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The Emerging Field of Medicines Authentication by Nuclear Quadrupole Resonance Spectroscopy

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Abstract We report the progress of a project at King's College London funded by UK Healthcare Charity, the Wellcome Trust, to develop a prototype, portable medicines authentication device based on nuclear quadrupole resonance spectroscopy (NQR) and employing innovative parametric data processing, for the non-invasive, non-destructive and quantitative inspection of packaged medicines. The first-generation system will target nitrogen- and/or chlorine-containing medicines in blister packs and bottles without the need to remove these items from their packaging. Initial proof of principle will be based around a database consisting of the NQR "fingerprints" of a select list of essential medicines as defined by the World Health Organization that are commonly counterfeited in developing countries. This database will be expanded as the project progresses, and as the device is taken up for commercialization.

1 Introduction: Counterfeit Medicines and NQR

Counterfeit medicines are a serious threat to human health, and the problem is a global one [1–6]. The scale of the problem is such that, in 2006, the World Health Organization (WHO) formed IMPACT—the International Medicinal Products Anti-Counterfeiting Taskforce (http://www.who.int/impact/about/en/). Although the focus of the above-mentioned activity is on deliberately counterfeited medicines, there is an additional health risk from substandard medicines—medicines that do not contain the correct amount of active ingredient due to poor quality assurance at point of manufacture, rather than via any deliberate act [7, 8]. At the present time, the most-often-applied method of detecting counterfeit medicines—so-called "medicines authentication"—is visual inspection of the packaging and contents;

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technological approaches to the problem include the application of any one of a wide range of techniques including liquid chromatography (LC), mass spectrometry (MS), X-ray powder diffraction (X-ray), terahertz (THz) spectroscopy and Raman (RS), infrared (IR) and near-infrared (NIR) spectroscopies [9–12]. All of these methods are best-suited for looking at individual pills or loose material (powder), whereas radiofrequency (RF) methods like NQR are uniquely suited to examining bulk quantities of pills, i.e., a full bottle or a complete blister pack, shipping cartons of bottles or packs; or drums of loose material. RF techniques are also inherently safe and can be used without any protective clothing and require only minimal training. In recognition of this, in 2010, the Wellcome Trust, the United Kingdom's largest healthcare charity, awarded the NQR group within the Division of Engineering, King's College London (KCL), £473,000 to develop a prototype portable quadrupole resonance-based medicines authentication device suitable for use anywhere in the world, but, most particularly, in developing countries.

The KCL NQR group, in common with other NQR groups [13–20], has been able to demonstrate that the method is quantitative, that different polymorphs of the same compound can be distinguished, and that there is no interference from the excipient or coating material. Approximately 50 % of atoms in the periodic table have quadrupolar nuclei, so signals could be acquired from the vast majority of medicines, the only caveat being that, when the quadrupolar isotope is in low abundance, such as ^{33}S and ^{17}O, inspection times will be longer. The problems posed by spurious signals, such as those observed from piezoelectric materials included as excipient in the pills or RF interference (RFI) are largely eliminated by cycling the phases of the RF pulses and then using the novel parametric data processing and classification techniques that have been developed by KCL in association with Lund University, Sweden, to deal with these problems [21]. The challenge for NQR in this application is in the low filling factors (ratio of sample volume to coil volume) that can be expected with blisters packs that contain a small number of pills/capsules across a surface area that may be large compared to the size of the pills/capsules—the standard configuration of packaging in most parts of the world. However, using RF methods, the contents of medicine bottles and blister packs can be examined through multiple layers of packaging without the need to remove the contents from cartons, plastic bags, drums or other additional packaging of various sizes and shapes.

Medicines authentication as a procedure can be reduced to the general question: do the contents of a packaged medicine match its label? Medicines authentication by NQR will be based around a database of NQR "fingerprints" of packaged medicines in specific, commercially available configurations that can be compared to signals returned from suspect packets of the same medicines.

2 Experimental

A range of different NQR measurements have been employed in this project. They can be divided into three types: (1) double-resonance methods (DR); (2) broadband excitation stochastic NQR methods (sNQR); and (3) conventional narrowband

excitation NQR methods. The first two types of measurement are employed primarily in the identification of NQR line frequencies (^{14}N or ^{35}Cl) for materials (medicines) for which the NQR line frequencies are not already known; the third for the estimation of the time constants of the identified NQR lines and for the detection pulse sequence to be used in the authentication.

2.1 Double-Resonance Methods (DR)

The current setup for DR measurements at KCL is a so-called double-resonance by cross-relaxation (d.r.c.r.) design [22] with pneumatic air cylinders for sample transfer. This system is used as a first-pass method of line-searching for ^{14}N NQR lines across the frequency range of 0.0–5.0 MHz. The polarizing and detecting magnet is a Bruker 19 MHz permanent magnet with pole diameter of 10 cm and pole gap of 2 cm. It provides a very good homogeneity of 1 in 10^6 over a large 2 cm cubic volume. A Tecmag APOLLO spectrometer supplies the RF pulses to measure ^{1}H NMR signals and also the TTL pulses to control the pneumatic system and the solenoid operation. The RF pulses were amplified using a TOMCO BT00500-Delta A 500 W power amplifier to give very short 90° pulses necessary for ^{1}H NMR of solids.

The system is controlled via software written using the MATLAB programming package, which allows for automated operation.

2.2 Broadband Excitation Stochastic NQR Methods (sNQR)

Broadband, low-power, so-called "stochastic" or "noise spectroscopy" methods [23] are also being investigated for locating ^{14}N and ^{35}Cl lines. Current capability gives a 200 kHz search bandwidth for ^{35}Cl and 100 kHz search bandwidth for ^{14}N NQR lines. The usual procedure for searching for ^{14}N NQR lines at KCL is to first identify a bandwidth to target using double-resonance methods, and then locate the lines with stochastic methods. In addition, stochastic methods can find lines that are not visible in DR spectra (e.g., ^{14}N lines "remote" from protons, or with time constants too long or too short to match up to sample transfer speeds). Stochastic methods can also give information on T_1 [24] while, at the same time, being less dependent on prior knowledge of T_1 for time-efficient signal accumulation, a useful property when, as in this case, the T_1s of the NQR lines of interest are, in the first instance, unknown.

In stochastic NMR/NQR spectroscopy narrow, wide excitation bandwidth RF pulses can generate FID (free induction decay) signals using low RF power. A long train of randomly phased, closely spaced pulses takes the place of the high-power RF pulses required in 'normal' RF spectroscopy to generate similar strength FID signals with a narrow pulse. Stochastic RF excitation should therefore be a convenient technique when searching for spectral lines and has been used for this purpose in solid state NMR spectroscopy.

