analytical chemistry

Feature

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Expanding the Analytical Toolbox: Pharmaceutical Application of Quantitative NMR

In response to the changing market pressures being applied to the pharmaceutical industry, a greater emphasis is being made to advance new drugs to market with minimal investment in early development stages. The use of quantitative NMR (q-NMR) has been shown to be a single point replacement for routine early development testing which previously combined elements of identity testing, chromatographic assay, moisture analysis, residual solvent analysis, and elemental analysis. This Feature will highlight the applications of q-NMR to early phase drug development testing and its efficient potency, solvent quantification, and relative response factor determinations.

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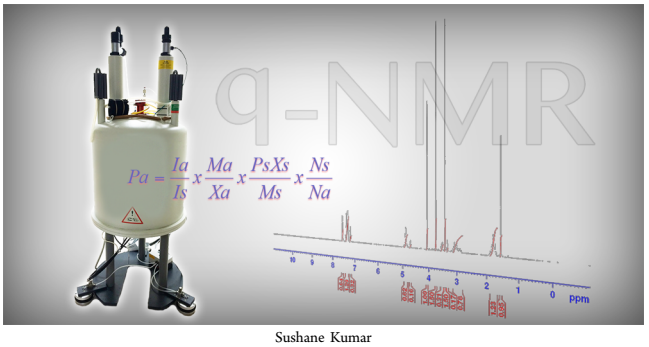

Sushane Kumar

Current pharmaceutical drug development focuses on the pursuit of new chemical and biological entities (NCEs, NBEs) to address unmet medical needs in a safe, efficient, and economical manner. This challenge is supported in every step of the way by analytical chemists who develop efficient test methods while maintaining lower cost structures. Early in product development, a primary task of the analytical chemist is the determination of potency for potential drugs. Traditionally in the pharmaceutical industry, potency determinations to evaluate the NCE have been centered on liquid chromatography platforms. The general rule in early development is that the purity/potency of the drug is simply the amount of active drug present minus the sum of any inactive substances, process impurities, and any degradation products present.[1]

Methods used in pharmaceutical drug development follow the "stages" of clinical and safety evaluations as each drug in development moves toward regulatory submission and approvals.[2,3] These molecules, are referred to as "active pharmaceutical ingredients or "API". The four major stages of pharmaceutical development are (a) Phase I is initial safety and clinical monitoring of the drug using healthy human subjects, (b) Phase II is the initial evaluation of the drug on a limited population of subjects affected with the indication the drug has been targeted to treat, (c) Phase III is evaluation of the drug on a statistically significant population of subjects affected with the indication the API has been targeted to treat, and (d) Phase IV is continuing evaluations post regulatory approval for additional indications and marketing. As late stage clinical studies now require an investment of several hundred million dollars, today's economics now drive the business concern toward minimal investment in the API until it has proven itself as a likely candidate for full development. For pre-Phase I and Phase I studies, developing fully characterized liquid chromatography (LC) methods is not always cost justified leaving the desire for many of the traditional method development and validation studies associated with LC development to be delayed. Yet, industry and regulatory needs require selective, accurate potency determinations from Discovery through Phase I. To address the needs of rapid, selective, and accurate potency determinations without requiring full development of traditional chromatographic methods, the industry is increasingly turning to the use of quantitative nuclear magnetic resonance (q-NMR) spectroscopy in early drug development stages.

As nuclear magnetic resonance spectrometry has improved, quantitative applications have followed suit.[4] Early studies with pharmaceuticals did not use today's high magnetic field instrumentation. Vinson and Kozak successfully determined aspirin potencies with a ^{1}H frequency of 60 MHz NMR.[5] Today, it is generally considered acceptable to use NMR instrumentation with ^{1}H frequencies of >200 MHz. Instruments of 400 MHz or greater are generally preferred to minimize method development time through selectivity and sensitivity advantages at higher field strengths. Generally, as the magnetic strength increases, greater resolution and sensitivity is achieved in the NMR experiment.

