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The use of heteronuclear multiple quantum spectra in the automatic analysis of NMR spectra of samples dissolved in liquid crystalline phases

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For spin systems with N^I and N^S interacting nuclei of magnetogyric ratio γ_I and γ_S respectively, it is possible to obtain multiple quantum spectra characterized by changes in the magnetic numbers m^X by $N^I, (N^I - 1), \dots, 0$ with $X = I, S$. The use of such heteronuclear MQ spectra as an aid in the automatic analysis of NMR spectra of both rigid and flexible molecules dissolved in liquid crystalline phases, is discussed. It has been found that when $I = {}^1\text{H}$ and $S = {}^{19}\text{F}$, the decoupling efficiency of the standard multipulse sequences, developed and optimized for heteronuclear decoupling in isotropic liquids, depends on the spin system and also on the overall orientation of the molecule when orienting mesophases are involved. These facts impose limitations on the applicability of a procedure which, when the decoupled heteronuclear MQ spectrum is obtained, significantly reduces the computing requirements of the automated spectral analysis.

1. Introduction

Multiple quantum NMR spectroscopy involving changes of the magnetic quantum number $|\Delta m| = N, (N - 1), (N - 2), \dots, 0$ for N spin $1/2$ interacting nuclei [1] has proved its utility as an aid in the analysis of very complex anisotropic ${}^1\text{H}$ SQ spectra [2]. The advantages stem from the fact that for a given spin system, MQ spectra contain very few lines with respect to the SQ spectrum and so it is quite easy to find parameter sets which fit the MQ frequencies. This can be done either by standard procedures or by an automatic procedure [3(a, b)] which (i) assigns lines to the $(N - 1)$ quantum refocused spectrum of an N spin $1/2$ system and (ii) refines and/or discards parameter sets using lower order MQR spectra. The correct assignment can be found using a *permutative* approach in which all the possible assignments of the k experimental lines to the calculated lines are tried. Such an approach requires a factorial function of k possible assignments. Even with modern computers, the CPU time required is close to being prohibitive. For this reason, the method can only

be applied to the analysis of $(N - 1)$ or, in the most favourable conditions, $(N - 2)$ Q spectra. On the other hand, for $N \geq 9$, spectral sensitivity and instrumental time can hinder the possibility of obtaining 7 or higher order MQ spectra even at moderately high fields; this fact reduces the application potential of the method [3(c)].

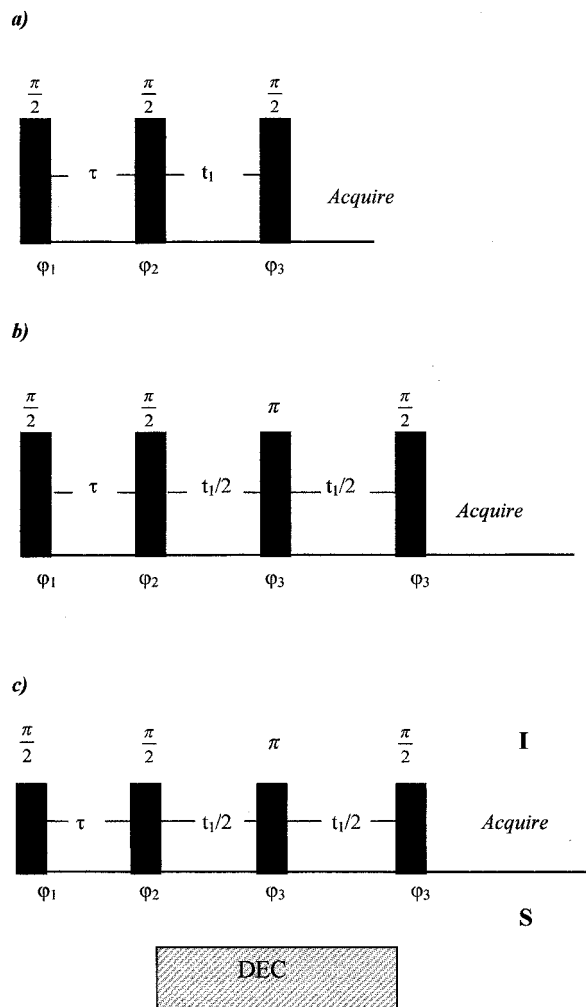
Heteronuclear multiple quantum (HMQ) spectroscopy [4] could offer an important advantage over the purely homonuclear case since it is possible to obtain all the spectral parameters by a suitable choice of lower order MQ spectra without having to resort to massive CPU time. Defining N^I and N^S , the number of nuclei I (typically ${}^1\text{H}$, the observed nucleus) and S, of magnetogyric ratios γ_I and γ_S respectively, it is convenient to introduce the quantity n^X defined as $n^X = n_{ij}^X = m_i^X - m_j^X$, with $X = I, S$ where m_i^X is the eigenvalue of $F_z^X = \sum N^X I_z^X$. Since m_i^I and m_i^S are *good* quantum numbers, it can be shown [4] that the $(n^S = 0, n^I = N^I)$ HMQ frequencies depend only on the heteronuclear spin-spin interactions D_{IS} and J_{IS} (the direct and indirect couplings, respectively) whereas the $(n^S = 0, n^I = N^I - p)$ HMQ spectra, with $p = 1, 2, \dots$, will comprise lines whose frequencies depend on both homo- and hetero-nuclear couplings and