One limitation of the sNQR technique is that it is an FID technique, and therefore is not suitable for very broad lines—the signal being lost under the deadtime. However, sNQR is not the only broadband excitation method available for this type

of NQR line frequency estimation. Frequency-swept pulses, such as WURST, may also be employed [25].

2.3 Conventional Narrowband Excitation NQR Methods

Time constants for lines found in the line-searching operations are determined using conventional "high-power" NQR and standard pulse sequences. The spectrometer setup used for this work is a Tecmag Redstone 0.1–125 MHz pulsed RF spectrometer with one transmit and one receive channel. The spectrometer is controlled via a PC running on the Windows 7 OS. The TX output of the Redstone across the frequency range of interest (0.4–35 MHz) is 0.391–0.431 V peak. This spectrometer is used in conjunction with a Tomco BT01000-AlphaS 1 kW 10–60 MHz pulsed RF amplifier. Although the lower end of the Tomco specification is 10 MHz, it works well at frequencies much lower than this, and in practice is routinely operated at 2.0–3.5 MHz.

The RF resonators used for this work are mostly of the conventional solenoidal design with either circular or rectangular cross sections with loaded Q values in the range of 60–160. To shorten the deadtime following the end of the pulse, a Q-switch is employed. The Q-Switch is of a KCL design similar in basic concept to that detailed in Ref. [26]. Time constants are determined across a number of temperatures across the range from -5 to $+35$ °C.

3 Results and Discussion

There are four steps in building an NQR fingerprint of a medicine: (1) identifying the NQR line frequencies; (2) selecting one or more of the lines to use for building the fingerprint; (3) characterizing the time constants of the selected line(s) at a range of temperatures; and (4) applying the knowledge gained to acquiring NQR signals from that medicine in a specific commercially available configuration. The final step provides the quantitative element of the authentication, the first three the qualitative. For the sake of brevity, the discussion of results will focus primarily on ^{14}N NQR. Procedurally, there is no difference between the generation of ^{14}N and ^{35}Cl NQR fingerprints, although the choice of NQR line to use for the fingerprint is simplified in the case of ^{35}Cl, as there is only one NQR line per crystallographically inequivalent chlorine site.

3.1 Generating NQR Characteristics of Medicines for a Fingerprint Database

All three types of NQR measurement described in Sect. 3, above, were employed in generating NQR characteristics of target medicines. The approach followed was to start with a double-resonance search of the range of 0.1–4.0 MHz to identify, within the $\pm$30 kHz resolution of the d.r.c.r. method employed at KCL, regions of the spectrum where the NQR lines can be found; to follow this with broadband sNQR searches of the regions identified—this step being particularly important to identify (a) instances where single features in the DR spectrum conceal multiple close NQR

lines; and (b) NQR lines arising from nitrogen distant from protons; and then, finally, use conventional narrowband NQR measurements to confirm as real any possible lines identified from the sNQR runs.

3.2 Example 1: Sulfamerazine

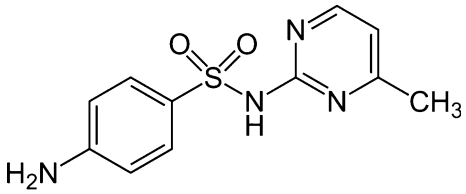
The viability of this approach was first tested using sulfamerazine, for which there are data in the literature with which the results can be compared [16]. Sulfamerazine, $C_{11}H_{12}N_4O_2S$, is a sulfonamide antibacterial. Sulfamerazine crystals are orthorhombic and of space group $Pn2_1a$ with $Z = 8$. The molecules are said to exist as dimers through hydrogen bonds formed by amide and pyrimidinyl nitrogen [16]. The structure is shown in Fig. 1.

Figure 2 shows the ^{1}H-^{14}N d.r.c.r. spectrum acquired at room temperature using the setup described in Sect. 3.1, above, and a powder sample of sulfamerazine. The sample was acquired from Sigma Aldrich. Figure 2a shows the first run from 0.0 to 4.0 MHz; Fig. 2b shows (lower trace) an expansion of the 1.8–3.3 MHz section of the spectrum. The broad features suggest that the ^{1}H-^{14}N system has saturated (time at low field $>>$ quadrupolar T_1 at this field); this was confirmed when this band of the spectrum was re-run with a shorter time spent in low field, producing noticeably narrower features (upper trace).

There is very good agreement between these results and those for sulfamerazine in Ref. [16] acquired using a double resonance by level crossing (d.r.l.c.) approach (described in Ref. [16]). The additional, sharp features at 250, 1,230 and 1,500 kHz in the d.r.c.r. spectrum presented in Fig. 2a not seen in the sulfamerazine d.r.l.c. spectrum in Ref. [16] may be attributed to two-proton relaxation flips, a common feature of cross-relaxation spectra that occur at or close to sub-harmonics of one or more of the NQR frequencies [22] and are not seen in level-crossing experiments. There are additional, small but reproducible dips at ca 2.8 and 4.0 MHz in Fig. 2 not found in the d.r.l.c. spectrum in Ref. [16]. However at this time, it is not known if these are real or artefacts.

Having identified regions of the spectrum to target in the search for lines, the next step in the approach is to locate the lines using a broadband NQR method—in this case stochastic NQR (sNQR). While sNQR signal intensity is influenced by the T_1 relaxation time and optimum sensitivity involves adjustment of the RF pulse width, separation and RF power, it is possible to set these sequence parameters such as to obtain signals for a range of T_1. Figure 3a shows the ^{14}N sNQR spectrum for sulfamerazine in the range of 2.9–3.1 MHz acquired using a small sample in a 3 cm^3 coil with a Q of ca 20; total measurement time was ca 8 h. This measurement