Application of q-NMR to pharmaceutical and biomedical compounds demonstrates the accuracy of q-NMR for potency and residual solvent applications.[6−15] The technique normally applies ^{1}H NMR for the method. This allows the technique to operate as a "universal" detector. Unlike molar absorptive differences in UV detection and ionization efficiency differences in mass spectrometry detection, protons in NMR spectrometry respond quantitatively from molecule to molecule assuming

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relaxation is accounted for. For the most part, ^{1}H NMR remains the primary technique for q-NMR; however, Martino et al. also showed q-NMR can be an effective tool using ^{19}F and ^{31}P NMR as well.[16]

As all work in the pharmaceutical industry must meet the appropriate regulatory standards, it is important to note that traditional NMR and q-NMR is accepted by the International Conference on Harmonization (ICH).[17] Malz and Jancke validated that q-NMR is robust to instrumental changes.[18] Bekizoglu et al. validated q-NMR for benzethonium chloride in grapefruit seed extracts.[19] Sharma et al. published a protocol for q-NMR applications with parental drugs.[20] Webster et al. challenged q-NMR with the traditional validation requirements applied in regulated industries to liquid chromatography methods to establish this technique as fully equivalent to liquid chromatography for early phase potency determinations.[21] These references confirm that q-NMR is an effective tool for rapid potency analysis. Additionally, q-NMR can be used for the determination of chromatographic response factors as demonstrated in our laboratory.[22]

IDEAL POTENCY EXPERIMENT

The lifeline of the pharmaceutical industry is the development of "new" chemical entities. Pharmaceutical scientists design molecules to meet specific structural, biological activity, and intellectual property requirements. Upon synthesis, these molecules need characterization for structural confirmation and purity. Often, there is no reference to standardize this material against. With limited access to fully characterized reference standards, potency determinations are generally done indirectly by subtraction.[1] Typically, the area percent of liquid chromatographic analysis is organic and inorganic impurities determined from water testing through Karl Fisher titration, residual solvents by gas chromatography or thermogravimetric analysis, residue on ignition, and elemental analysis. The equation employed is shown in eq 1:

$$\%\ \text{potency} = \frac{100 - \%\ \text{related} - \%\ \text{enantiomer}}{100} \times \frac{100 - \%\ \text{water} - \%\ \text{others}}{100} \times \frac{\text{MW}_{\text{active}}}{\text{MW}_{\text{salt}}} \times 100 \quad (1)$$

As we will see, this equation is readily replaced by a single q-NMR determination.

The question that may come to mind for rapid potency determinations is "why not quantitative UV−Vis spectrophotometry, quantitative infrared, or mass spectrometry." Primary points for this discussion are three: the technique must be "rapid" and selective for the drug being characterized in the presence of like impurities and the technique must be quantitative without the availability of a characterized standard of the specific drug. UV−vis spectrophotometry is rapid but is not selective for like impurities and needs a characterized drug standard. Infrared and mass spectrometry can be selective for like impurities but require a characterized standard for quantification. Only ^{1}H NMR readily fits all three of these criteria and is available in most pharmaceutical laboratories.

One of the first applications in the pharmaceutical industry for q-NMR was rapid potency determinations. Historically, early batches of the drug under development were made to begin early safety GLP studies and later batches were made with full GMP release testing and specifications for human clinical trials. Manufacturers want to minimize the number of batches of a drug in development. Thus, the challenge became how to test and release the same lot of material for GLP applications and then use the same lot for GMP applications. Some considerations are that (a) duplicate testing is not allowed on the same lot and (b) GMP release requires fully developed methods to be employed as well as release specifications. The extra requirements for GMP do not allow a rapid release as needed for GLP studies.

It was soon realized that q-NMR enabled a quick solution to this problem. By using q-NMR for the GLP release, the scientist had a rapid determination of potency and spectroscopic confirmation of structure. The q-NMR method does not conflict with the GMP release for it is not a method employed in GMP testing so no duplicate testing of the drug batch occurs. Manufacturers could reduce costs by only producing a single lot for initial GLP and GMP applications. The lot would be produced and tested by q-NMR for GLP applications and after the GMP methods were finalized and validated, along with specifications for release, the batch was tested for GMP release as well. Subsequent batches produced would simply be tested under the GMP protocol.

