T_1	longitudinal relaxation time
USP	United States Pharmacopoeia
WATERGATE	water suppression by gradient-tailor excitation

1. Introduction

^{1}H and ^{13}C NMR spectroscopy is routinely used for the elucidation of structures of newly synthesized compounds, natural products and semi-synthesized compounds. The constitution, configuration and conformation of small molecules, polymers, peptides, proteins, sugars, or nucleotides, can be elucidated by utilizing two-dimensional techniques such as COSY, HMBC, HSQC, TOCSY, NOESY, and ROESY experiments. Additionally, ^{19}F, ^{15}N, and ^{31}P NMR can be employed in structure elucidation.

According to the definition of the CCQM (Comité Consultatif pour la Quantité de Matière), NMR spectroscopy is a primary method of measurement [1,2]. Thus, it can be used for quantification purposes. As early as 1963 the first quantitative NMR measurements (qNMR) were already performed on a commercial preparation of analgesics by Hollis who quantified the content of the ingredients with a deviation of 1.1% for aspirin, 2.2% for phenacetin and 3.2% for caffeine [3]. And in the 1980s the German Pharmacopoeia DAB9 characterized the composition of gentamicin by means of ^{1}H NMR spectroscopy [4]. These examples clearly demonstrate the applicability of ^{1}H or ^{13}C NMR spectroscopy for the identification and quality assessment of drugs and excipients.

Nowadays qNMR is a well established technique in many areas such as drugs [5], excipients [6], vaccines [7], natural products [8,9], peptides [10], agrochemicals [11,12], food and beverages [13–15], metabolic profiling/fingerprinting of plant extracts and tinctures [16] as well as body fluids, e.g. metabolomics for diagnostic of diseases and drug treatment control [17–21], combinatorial chemistry [22], and on-flow monitoring of reaction processes [23]. ^{1}H and ^{13}C, as well as ^{19}F and ^{31}P NMR spectroscopy can be used for quantification purposes.

In the quality evaluation of drugs NMR spectroscopy has utility in various fields, such as

- Identification of drugs,
- Determination of the composition of multicomponent drugs,
- Determination of the isomeric composition: the ratio of diastereomers and/or the enantiomeric excess (ee) of chiral drugs by means of chiral additives, e.g. chiral solvating agents or chiral shift reagents,
- Determination of the level of impurities and elucidation of their structure,
- Observation of the course of degradation/decomposition of a drug,
- Evaluation of the content of residual solvents,
- Determination of the molar ratios of (protonated) basic drugs and (deprotonated) organic acids in respective salts,
- Assay,
- Counterfeit analysis.

Even though one- and two-dimensional NMR spectroscopy and qNMR are capable of the quality evaluation of drugs the number of applications in international pharmacopoeias, e.g. the European Pharmacopoeia (PhEur) [24] and United States Pharmacopoeia (USP) [25], is limited. While the pharmaceutical companies extensively apply qNMR in drug discovery and development they mostly use HPLC in routine quality analysis rather than qNMR. Currently the following examples can be found.

For identification: Buserelin, Goserelin, Tobramycin (PhEur 6.0), Hydrocortisone Sodium Phosphate (British Pharmacopoeia 1998), Amylnitrite isomers (USP 33), unfractionated Heparin Sodium and Calcium (USP33, PhEur 6.6), Heparins of low-molecular-mass (USP33, PhEur 6.0), Haemophilus Type b Conjugate Vaccine, Meningococcal Group C Conjugate Vaccine, and Pneumococcal Polysaccharide Conjugate Vaccine (adsorbed) (PhEur 6.0), Salmon oil farmed (PhEur 6.0).

For tests: Poloxamer: ratio of oxypropylene/oxyethylene(PhEur 6.0); Hydroxypropylbetadex: molar substitution (PhEur 6.0); Lauromacrogol 400 known as polidocanol 9 or macrogol 9 lauryl ether: average chain length of fatty alcohol and average number of moles of ethylene oxide (PhEur 6.0), Orphenadrine citrate: *meta*/*para* isomer (USP 33), Medronic acid for radiopharmaceutical preparation: impurity profiling (PhEur 6.5).

For assay: Amylnitrite isomers (USP33).

Recently, qNMR became the leading method for the purity analysis of unfractionated heparin sodium and calcium in the USP which was deliberately contaminated with anaphylactoid oversulfated chondroitin sulphate (OSCS). No other method, neither HPLC nor CZE or biological assays, was capable of limiting this impurity to less than 0.1% in the first stage of monograph revision (see Ref. [25] and discussion in Section 6). In the second stage of revision qNMR is used in the identity paragraph of the monograph limiting simultaneously OSCS, dermatan sulphate and other accompanying glucosaminoglycans to 0.1% in addition to residual solvents. This example clearly shows the potential of qNMR.

With respect to counterfeit and substandard drugs which are increasingly appearing on the American and European market, qNMR can be used as an orthogonal method to HPLC, CZE, and other separation methods which are developed and validated for one defined production pathway [26]. Since contaminants of an ac-