Fig. 1 Sulfamerazine



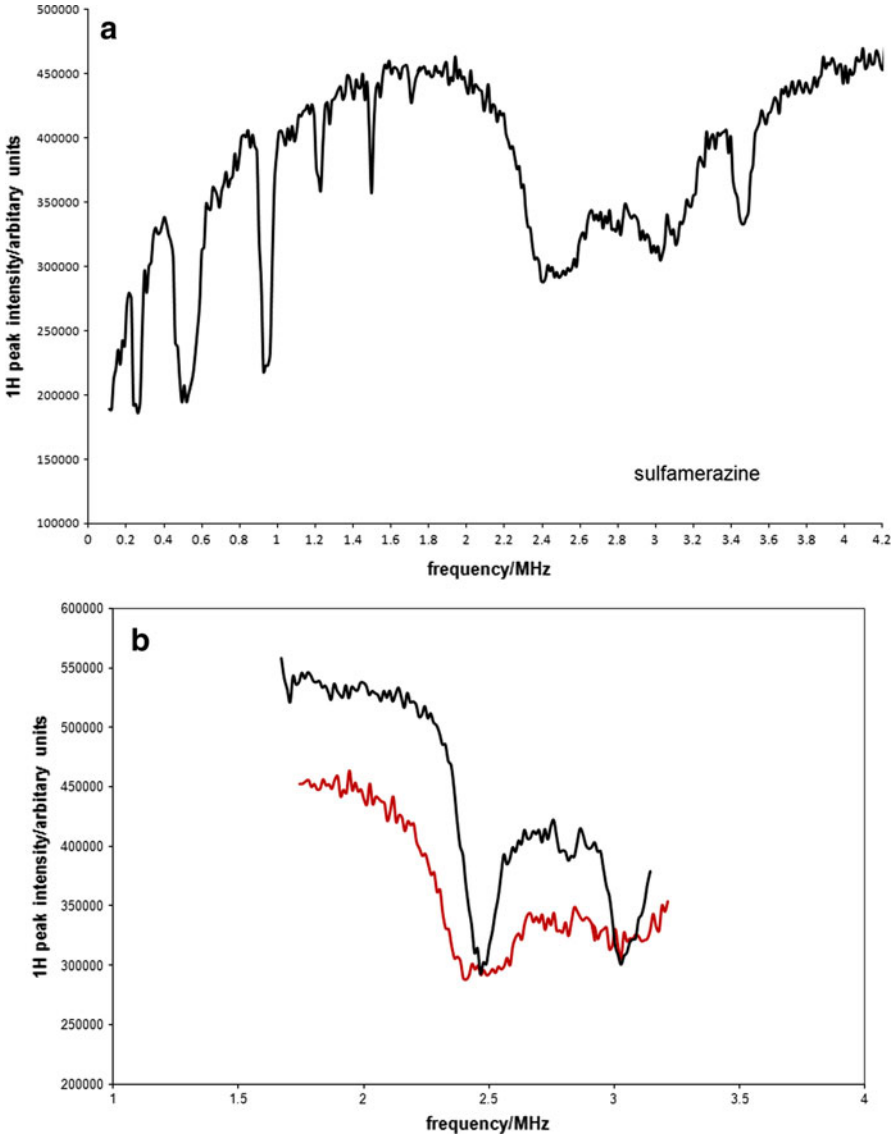


Fig. 2 **a** ^{1}H-^{14}N Double resonance by cross relaxation (d.r.c.r.) 0.0–4.0 MHz spectrum of the material sulfamerazine at room temperature; **b** *lower trace* expansion of the 1.8–3.3 MHz region of the trace in **a**; *upper trace* repeat of this frequency band with a shorter contact time

clearly shows that the single feature at ca 3 MHz in the d.r.c.r. spectrum "conceals" a multitude of lines—something that could easily be missed if a narrowband conventional NQR measurement was employed directly to identify the "line" expected in this region. Figure 3b shows the ^{14}N NQR spectrum of the sample over the same region acquired in the same coil using a conventional single-pulse NQR acquisition. This latter spectrum was acquired once the T_1s of the lines had been

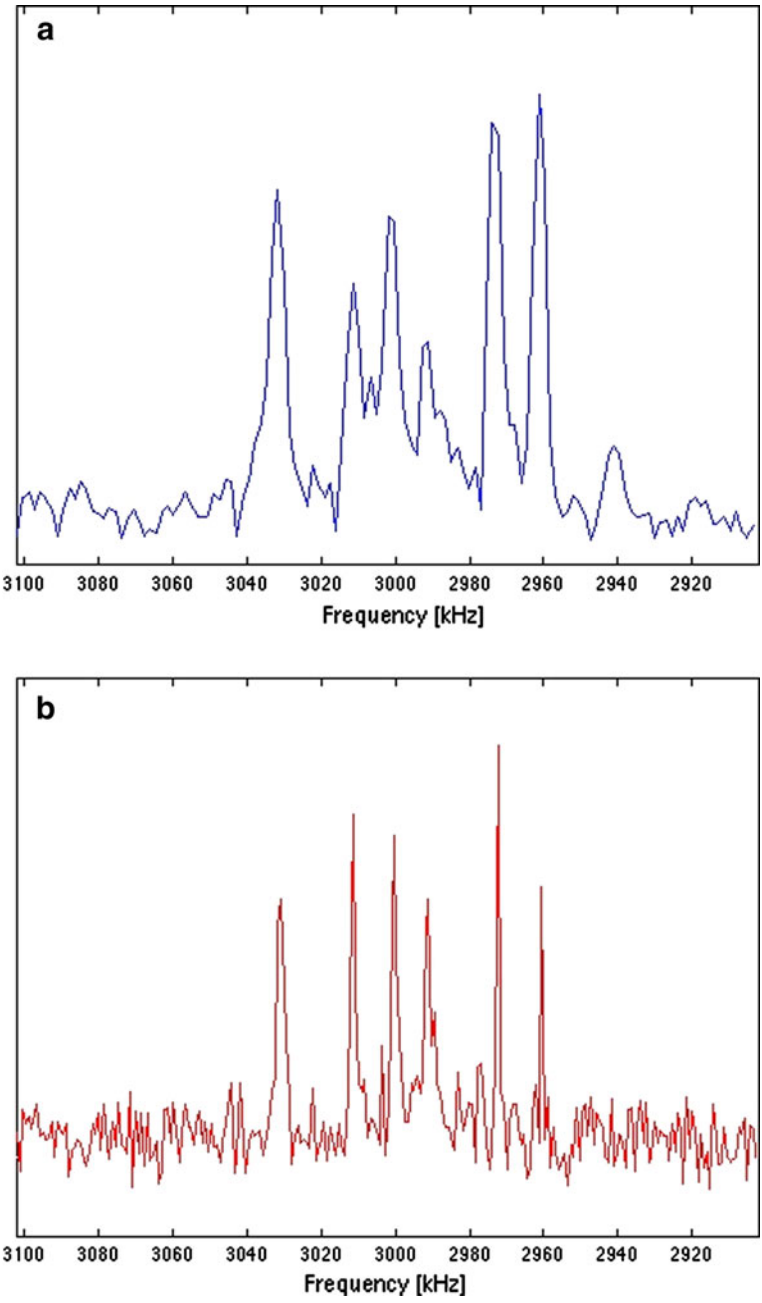


Fig. 3 **a** ^{14}N Stochastic NQR (sNQR) spectrum of sulfamerazine in the range 2.9–3.1 MHz (measurement time ca 8 h); **b** conventional single pulse 14 N NQR spectrum of the sulfamerazine sample across the same range acquired once the T_1s of the individual lines had been determined (measurement time ca 50 min). In both cases, the sample was a few grammes of powder in a 3 cm^3 coil, loaded Q of ca. 20. Using a small coil allowed sufficient RF power to be used for a short (10 μs) 90°$_{\text{eff}}$ pulse to be employed for the conventional measurement