POTENCY BY Q-NMR (CALCULATION)

NMR spectroscopy is by definition a quantitative tool. It uses the integration of resonance signals that are proportional to the number of nuclei present (assuming similar relaxation, minimal nuclear Overhauser energy (NOE), uniform excitation). Thus, each proton has a "universal" response and there is no need for relative response factors. The technique is recognized by the U.S. Pharmacopeia as a primary analytical method. While many view q-NMR as a new application, it has, in fact, been available for almost as long as NMR itself. The first literature reference for q-NMR was published in 1954.[23] Unlike many analytical techniques in use today, q-NMR is a "green" analytical technique, as its use does not contribute additional CO_2 to the atmosphere.

The q-NMR potency determination is based on eq 2:

$$\text{Pa} = \frac{\text{Ia}}{\text{Is}} \times \frac{\text{Ma}}{\text{Xa}} \times \frac{\text{PsXs}}{\text{Ms}} \times \frac{\text{Ns}}{\text{Na}} \quad (2)$$

where Pa, Ps = potency of analyte/standard; Ia, Is = integral of analyte/standard from NMR spectrum; Na, Ns = number of protons from analyte/standard; Ma, Ms = molecular weight of analyte/standard; Xa, Xs = weight of analyte/standard.

Thus, the potency or purity of the chemical entity is determined normalizing the response of the protons present to those of a characterized, internal standard. The ideal internal standard for q-NMR is a singlet that is near the analyte integral being targeted. More important than being a singlet, the internal standard response must be in a region that is "clean" of other responses from the chemical entity and the sample in general. For pharmaceutical investigations, the internal standard used for the q-NMR experiment should be a certified material or standardized as a secondary standard. Recently, commercially available standards certified for q-NMR use have become readily available. (Table 1) Prior to this, the chosen internal standard was commonly standardized against NIST grade benzoic acid.

There are several criteria that must be met for a compound to be an appropriate internal standard for q-NMR experiments. A suitable internal standard should (a) have signal(s) which do not interfere with other signals in the spectrum and vice versa, (b) be available in pure form, (c) be soluble in different NMR

Table 1. Commonly Used q-NMR Internal Standards

standard	approximate chemical shift (ppm)
duroquinone	2
dimethylsulfone	3.2
maleic acid	6.2
benzoic acid	7.4−8.2
3,5-dinitrobenzoic acid	9.2

solvents, (d) be easily weighable, (e) nonvolatile, (f) be nonreactive, (g) be long-term stable, and (h) have optimal molecular weight.[24] Besides the obvious requirements, such as being nonreactive, the most essential requirement is that the signal(s) of the internal standard should have unique and isolated chemical shifts. The most commonly used internal standard in natural product analysis is maleic acid which has an olefinic signal near 6 ppm. A large number of compounds do not have alkene functional groups; therefore, the signal of maleic acid does not interfere with the analyte peaks. The ^{1}H NMR of maleic acid with benzoic acid in DMSO-d_6 (Figure 1)

