The use of quantitative proton nuclear magnetic resonance (^{1}H qNMR) in the purity determination of established and novel ingredients

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Introduction

Nuclear magnetic resonance (NMR) spectroscopy is an important analytical technique commonly used for structure elucidation of small organic compounds. However, due to its potential to provide quantitative information, even within complex mixtures, proton (^{1}H) NMR has gained increasing importance over the years (Table 1).

The fundamental principal of qNMR lies with the intensity of a signal being directly proportional to the number of nuclei responsible for that particular resonance; therefore, analyte quantification – relative and absolute – can be determined by measuring the area under the signals (i.e. integral) without a need for calibration to determine response factors as in other analytical techniques, such as high-performance liquid chromatography (HPLC) or gas chromatography (GC).

Despite being a versatile and robust technique, a few considerations should be highlighted in order to get accurate qNMR measurements (Figure 1). When available, the use of a larger amount of sample (10 mg) is advisable, as weighing is the largest source of error in qNMR. The use of a micro-balance is recommended as a less sensitive balance may contribute to a higher degree of uncertainty. However, the amount of sample available for new synthetic molecules and new psychoactive substances is often limited, which may lead to inaccurate results.

Industry	Relative quantitation	Absolute quantitation
Pharmaceuticals	Polymer characterisation (internal molar ratios)	Purity of active pharmaceutical ingredients Quantification of substances in raw materials and finished products Residual solvent testing
Organic synthesis and drug discovery	Structural isomer ratio	Purity of new synthetic molecules
Dietary and food supplements	Carbohydrate linkage ratios	Purity of novel ingredients/dietary supplements Residual solvent testing
Polymers and biomaterials	Biopolymer molar ratios	Residual solvent testing
Forensics	Unknown mixture analysis	Quantification of new psychoactive substances

Table 1: Examples of quantitative applications of NMR.

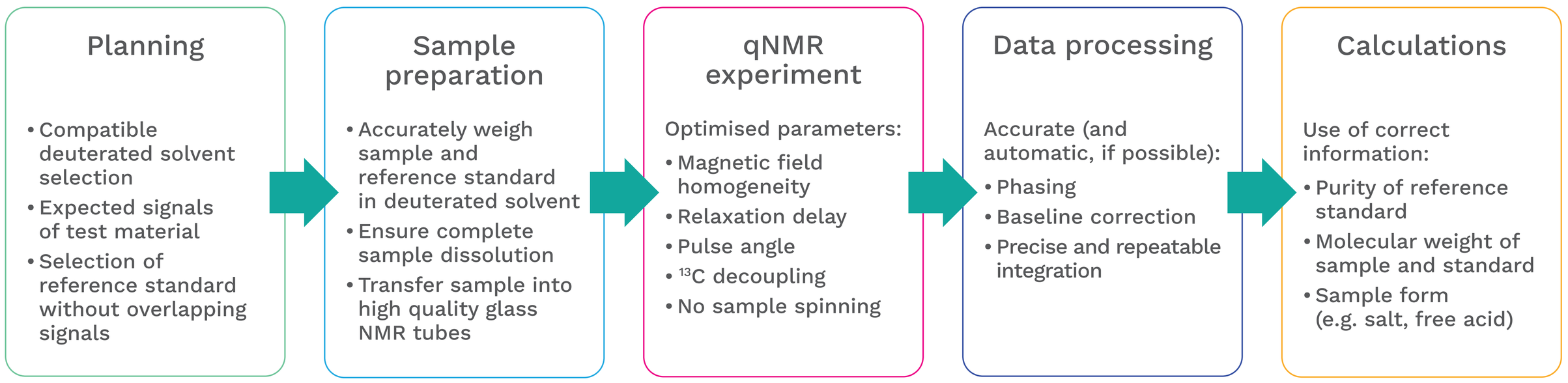

Figure 1: General process of qNMR.

Objectives

To develop a robust method of purity determination to be applied to a range of existing and novel ingredients, some of which will be sample limited, using ^{1}H qNMR spectroscopy.

Material and methods

After careful planning regarding solubility and chemical compatibility, a small amount (2 mg and 6 mg) of two certified reference standards (caffeine 99.9% w/w purity, Sigma-Aldrich; methyl 3,5-dinitrobenzoate 99.71% w/w purity, Sigma-Aldrich) was accurately weighed and dissolved in ca. 1 mL of deuterated chloroform. An aliquot of the solution was transferred to an NMR tube (5 mm, Wilmad). Sample preparations were conducted in duplicate. Data was acquired using a Bruker NEO 600 MHz NMR spectrometer, using a quantitative proton acquisition program with long relaxation delay (60 s), ^{13}C decoupling enabled and the data collected without sample spinning.

Results

Although small sample amounts were used, good agreement between replicate preparations and with the expected certificate result were observed (Table 2).

Magnet homogeneity and consistent data processing was shown to be critical in achieving good agreement between replicates.

This approach was applied to determine the purity of established and novel compounds.