individually estimated; those T_1s were relatively short (e.g., 7 ms at 293 K for the 3.033 MHz line) and so the total measurement time was only ca 50 min. Using a small coil allowed sufficient RF power to be used for a short (10 μs) $90°_{eff}$ pulse to be employed for the conventional measurement. There is excellent agreement between the two spectra (a) and (b) showing that the sNQR is a good indicator of the true spectrum. Line assignments are beyond the scope of this current work, but the multiplicity of lines relative to the crystal structure suggests that lines from all four crystallographically inequivalent nitrogen have been identified.

The sulfamerazine measurements were not pursued beyond testing the viability of the approach adopted for generating NQR characteristics of medicines and comparing these results with those obtained by other authors. This was primarily because sulfamerazine is not on the WHO list of essential medicines.[1] However, the next example features a medicine—ampicillin—that is on the WHO list, and this work has been pursued to the point of obtaining signals from the genuine medicine inside blister packs.

3.3 Example 2: Ampicillin Trihydrate

Ampicillin, $C_{16}H_{19}N_3O_4S$, is a beta-lactam antibiotic. It is on the WHO model list of essential medicines. The trihydrate crystals—the form found in pharmaceutical formulations—are of space group $P2_12_12_1$, and $Z = 4$ [29]. The amine and amide nitrogen are both involved in hydrogen bonding. There is no chlorine in the molecule, making ^{14}N NQR the approach of choice. The structure is shown in Fig. 4.

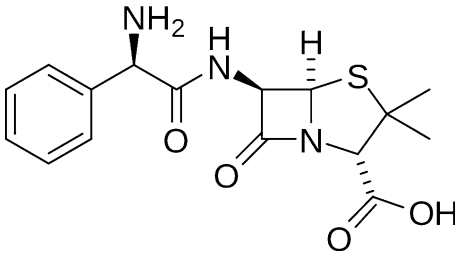
Figure 5 shows the ^{1}H-^{14}N d.r.c.r. spectrum of ampicillin across the range of 0.0–4.7 MHz (courtesy of Dr. David Stephenson, University of the West Indies). The sample used was the anhydrous material, as the relaxation times of the trihydrate material proved too short in comparison with the cycle time of the DR instrument. ^{14}N Stochastic NQR measurements on the trihydrate material in the regions identified in the d.r.c.r. spectrum of the anhydrous material did not reveal any multiplicity of lines, but did confirm that the spectrum of the anhydrous material was a good guide to the location of the lines in the trihydrate. Table 1 shows the tentative groupings of the lines identified in the d.r.c.r. spectrum of the anhydrous material, and the sNQR spectrum and subsequent narrowband conventional ^{14}N NQR measurements of the trihydrate, with corresponding quadrupolar coupling constants and asymmetry parameters.

Again, line assignments are outside the scope of this work, but comparison with the literature suggests that sites I and II in Table 1 are most likely to be the amide and amine nitrogen and site III therefore the beta-lactam nitrogen. The 3,033 MHz line of the trihydrate was selected for full characterization. Table 2 shows a selection of time constants at 293 K for the 3,033 kHz line, as well as the equivalent time constants for the sulfamerazine line which shares the same frequency at 293 K (discussed in Sect. 3.5 below).

[1] Available to download at: http://www.who.int/medicines/publications/essentialmedicines/en/index.html.

The NQR behavior of the line immediately suggested that the pulse sequence to use for maximizing the signal-to-noise ratio per unit time was the so-called pulsed-spin locking (PSL) AKA spin-locked spin-echo (SLSE) multiple-pulse sequence similar in form to the familiar Carr–Purcell–Meiboom–Gill (CPMG) sequence of

Fig. 4 Ampicillin



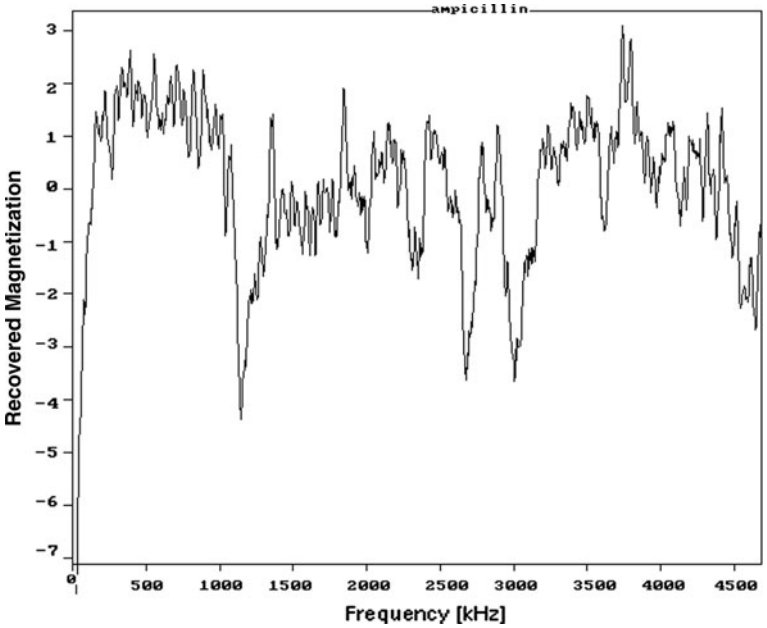


Fig. 5 ^{1}H-^{14}N Double resonance by cross relaxation (d.r.c.r.) 0.0–4.7 MHz spectrum of the material anhydrous ampicillin at room temperature (courtesy of Dr David Stephenson)

Table 1 ^{14}N NQR frequencies for ampicillin trihydrate by pure and double-resonance (frequencies in italics) NQR at room temperature

kHz	Site I	Site II	Site III
ν_+	3,033	*2,973*	*1,426*
ν_-	2,673	*2,326*	*1,160*
ν_0	*394*	*647*	*253*
Q_{cc}	3,805	3,533	1,724
η	0.28	0.55	0.46

Table 2 Summary of frequency and time constants for ampicillin trihydrate and sulfamerazine at 3,033 kHz and 293 K

Compound	Resonant frequency/MHz	FWHM/Hz	T_2*/ms	T_{2e}/ms[a]	T_1/ms	Temp. Coeff.
Sulfamerazine	3.0337	2,278	0.134	5.6	8	−310 Hz/K
Ampicillin $3H_2O$	3.0335	1,927	0.166	10.5	600	−80 Hz/K

nuclear magnetic resonance (NMR): $P1_{\pm\phi} - \tau 1 - (-P2_{(\phi+90)} - \tau 2 -)n$, where $P1$, $P2$ are the pulses with phase $\pm\phi = 0°$, 180° and $(\phi+90) = 90°$, and $\tau 1$ and $\tau 2$ two delays where $\tau 2 \sim 2 \times \tau 1$.