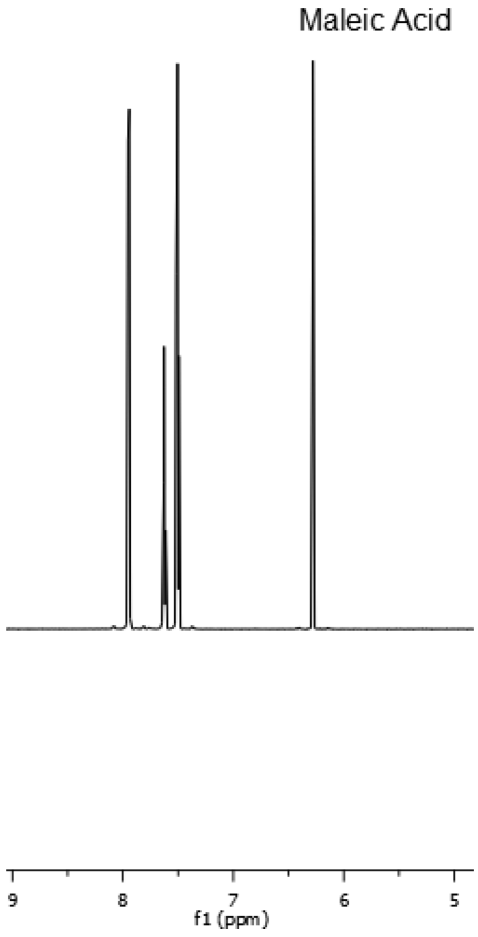


Figure 1. NMR spectrum of maleic acid in benzoic acid standard.

shows that its signal at 6.3 ppm is clearly separated from the aromatic signals of benzoic acid. A large number of drugs possess aromatic moieties, thus making maleic acid a suitable internal standard for these drugs. The signals before 5 ppm in Figure 1 are for residual solvent and residual HOD and are not illustrated in the figure. However, for olefinic analytes, maleic acid is not suitable.

There is no universal internal standard. An internal standard is chosen based on the structure of the analyte. Nonaromatic analytes have a choice of internal standards with aromatic functionality, such as 3,4,5-trichloropyridine, 1,4-dinitrobenezene, and 2,4,6-triiodophenol. For analytes with ketone functionality or other functionality having NMR peaks around 2 ppm, the internal standard with signal around 1 ppm, such as 2,2-dimethylmalonic acid, can be chosen. Almost all internal standards employed in q-NMR are solids as one of the criteria is nonvolatility. However, 1,4-dioxane with a bp of 101 °C and *tert*-butyl alcohol with a bp of 82 °C have been used successfully. Even though bistrimethylsilylbenzene is not a widely used internal standard, its methyl signal around 0 ppm does not interfere with almost all the natural product compounds and other analytes.

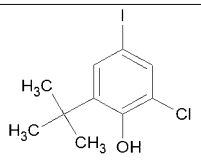
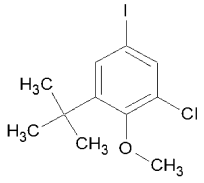
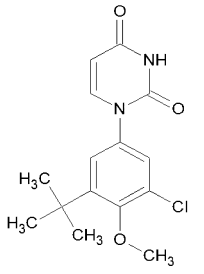
A deuterated analogue of this compound, bistrimethylsilylbenzene-d_4, in which all aromatic protons have been replaced with deuterium atoms, is also available for q-NMR. However, the nondeuterated residual material will still have aromatic signals, thus making it unsuitable for aromatic analytes. Several residual solvents have also been used as internal standards in cases where the solvent signals of nondeuterated residual solvents do not interfere with the signals of the analyte.[25,26] Several natural products have been analyzed using residual solvents as the internal standards.[27] The use of residual solvents has an advantage of keeping the sample clean in case it needs to be retrieved. On the other hand, the amount of residual solvent varies from bottle to bottle and from batch to batch. Therefore, the use of residual solvent as internal standard should be restricted to the same bottle of solvent for a set of experiments. The use of these solvents implies that nonvolatility is not an essential criterion for internal standards.

The q-NMR technique relies on the measured amount of the internal standard. However, electronic reference signals synthesized by an electronic device can be used instead of an internal standard for quantitative purpose. The most common method, Electronic REference To access In vivo Concentrations (ERETIC) method, is available on all major NMR spectrophotometers.[28] The major advantage of this method is that an internal standard is not added to the NMR sample, and it remains clean. ERETIC has been evaluated by several studies. In one study, concentrations of sodium lactate were determined by the ERETIC method and the results were compared with trimethylamine hydrochloride as internal standard.[29] The results were slightly favorable for the ERETIC method. This method was also compared with an internal standard, (trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) in Proton HR-MAS spectroscopy of prostate tissue.[30] The results showed the ERETIC method to be superior to the internal standard method. QUANTAS (QUANTtification by Artfician Signal) is another software-based protocol for concentration measurement by NMR.[31] In a similar approach, a mathematically generated signal was utilized in postacquisition stage. This artificial Signal Insertion for Calculation of Concentration Observed (aSICCO) can be used for validation of isolated metabolites from drug metabolites studies as analytical standards.[32] Recently, pulse length based concentration determination (PULCON) methodology has been successfully used for purity determinations of pharmaceutical reference materials.[33]