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on chemical shifts. Since nuclei with different γ values are observed separately, some gain can be achieved (there are fewer transitions in, for example, a ^1H HMQ spectrum of a heteronuclear spin system than in the same order MQ spectrum of an equivalent, purely homonuclear case), but such gain is not enough since for $p = N^S$, or higher, the number of transitions is still so high that the permutative procedure cannot be applied. The case in which hetero-decoupling is applied to HMQ spectra would be more interesting since the spectra will depend only on the homonuclear dipolar interactions and thus contain fewer transitions.

Many pulse sequences have been developed to improve the efficiency of heteronuclear decoupling in liquids; for example, decoupling sequences based on composite pulses with phase-modulation, such as WALTZ [5], GARP [6] and DIPSI [7], have proved to be very successful. These broadband decoupling sequences were specifically designed for isotropic liquids, and so their application in heteronuclear decoupling for liquid crystals is far from satisfactory mainly because of the existence of large dipolar interactions in these systems. In principle, the simplest method of heteronuclear decoupling is to apply a high power continuous wave (CW) radio-frequency irradiation whose field must increase with increasing strength of the homonuclear and heteronuclear interactions present in the ordered media. Unfortunately, the heating caused by the required high RF power introduces thermal gradients in liquid crystalline samples; the ensuing broadening of the outer lines in the high resolution spectra makes the SQ spectra unmanageable even if the magnitude of the dipolar interactions is significantly smaller than in the solid.

Alternatively, by adding a π pulse in the centre of the t_1 period of the pulse sequence shown in figure 1 [8, 3(b)], ^1H spin-echo HMQ spectra can be obtained; not only chemical shifts, but in some cases heteronuclear spin-spin interactions also, can be removed. The latter was the case for 3-phenylpro-1-yne [3(b)] and ethylbenzene [3(c)] for which the analysis of the SQ spectra was possible only after having analysed the ^1H spin-echo HMQ spectra of deuterated isotopomers. The crucial point of the procedure is the effectiveness of the heteronuclear decoupling as evident in the case of $\beta\beta\beta$ -trideuteroethylbenzene (for which the decoupling is effective) and of $\alpha\alpha$ -dideuteroethylbenzene (for which the decoupling is not effective). In both cases the S nuclei are deuterium nuclei which have a relatively low magnetogyric ratio ($\gamma_{^1\text{H}}/\gamma_{^2\text{H}} \cong 6$); D_{HD} and D_{DD} can be small and have very similar values and so the decoupling is likely to be effective (see the following paragraph). When S are ^{19}F nuclei the situation is more troublesome, since in this case, due to the larger D_{HF} and D_{FF} couplings (now



DEC = WALTZ-16, GARP, DIPSI

Figure 1. Pulse sequences used to obtain: (a) ($n^S = 0, n^I$) heteronuclear multiple quantum spectra; (b) ($n^S = 0, n^I$) ^1H spin-echo heteronuclear multiple quantum spectra; (c) heteronuclear multiple quantum spectra decoupled using a multipulse broadband decoupling sequence with chemical shift refocusing. Since $n^S = 0$, no pulse is applied on the S channel—not reported for case (a) and (b). I refers to the observed nucleus.

$\gamma_{^{19}\text{F}}$ is very similar to $\gamma_{^1\text{H}}$) the decoupling efficiency of this method is expected to be very low. Thus, more complex spectra will be obtained in which the transition frequencies will depend in a complex way on homo- and hetero-nuclear couplings. On the other hand, an increasing number of fluorinated molecules have been synthesized and tested for mesogenic properties [9]; knowing the full set of dipolar couplings from the analysis of their SQ spectra, conformational equilibria present in the molecules could be studied and the interplay between shape-dependent short range and long range electrostatic interactions investigated [9(a, b)].