Figure 6 shows the result of measurements in a cylindrical solenoid, volume 285 cm^3, loaded Q of ca. 80, at 3,033 kHz with and without a 22 g sample of ampicillin trihydrate powder (Sigma Aldrich) present. The sequence was a PSL sequence of the type described above, $P1 = P2 = 50$ μs, $\tau 1 = 175$ μs s, $\tau 2 = 274$ μs, $n = 128$, with 1 s repeat between scans with (a) 240 scans per measurement, each measurement repeated 12 times with and without a sample in the coil (ca 4 min measurement time per measurement); and (b) 120 scans per measurement, each measurement repeated 12 times with and without a sample in the coil (ca 2 min measurement time per measurement). Each point in the plots is the sum of the magnitude of all the time domain data points for each measurement.

^{14}N NQR signals acquired with packaged ampicillin trihydrate are included in the next section.

3.4 Testing Suitable Resonator Designs

From a technical standpoint, it is difficult to cover the full 0.0–5.5 MHz frequency range of ^{14}N NQR lines with a single coil in a system that is intended for a non-expert user given the attendant requirement for some form of auto-tuning with both switched, fixed capacitance and some form of a motor-driven variable capacitor for fine tuning and matching. In addition, coil performance would be compromised at all frequencies. Instead, the approach adopted in this project has been to equip the system with a primary coil that can cover one band within that range when coupled to an auto-tuner that uses motor-driven 0–125 pF variable capacitors, but no switched, fixed capacitance. The auto-tuner employed by KCL has been designed specifically for this project and the proposed prototype portable device; however, there are also schemes in the literature (e.g., Ref. [27]). Coils to cover other bands within the range and designed to operate with the same auto-tuner will be provided with the prototype, but it is anticipated that the prototype will be used largely with the primary coil alone. It was therefore essential that the tuning range of the primary coil cover a part of the ^{14}N NQR spectrum over which as broad a selection of medicines as possible have ^{14}N NQR lines.

To determine what frequency range the primary coil should cover, a survey of ^{14}N NQR line frequencies in medicines was carried out. This consisted partly of a literature search, and partly of measurements done at KCL to determine ^{14}N NQR line frequencies at room temperature in a number of medicines and other

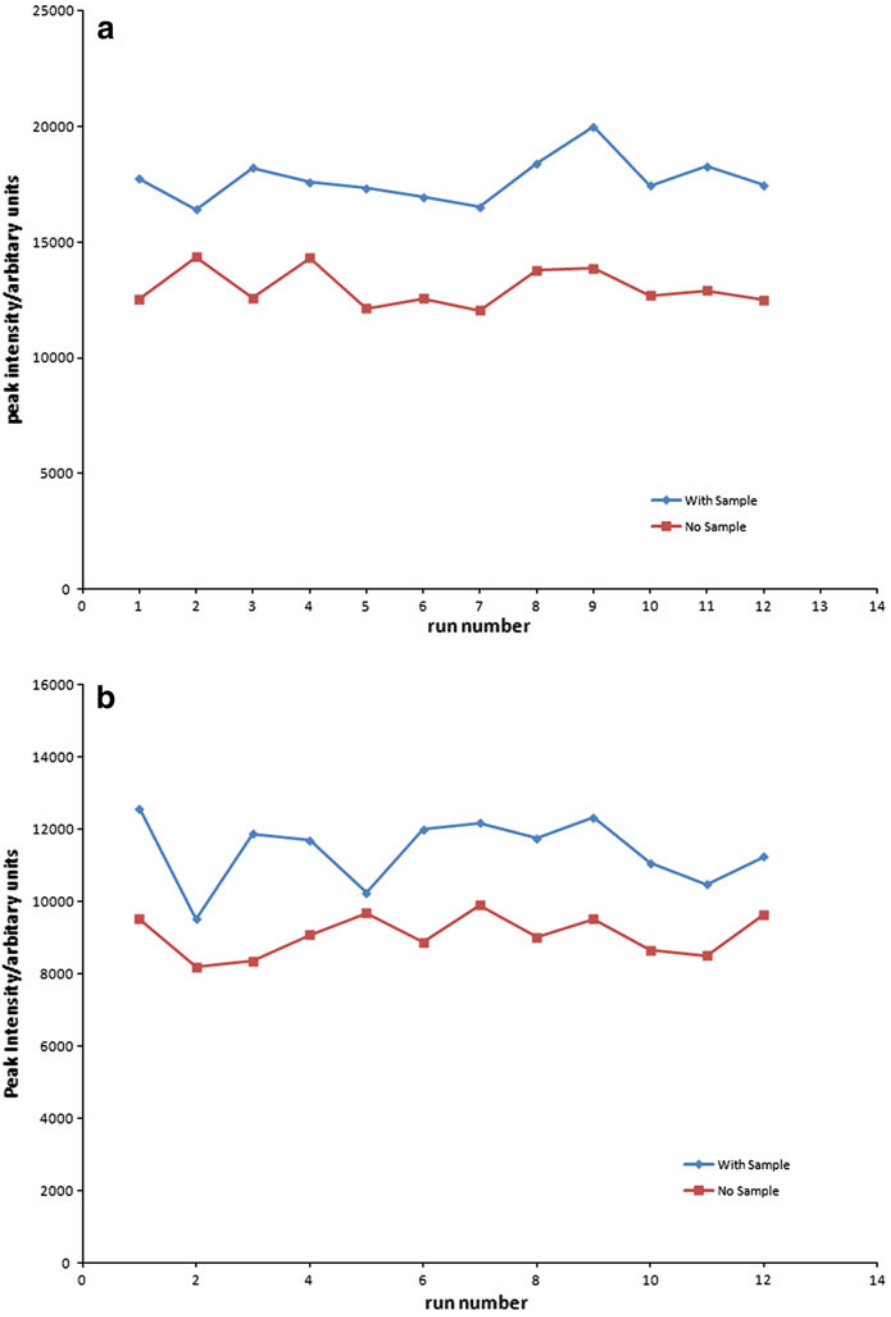


Fig. 6 Measurements in a cylindrical solenoid at 3,033 kHz with and without a sample of ampicillin trihydrate powder (Sigma Aldrich) present. The sequence was a PSL sequence, timings in the text, with **a** 240 scans per measurement, each measurement repeated 12 times with and without a sample in the coil (ca. 4 min measurement time per measurement); and b) 120 scans per measurement, each measurement repeated 12 times with and without a sample in the coil (ca. 2 min measurement time per measurement)