SELECTIVITY OF Q-NMR

For q-NMR to be successfully applied to drug development, it must be selective to small changes in structures for impurities. Table 2 illustrates the structures of a hypothetical mix of three similar compounds that would have similar elution characteristics in LC. The only difference between Compound 1 and Compound 2 is the methyl substitution on the phenolic hydrogen. Compound 3 is a combined product of the two and would post a similar NMR spectrum. However, as seen in Figure 2, the mixture would be easily resolved in a typical NMR experiment using signals such as the methyl signal of the methoxy group of Compound 2 around 3.7 ppm. Selectivity is seldom an issue in NMR, and no more so than chromatographic methods are routinely challenged with.

Table 2. Selectivity Examples

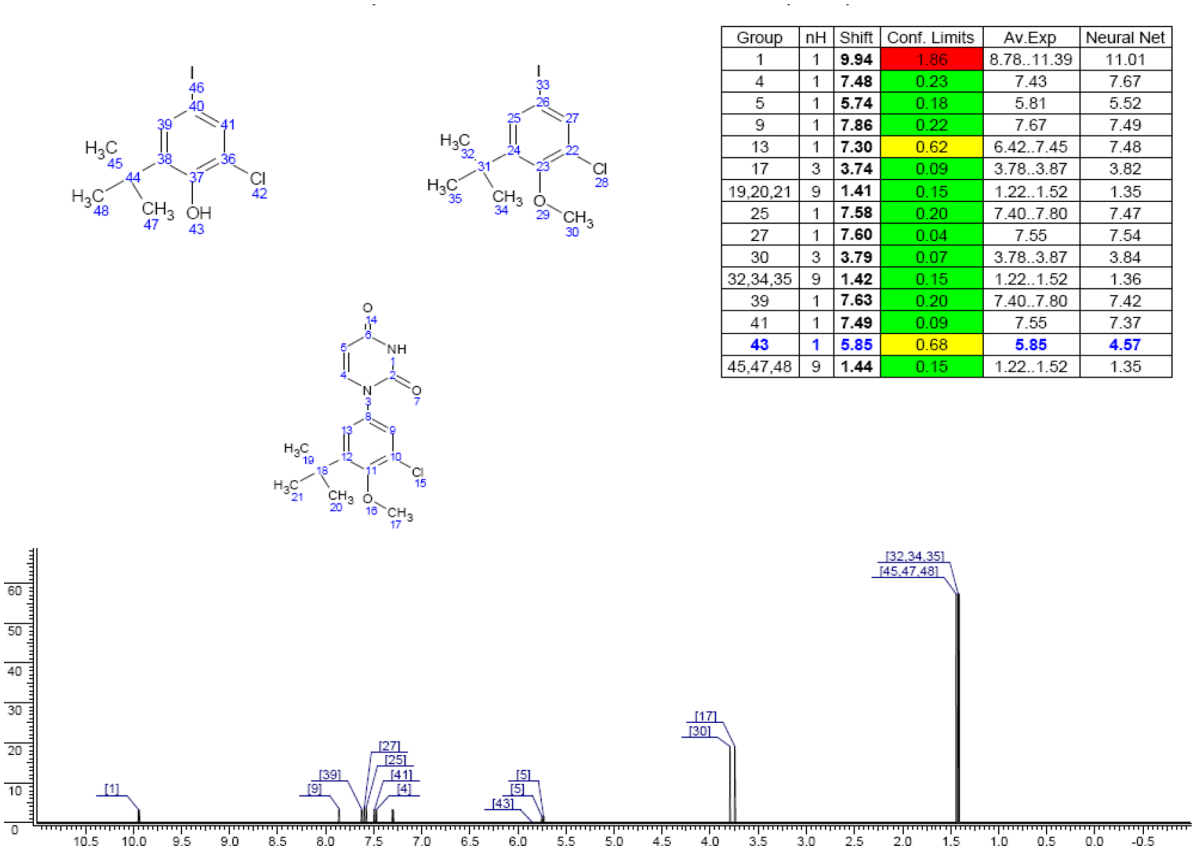
Compound	Structure
1	
2	
3	

OPERATING PARAMETERS

Representative operating parameters for the q-NMR experiment are listed in Table 3. Slight changes may be needed depending on field strength and type of hardware utilized. Generally, one would want to run reasonable quality data prior to any analysis to minimize the impact on quantitation such experimental aspects as shim, tuning, solvent, solvent suppression, etc. There is no hard and fast rule about quantitating with solvent suppression except to use the further signal away from the solvent, as this is likely to be the least affected. Phase and baseline correction are likely to be the most important experimental factors for routine analysis. For sample preparation, solubility in deuterated solvent and selection of an internal sample that has distinct, resolved signals from the analytes present is the key.

VALIDATION OF Q-NMR

From a regulatory standing, traditional and quantitative NMR is accepted by the International Conference on Harmonization



Group	nH	Shift	Conf. Limits	Av.Exp	Neural Net
1	1	**9.94**	1.86	8.78..11.39	11.01
4	1	**7.48**	0.23	7.43	7.67
5	1	**5.74**	0.18	5.81	5.52
9	1	**7.86**	0.22	7.67	7.49
13	1	**7.30**	0.62	6.42..7.45	7.48
17	3	**3.74**	0.09	3.78..3.87	3.82
19,20,21	9	**1.41**	0.15	1.22..1.52	1.35