In this paper, results from five fluorinated molecules (1,2-dichloro-4-fluoro-5-nitrobenzene **I**, fluorobenzene **II**, 1,2-difluorobenzene **III**, α,α,α -trifluoromethoxybenzene **IV** and α,α,α -trifluoromethylbenzene **V** dissolved in a nematic solvent are presented. The efficiency of the spin-echo MQ and of some decoupling pulse sequences in removing the coupling with non-resonant nuclei is discussed and the difficulties and limitations which arise when decoupling is not effective are highlighted.

2. Heteronuclear multiple quantum spectra

The Hamiltonian describing the heteronuclear spin system when $I_I = I_S = 1/2$, can be written as

$$H = H_Z^I + H_Z^S + H_I^{II} + H_I^{SS} + H^{IS}. \quad (1)$$

Here, the first and second terms represent the Zeeman interactions of nuclei I and S with the external magnetic field; the third and fourth terms express the dipolar (direct and indirect) interactions within nuclei I and S respectively; the last term

$$H^{IS} = - \sum_i^{N^I} \sum_j^{N^S} 2T_{ij} I_{z_i}^I I_{z_j}^S \quad (2)$$

with $T_{ij} = 1/2(J_{ij} + 2D_{ij})$ accounts for the heteronuclear couplings. Note that H^{IS} does not contain *flip-flop* operators; and thus, the total magnetic quantum number of each species is a good quantum number to describe the free evolution of the spin system. The implications of this are far reaching since (i) the density matrix operator ρ describing the spin system at time τ can be written as $\rho(\tau) = \sum_{n^I} \sum_{n^S} \rho_{n^I n^S}(\tau)$, and (ii) $[I_{z_i}^I; \rho_{n^I n^S}] = n^I \rho_{n^I n^S}$ and $[I_{z_j}^S; \rho_{n^I n^S}] = n^S \rho_{n^I n^S}$. Thus it is possible to separate spectra characterized by each pair of indices (n^S, n^I) by properly setting pulse phase shifts in the pulse sequences of figure 1 [4]. If $N^S = 1$ (cases **I** and **II**), equation [1] reduces to $H = H_Z^I + H_I^{II} + H^{IS}$.

2.1. HMQ spectra

Spectra characterized by $n^S = 0$ and by the highest values of n^I should give: (i) for $n^I = N^I$, one pair of lines with splitting $2|\sum_i T_{iS}|$; (ii) for $n^I = (N^I - 1)$, k pairs of lines with splitting proportional to a linear combination of all but one of the IS couplings (k depends on the number and symmetry of the I sub-spin system). The m independent T_{iS} couplings, sign included, can be determined by the m different splitting observed in the spectrum.

Molecule **I** (a three spin ABX system) is the simplest heteronuclear $^1H/^{19}F$ case in which HMQ coherences can be excited and the evolution of the system under the effect of RF(α, φ) pulses (with α the tip angle and φ the phase of the pulse) and the free precession Hamiltonian can be calculated analytically. Full density

matrix calculations have been performed following the pulse sequence in figure 1(a) (no pulses on S since $n^S = 0$) and the results experimentally verified. HMQ spectra were obtained using the pulse sequence in figure 1(a).

2.2. Spin-echo HMQ spectra

Adding a π pulse in the middle of the time interval t_1 of figure 1(a), 1H spin-echo heteronuclear multiple quantum spectra can be recorded. Heteronuclear decoupling will occur if $[H', H] = 0$ where $H' = -H_Z^I + H_I^{II} - H^{IS}$. The condition will occur (i) in the weak coupling limit when chemical shift differences for nuclei I greatly exceed the spin couplings, or (ii) when S and I spins form fully magnetically equivalent sets, i.e. the same chemical shifts and identical heteronuclear couplings. In practice, neither condition is exactly fulfilled for anisotropic spectra; however, when the heteronuclear couplings with non-isochronous I nuclei subsets are identical, H_I^{II} will nearly commute with H_Z^I and H^{IS} even if the I nuclei do not form a fully magnetically equivalent set. The decoupling will also be effective in this case. If $[H', H] \neq 0$, a more complex analysis is needed and heteronuclear couplings will affect the spin-echo spectrum [8(a, b)]. This behaviour is well known and has been investigated for 1H spin-echo single quantum spectra [8(c, d)]; in this paper, the analysis will be extended to multiple quantum cases.