compounds that contain nitrogen functional groups commonly found in medicines. Figure 7 shows a histogram of the occurrence of ^{14}N NQR lines in 45 medicines grouped by frequency. This survey, while not exhaustive, suggested that a range of 2.3–3.7 MHz was the best choice for the primary coil. This is easily realizable with a coil of inductance ca 25–30 μH and the 0–125 pF variable capacitors of the autotuner.

Having decided on the frequency range of the resonator, the next thing to determine was its geometry. Experiments revealed that the aluminium foil coating one side of a blister pack affects the performance of an RF resonator in inverse proportion to the RF excitation frequency. This can be correlated with the skin depth relationship between frequency and the thickness of the aluminium foil (standard thicknesses for the foil of blister packs are 25, 30 and 35 μm). Below around 10–15 MHz, the skin depth in aluminium is greater than the thickness of the aluminium foil of a blister pack. As a consequence, below 5 MHz, it would be expected that the presence of the foil in the resonator would have a lossy affect on the performance of the resonator equivalent to the introduction of a resistive load into the circuit. This proves to be the case.

However, measurements at KCL have also shown that the effect is least when the plane of the blister pack is parallel to the direction of the B_1 field, and greatest when the plane of the blister pack is perpendicular to the direction of the B_1 field, as illustrated in Fig. 8, and in the results presented in Tables 3 and 4.

These results show that the preferred orientation at all frequencies for minimum effect is with the plane of the blister pack parallel to the direction of the B_1 field. The easiest way to ensure this is to use a volume resonator of some design into which the blister pack can be "dropped". At the same time, there is the need to maximize filling factor when the sample consists of pills contained within a blister pack. This immediately suggests a design of resonator that matches in proportion a

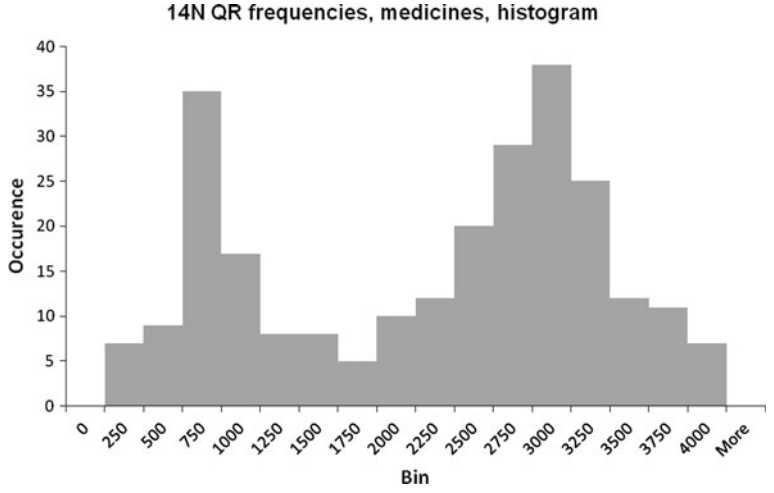


Fig. 7 Histogram showing the distribution of ^{14}N NQR frequencies in 45 different medicines and their polymorphs (data from KCL and courtesy of Josef Stefan Institute and University of Ljubljana, Slovenia)

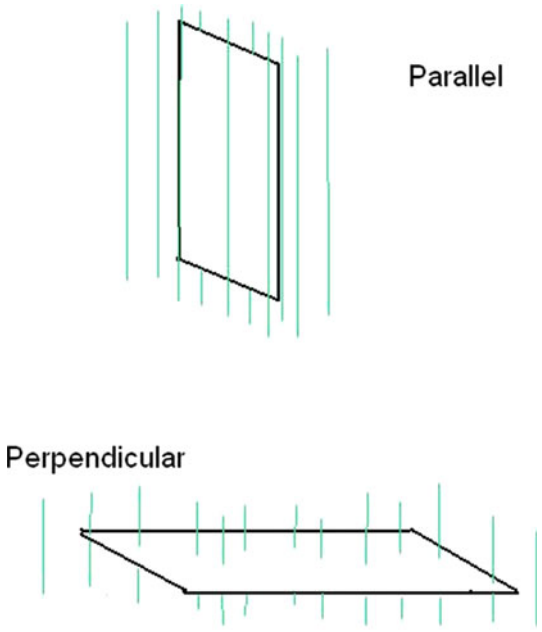


Fig. 8 Two possible orientations of a medicine blister pack with respect to the direction of the B_1 field

Table 3 Measuring the Q of the surface resonators with and without a blister pack present

Type of coil/resonator	Measured quality factor (Q) ± 5		
	Without blister pack	With blister pack[b]	
		Parallel to B_1[a]	Perpendicular to B_1[a]
34 MHz Meander line	100	–	98
3.6 MHz Spiral	67	67	36
1 MHz Spiral	74	–	27

[a] The direction of the B_1 field above a surface coil runs perpendicular to plane of the resonator

[b] Blister pack 125 × 43 × 5 mm; aluminium foil on one side only, thickness of 20–30 μm

Table 4 Measuring the Q of the volume resonators with and without a blister pack present

Type of coil/resonator	Measured quality factor (Q) ± 5		
	Without blister pack	With blister pack	
		Parallel to B_1[a]	Perpendicular to B_1[a]
34 MHz Resonator	107	104	89
1 MHz Resonator	173	116	27

[a] The direction of the B_1 field inside the resonator runs perpendicular to the resonator's cross section

cardboard packet containing one or more blister packs—i.e., a long narrow box. At ^{14}N frequencies, this would suggest a solenoid consisting of wire wound on an appropriate plastic former.