A ^{1}H NMR spectrum of a dietary supplement (apigenin) using a qNMR experiment is shown in Figure 2. In this instance, the percentage difference between two replicates was 0.4%.

	Calculated purity (2 mg mass)		Calculated purity (6 mg mass)	
	Analyst 1	**Analyst 2**	**Analyst 1**	**Analyst 2**
Replicate 1 (%, w/w)	99.3	99.8	99.7	99.8
Replicate 2 (%, w/w)	98.1	100.4	100.0	100.0
Average (%, w/w)	99.1	100.1	99.9	99.9
% diff between replicates	0.5	0.6	0.3	0.2
% diff from CoA	0.6	0.4	0.2	0.2

Table 2. Method development results.

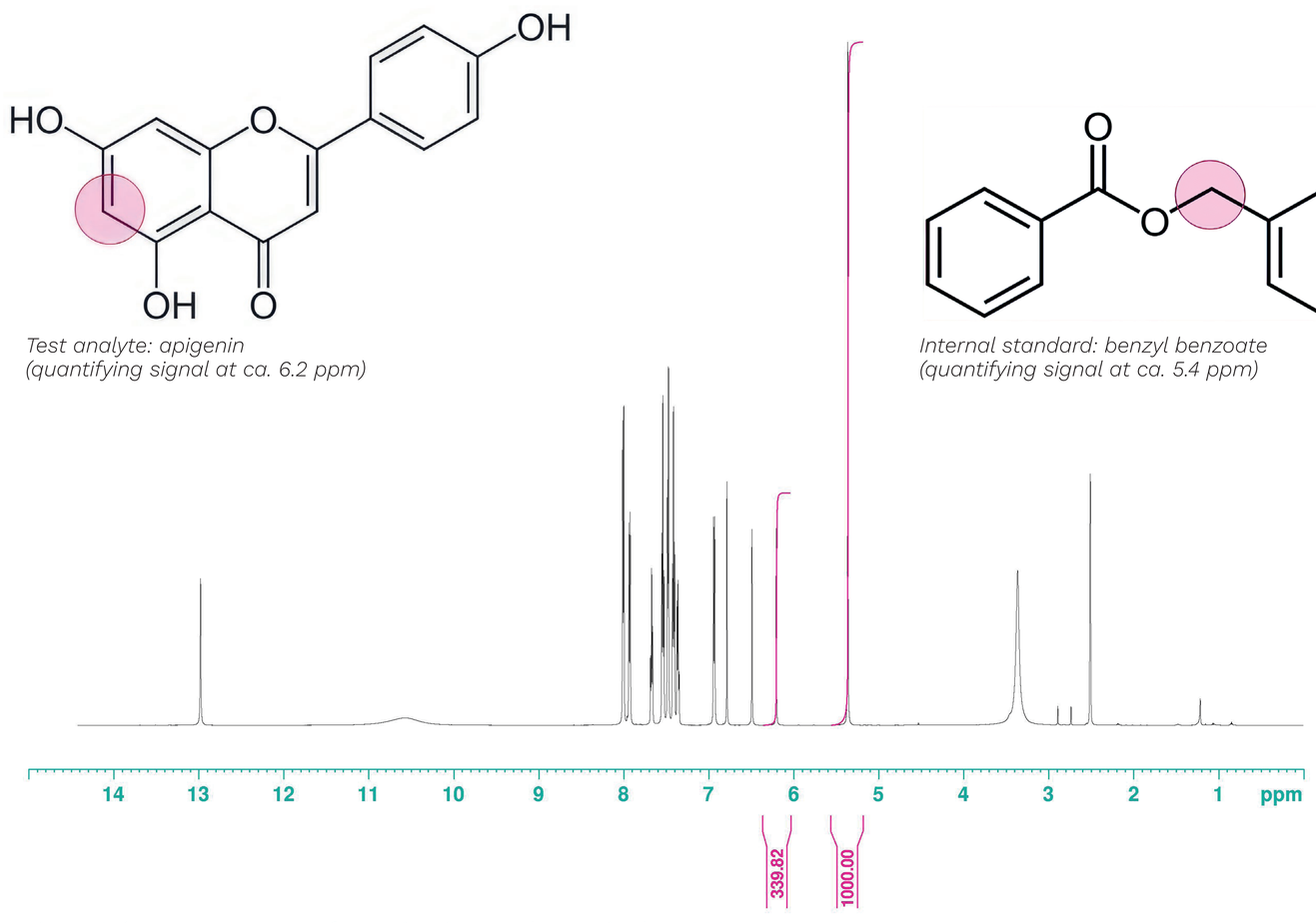

Figure 2. ^{1}H NMR spectrum of an apigenin sample acquired on 600 MHz NMR.

Quantitative NMR has been shown as a reliable, accurate and quick means of calculating purity for a wide range of molecules, and is particularly useful in instances where certified reference standards for the analyte of interest are not available (e.g. new synthetic active pharmaceutical ingredients, new psychoactive substances), or are prohibitively expensive.

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