25	1	**7.58**	0.20	7.40..7.80	7.47
27	1	**7.60**	0.04	7.55	7.54
30	3	**3.79**	0.07	3.78..3.87	3.84
32,34,35	9	**1.42**	0.15	1.22..1.52	1.36
39	1	**7.63**	0.20	7.40..7.80	7.42
41	1	**7.49**	0.09	7.55	7.37
43	**1**	**5.85**	0.68	**5.85**	**4.57**
45,47,48	9	**1.44**	0.15	1.22..1.52	1.35

Figure 2. NMR selectivity required for potency determinations.

Table 3. q-NMR Instrument Conditions

field strength	≥400−600 MHz	≥700 MHz
nucleus	^{1}H	^{1}H
temperature	regulated, typically 25−30 °C	regulated, typically 25−30 °C
number of scans	≥32	≥4
flip angle	≤45°	≤45°
spectral width (sw)	≥16 ppm	≥16 ppm
relaxation delay (D1)	>30 s	>30 s
solvent	DMSO-d_6, 1% D_2O in DMSO-d_6, $CDCl_3$, CD_3OD, CD_3CN	DMSO-d_6, 1% D2O in DMSO-d_6, $CDCl_3$, CD_3OD, CD_3CN
acquisition time	≥3 s	≥3 s

(ICH).[14] Maniara et al.[34] showed that q-NMR can be effectively applied for both the major component and the impurities in a drug substance by demonstrating that impurities at the 0.1% level or higher could be quantified by NMR with a sensitivity, speed, precision, and accuracy similar to HPLC. Several investigators added to the confirmation that q-NMR is a valid technique for pharmaceutical analysis investigations and the validation parameters are found in the literature.[18−21] The use of q-NMR was challenged through the use of traditional validation requirements applied in regulated industries to liquid chromatography methods and established q-NMR as fully equivalent to liquid chromatography for early phase potency determinations.

RESIDUAL SOLVENTS IN DRUGS

NMR spectral assignments of common ICH class 1 and class 2 solvents in a variety of deuterated solvents has been reported by Jones et al.[35] This work can be applied to evaluate the levels of these samples in the pharmaceutical formulation as well as traditional residual solvent levels in the API. Coupling this to the potency experiment, analysts can rapidly combine several required limit tests into a single q-NMR experiment. Additional applications can be the use of q-NMR for determining the completeness of reaction synthesis, drying steps, and solvate ratios.