For the ^{14}N NQR work, a solenoid with a rectangular cross section ("RCS solenoid"), pictured in Fig. 9, was constructed to operate between ca. 2 and 4 MHz. The dimensions are as follows: outside diameter (OD) of the enameled copper wire, 0.5; cross section of coil, 120 × 40 mm; height, 85 mm; spacing between turns (wire center to wire center), 4 mm; number of turns, 21; measured inductance (L), 24 μH; and measured Q (unloaded) @ 3.03 MHz, 84. In practice, the tuning range with the 5–125 pF vacuum variable capacitors proved to be 2.4–5.0 MHz, which was adequate for these studies. The B_1 field measurements showed a 38 % drop-off in B_1 field strength from the center to the edges of the coil, but this did not materially affect the measurements. A version of the RCS solenoid with windings of variable pitch to give a more even B_1 field distribution [13, 28] has been constructed and is currently being tested.

This resonator was tested with ampicillin at 3,033 kHz. Packs of ampicillin capsules BP (Kent Pharmaceuticals) in both the 250 and 500 mg capsule size were bought from pharmaceutical wholesalers. For testing the RCS solenoid, the 500 mg capsules were used. A standard pack contains two blister packs, each holding 14 capsules, giving a total of (28 × 500 mg =) 14 g of ampicillin trihydrate—more than adequate for the purposes of these measurements. One of the packs used, opened to show the arrangement of the blister packs inside is illustrated in Fig. 10.

Measurements were carried out with (a) the standard pack, with the capsules held in the blister packs as shown; (b) loose capsules removed from the pack and positioned in the center of the coil; and (c) the emptied pack (i.e., no sample present, but retaining the emptied blister packs and cardboard packaging).

The pulse sequence used for the measurements was a CPMG-type multiple-pulse sequence of the form: $P1_{\pm\phi} - \tau 1 - (-P2_{(\phi+90)} - \tau 2 -)n$, with P1 = P2 = pulse

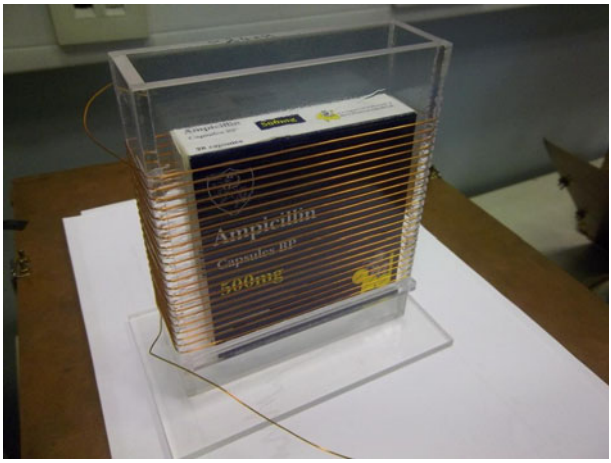


Fig. 9 ^{14}N Rectangular cross section solenoid built to accommodate blister packs inside their packaging and operate between 2 and 4 MHz

Fig. 10 Standard pack of ampicillin 500 mg capsules opened to illustrate the arrangement of the blister packs inside

duration = 70 μs (optimized for the pulse power used), $\tau 1$ = first delay = 155 μs, $\tau 2$ = total time between the pulses in the train = 512 μs, n = number of loops = 64. The receiver deadtime was set to 190 μs and acquisition time 256 μs, thus $\tau 1$ was set such that the peak of the echo coincided with the end of the receiver deadtime, resulting is a "half-echo", rather than the full-echo being acquired, as illustrated in Fig. 11. This signal was then processed with a conventional FT, as if it were an FID decay. The time between scans was 1 s;

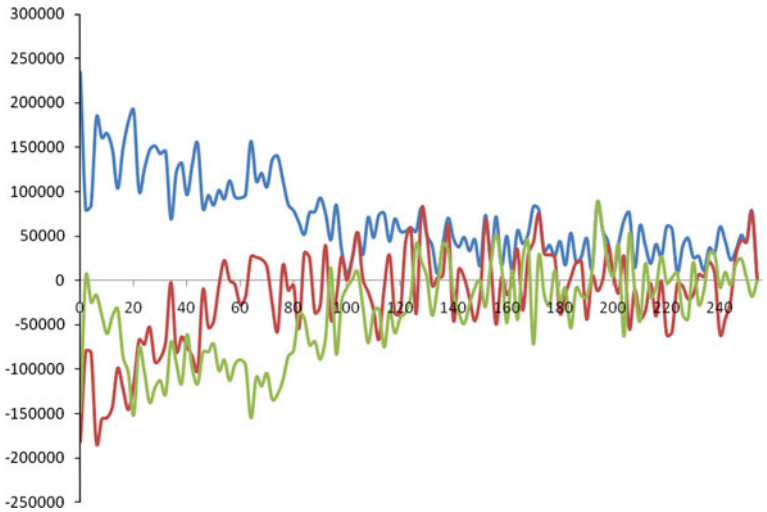


Fig. 11 Time domain ^{14}N NQR "half-echo" response from loose capsules of ampicillin trihydrate placed inside the RCS solenoid. Excitation frequency = 3,036.5 kHz (resonance: 3,033.7 kHz). Timings in the text. *Blue* magnitude, *red* real, *green* imaginary (color figure online)

4,000 scans were acquired and summed for each measurement (a total of 256,000 half-echoes). Returned SNR was ca. 17:1, far above that required in a practical device, but at a level allowing examination of the returned signal by visual inspection. Phase cycling was used to eliminate pulse-breakthrough and other artefacts. Experiments were carried out in a temperature-stabilized room, with the excitation frequency set to 3,036.5 kHz, while the resonant frequency was 3,033.7 kHz. Working off-resonance, while sub-optimal for returned SNR, allowed for an unequivocal assignment of any returned signal to ampicillin.

Figure 12 shows the results of these measurements. It is clear from Fig. 12 that there is no difficultly in acquiring signals from the ampicillin trihydrate in the capsules even when the capsules are held inside blister packs, or in differentiating empty packaging from packaging containing genuine samples. In this experiment, the loose capsules were simply placed together towards the center of the length of the coil, and, so, received a more constant B_1 field than the capsules in the blister packs, which stretched from the top to the bottom of the coil. The reduced SNR for the loose capsules *cf* that for the capsules in the blister packs can be accounted for by the fact that the pulse duration was optimized for the capsules in the blister packs. It is also possible that the metal foil acts to reduce the B_1 field inhomogeneities. However, as the field distribution across the whole of the coil was less than ± 20 %, from a comparison of (b) and (c) it can be said that the signal from the capsules in the blister packs is consistent with all the capsules contributing to the signal, i.e., that there is little or no attenuation of the signal due to eddy currents within the metal foil of the blister pack, even for the case of the capsules that are "sandwiched" between two—unconnected—sheets of metal foil (the top capsules in Fig. 10). This is a significant result, showing that the system is suitable for examining both single blister packs and stacks of blister packs at this frequency.