RELATIVE RESPONSE FACTORS

Impurities in pharmaceutical development are often estimated using an area-normalization approach until a well-characterized impurity reference standard is produced. At the early stage of drug development, processes are still changing and reference standards for impurities are limited, not readily available, or have uncertain purity. Generating these standards is a cost that most companies feel is not warranted at early stages; so, most impurities are usually not well characterized or available in sufficient quantities.

While area-normalization is feasible for early stage investigations, there are times, such as for stability investigations, where the actual relative response factor of the API is needed. When a standard of known purity is available for both the impurity and the API, the response factor for each standard is established by normalizing the response factor at a specific wavelength by the mass used to produce this absorbance. The relative response factor (RRF) is the ratio of the response factor of the impurity of interest to the response factor of the API at a specific wavelength. When standards are not available, an alternative procedure to normalize the absorbance by mass must be used. Nussmaum et al. first showed that using chemiluminescent nitrogen detector (CLND) as a mass detector, the response factors for impurities in Fluoxteine HCl (Prozac) could be established using nitrogen specific detection.[36] Jackson et al. expanded this application to several compounds of pharmaceutical interest.[37] Subsequently, Sun et al. showed that the charged aerosol detector (CAD) also provided a suitable mass response for determining the relative response factors for the liquid chromatographic analysis of paclitaxel related substances.[38]

Webster et al. showed that NMR can also be used to establish relative response factors.[22] Because the protons observed in q-NMR basically all respond the same within the sample, NMR spectrometry becomes a virtual universal mass detector for the relative response factor application. NMR also has the advantage over CAD and CLND in that there are fewer restrictions on sample preparation and analysis.

NATURAL PRODUCT/BIOLOGICAL APPLICATIONS

On several occasions the pharmaceutical industry utilizes components of natural products as potential target drugs. Extracted active material invariably contains a variety of known and unknown compounds in varying proportions. The first challenge is to identify the structure of the active drug and structures of as many additional components as possible. The second challenge is to quantify the active ingredient in the mixture to determine the potency. Chromatographic techniques are simply not suitable for the purpose of identification. Even potency determination of the active material by chromatographic techniques can be difficult if pure samples of the active material and accompanying components are not available. NMR is useful in structure elucidation of active material and other known components. It is also capable of providing clues toward characterizations of unknown components. Quantitative NMR with a known amount of internal standard is capable of determining potency of the active material even in complex mixtures, as long as at least one NMR peak of the active material and at least one NMR peak of the internal standard are isolated in the spectrum. The isolation of these peaks is essential for the q-NMR technique, even if the rest of the NMR spectrum of the mixture is complex or it has overlapping peaks from different components. Quantification of the amount of the active material by taking the ratios of the areas of the isolated peaks of the active material and the internal standard leads to potency of the drug from the known amount of the mixture. Pauli et al. have provided the details of the data-acquisition, postacquisition processing parameters, and conditions for the q-NMR experiments suitable for potency elucidation of natural products and have reviewed a large number of studies for analysis of a variety of natural products and metabolites.[14,15]

Because of the ability of q-NMR to quantify compounds in a complex mixture without the requirement of having a standard sample of the analyte, this technique has been used for analysis of mixtures obtained from plants in the food industry and wine

industry.[39−41] There are numerous examples of the use of q-NMR in mixtures such as bird repellants containing garlic oil,[42] cyanobacterial extracts containing cyanophycin,[43] and marine matrixes of shellfish containing milligram quantities of phytotoxic domoic acid.[44] Several trilactones have been analyzed quantitatively in *Ginko biloba* using q-NMR over 20 years ago.[45] Since then several similar studies have been done for analyzing these and other components in *Ginko biloba*. A comparison of q-NMR with GC and HPLC for the determination of purity of and impurities in two agrochemicals, 2,4-dichlorophenoxyacetic acid and sodium 2,2-dichloropropionate, showed better accuracy and precision in the q-NMR method.[46] Several metabolic pathways in plants have also been studied by q-NMR. Simultaneous quantification of ethanol, acetic acid, malic acid, lactic acid, and succinic acid, proline, and alanine metabolites of fermentation of wine was done using q-NMR.[47] This study also included monitoring of proline to arginine ratio during fermentation. The examples cited in this article are just a few representative examples among a vast number of q-NMR studies in the food and drug industry.

NMR spectrometry has been successfully applied in quantitative analysis in the study of metabolites. It is not necessary to have reference samples of the metabolites. The methodology employed is quick and can be applied to complex mixtures. However, at least one NMR signal of a metabolite should be in isolation. Use of a high-field NMR spectrometer, >400 MHz provides high resolution and potentially separates the signals so that more metabolites can be analyzed. With higher resolution in hand, quantitative analysis of metabolites is possible in biological fluids such as urine and blood plasma. Therefore, these techniques lend themselves to metabolic and toxicological studies of drugs not only in pharmaceutical laboratories but also in forensic laboratories. Since the urine and plasma samples are aqueous based samples, solvent suppression is employed during acquisition of NMR signals. Barding, Salditos, and Larive have provided a review of q-NMR for bioanalysis and metabolomics along with sample preparation of biofluids, data-acquisition parameters including solvent suppression techniques, and postacquisition processing parameters suitable for metabolic studies.[48]

q-NMR spectrometry was utilized to quantify metabolites present in cultured mouse fibroblast cells 3T6 in their native and after treatment with an inhibitor of the fibroblast growth factor receptor.[49] In this study the q-NMR technique allowed identification and quantification of 40 different metabolites of nmol/mg of protein level. In a similar study, nine lipid species were identified and quantified by q-NMR of blood plasma or serum lipid extracts.[50] This method has also been used to identify unusual lipids in the blood of patients with inborn errors of lipid metabolism, thus making this method a possible clinical diagnostic tool. In another study, hippurate, formate, and 4-cresol sulfate, three urinary metabolites, were quantified by q-NMR to differentiate between Crohn's disease and ulcerative colitis patients as the level of these three metabolite amounts present in the urine differed.[51] Urine is a readily available biofluid with little presence of proteins and lipids, which makes it a preferred medium for metabolic study. However, it contains a very large number, upward of hundreds, of metabolites. No single technique or methodology can identify and quantify this large number of metabolites. Bouatra et al. have employed q-NMR, gas chromatography (GC), liquid chromatography−mass spectrometry (LC−MS), direct flow injection mass spectrometry (DFI/LC-MS/MS), inductively coupled plasma mass spectrometry (ICPMS), and high-pressure liquid chromatography (HPLC) on multiple urine samples to identify 445 and quantify 378 metabolite or metabolite species.[52] Of these metabolites, q-NMR was able to identify and quantify 209 species, the largest number obtained between any of the other techniques employed.

COST COMPARISON

Because the q-NMR experiment is often run on existing NMR instrumentation, the additional cost of running q-NMR to traditional LC based potency determinations is quite favorable. Using standard industry costs shown in Table 4, q-NMR is more economical for a "first sample" tested for a new drug in development. For each additional sample the economics are comparable. The additional advantage is that q-NMR can post the potency results in terms of hours to 1−2 days. The LC based determination typically runs 2 days to a week to post a verified result.

Table 4. q-NMR Cost Comparison

	q-NMR	LC + TGA
labor	$440	$2 940
chemicals	$ 87 (DMSO-d_6, D_2O, internal standards)	$196 (ACN, H_2O, MeOH, TFA)
TGA (thermogravimetric analysis) testing		$490
total	**$527**	**$3 626**
cost of additional samples	$457	$477

CONCLUSION

Quantitative NMR spectrometry is gaining grounds in the pharmaceutical industry as an alternative technique to chromatography for determination of drug potency, especially in the early stage of drug discovery. The distinct advantages of q-NMR are (1) its ability to determine potency without having a standard, (2) it is quicker, and (3) it is more economical. While simultaneously providing qualitative information, q-NMR is also useful in quantifying compounds of interest in complex natural product mixtures and in metabolic studies.

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Notes

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The authors declare no competing financial interest.

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