3.5 Overlapping Lines in Different Materials

Before moving to a summary of the findings of the NQR studies in this first stage of the project, there is one last point to consider in building NQR fingerprints of medicines: cases where NQR lines from different medicines occur at the same frequency at a given temperature (or within the uncertainty of a temperature determination). In the course of measuring line frequencies to produce the data represented in Fig. 7, above, it was revealed that just such a situation arises with ampicillin trihydrate and sulfamerazine at 293 K: both materials have lines at 3,033 kHz at this temperature. The short answer of what to do in such a situation is to build the fingerprints using different lines where there is no overlap, but situations may arise where this is not possible. Table 2, above, shows a comparison of the time constants at 293 K and the temperature coefficients for the two coincidental lines of ampicillin trihydrate and sulfamerazine. The coincidence of the resonant frequencies at this temperature aside, the lines display very different behavior, reflecting the very different environments of the nitrogen in the respective structures and crystals. This at once illustrates a means of discriminating between signals from the two materials even at this frequency—via the use of parametric data processing [21]—and the sensitivity of NQR to changes in structure in solids, something that is further

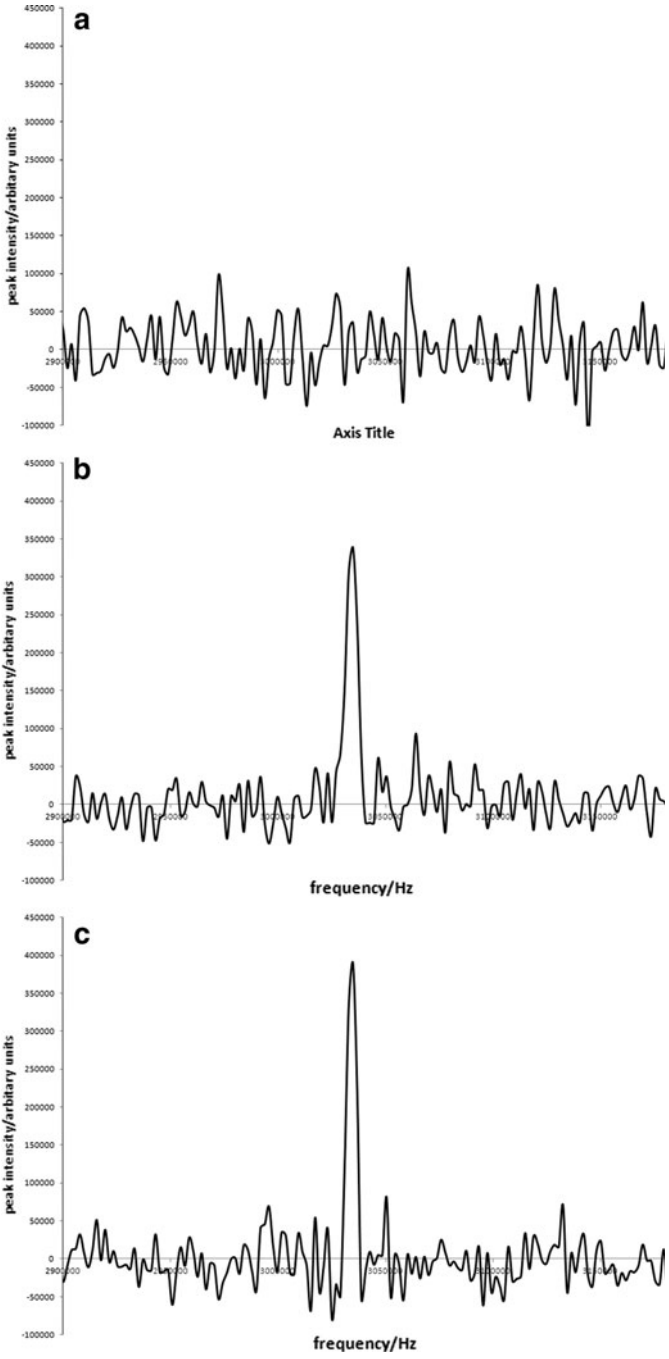


Fig. 12 ^{14}N NQR spectra returned at 3,036.5 kHz using a PSL-type sequence and different samples inside the RCS solenoid illustrated in Fig. 8. Sequence timings given in the text; **a** empty packaging; **b** loose capsules; and **c** Standard pack, with capsules held in blister packs as illustrated in Fig. 9

illustrated in the ability of the technique to discriminate between polymorphs of the same material [13, 16]. This result further suggests that as the database is built up—as the number of coincidental resonant frequencies between lines in different medicines at specific temperatures grows—the technique will retain its powers of discrimination.

4 Conclusions

The findings of the NQR studies in this first stage of the project can be summarized:

a. It is possible to target a wide range of medicines utilizing the tuning range of a single RF resonator and off-the-shelf trimmer capacitors, even at ^{14}N NQR frequencies, opening up the opportunity to establish proof of concept for using NQR as a tool in medicines authentication to be accomplished with the minimum of hardware technical complexity; the bands selected for the project, based on experiment and a study of the literature, are, for ^{14}N: 2.3–3.7 MHz, and for ^{35}Cl: 33.0–37.0 MHz. Note: this does not preclude utilising other frequency bands in a production model device to expand the range of medicines to be authenticated.
b. A useful number of medicines on the WHO model list of essential medicines have NQR lines within the chosen frequency bands, which, again supports the viability of this approach in establishing proof of concept for using NQR as a tool in medicines authentication.
c. At the same time, the frequencies and time constants of the NQR responses of the pharmaceutical compounds studied, despite similarities in structure, are sufficiently separated that they cannot be confused, or contribute to false alarms, provided that multiple characteristics of the NQR responses are compared, rather than relying on line frequency alone.
d. The separation in frequencies and/or other time constants of the pharmaceutical compounds studied is well-defined enough to be exploited by parametric signal processing approaches.
e. There is no technical barrier to acquiring ^{14}N signals from medicines inside pills inside blister packs. This has been demonstrated with measurements made on blister packs containing capsules of the antibiotic ampicillin trihydrate (^{14}N NQR resonance chosen for the measurements: 3,034 kHz) inside a rectangular cross section solenoid.

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