Not so well known are the effects which derive from the presence of the H^{SS} term. For molecule **I**, the two quantum coherences calculated when $n^S = 0$ at t_1 with the pulse sequence in figure 1(a), are expressed by the elements $\sigma_{16}(t_1) = -C_{16} \exp(-2i\varphi E_{16} t_1)$ and $\sigma_{38}(t_1) = -C_{38} \exp(-2i\varphi E_{38} t_1)$ where the coefficients C_{ij} depend on the spectral parameters and time delay τ . After a few more elementary but cumbersome calculations, the density matrix reported in figure 2 was obtained at t_1 with the pulse sequence in figure 1(b) when $n^S = 0$. It is important to notice that each element of the density matrix depends only on the parameters T_{HH} ; hence, at least for an AMX spin system, the pulse sequence in figure 1(b) can produce effective decoupling from the X nucleus.

2.3. $\{^{19}F\}$ - 1H HMQ spectra

A low power broadband decoupling pulse sequence can be used as an alternative and more efficient way than the soft refocusing π pulse to remove the heteronuclear interactions. We have performed a series of experiments introducing different decoupling sequences such as WALTZ-16, DIPSI and GARP during the MQ evolution time t_1 , while the π pulse on the protons will only refocus the chemical shift difference. We will refer to such spectra obtained using the pulse sequence of figure 1(c) by the acronym HMQR (R for refocused).

$$\sigma(t_1) = \begin{pmatrix} C_{11} & -iC_{12}\exp(i\varphi)\exp(-iT_{\text{int}}t) & 0 & -iC_{14}\exp(i\varphi)\exp(-iT_{\text{int}}t) & 0 & C_{16}\exp(2i\varphi) & 0 & 0 \\ iC_{21}\exp(i\varphi)\exp(iT_{\text{int}}t) & C_{22} & 0 & -C_{24} & 0 & iC_{26}\exp(i\varphi)\exp(iT_{\text{int}}t) & 0 & 0 \\ 0 & 0 & C_{33} & 0 & -iC_{35}\exp(i\varphi)\exp(-iT_{\text{int}}t) & 0 & -iC_{37}\exp(i\varphi)\exp(-iT_{\text{int}}t) & C_{38}\exp(2i\varphi) \\ -iC_{41}\exp(-i\varphi)\exp(iT_{\text{int}}t) & C_{42} & 0 & C_{44} & 0 & -iC_{46}\exp(i\varphi)\exp(-iT_{\text{int}}t) & 0 & 0 \\ 0 & 0 & iC_{53}\exp(i\varphi)\exp(iT_{\text{int}}t) & 0 & C_{55} & 0 & -C_{57} & iC_{58}\exp(i\varphi)\exp(iT_{\text{int}}t) \\ -C_{61}\exp(-2i\varphi) & -iC_{62}\exp(-i\varphi)\exp(-iT_{\text{int}}t) & 0 & -iC_{64}\exp(i\varphi)\exp(-iT_{\text{int}}t) & 0 & C_{66} & 0 & 0 \\ 0 & 0 & -iC_{73}\exp(-i\varphi)\exp(iT_{\text{int}}t) & 0 & C_{75} & 0 & C_{77} & iC_{78}\exp(i\varphi)\exp(iT_{\text{int}}t) \\ 0 & 0 & -C_{83}\exp(-2i\varphi) & 0 & -iC_{85}\exp(-i\varphi)\exp(-iT_{\text{int}}t) & 0 & -iC_{87}\exp(i\varphi)\exp(-iT_{\text{int}}t) & C_{88} \end{pmatrix}$$

Figure 2. Density matrix elements calculated at time delay t_1 of pulse sequence of figure 1 (b) for an ABX spin system.

3. Experimental

The following solutions were prepared and HMQ, ^1H spin-echo and $\{^{19}\text{F}\}$ - ^1H MQR spectra recorded:

- (i) For a 10 wt % 1,2-dichloro-4-fluoro-5-nitrobenzene (Aldrich) solution in ZLI1132 (Merck) the H2Q (**Ia**), H0Q (**Ib**) and ($n^S = 0, n^I = 1$) ^1H spin-echo HMQ (**Ic**) spectra were recorded. The total acquisition time was 1 h 50 min for each experiment. The quantum order was selected using the pulsed field gradient technique [10].
- (ii) For a 10 wt % fluorobenzene (Aldrich) solution in ZLI1132 the H5Q (**IIa**), H4Q (**IIb**), ($n^S = 0, n^I = 4$) ^1H spin-echo HMQ (**IIc**) and the $\{^{19}\text{F}\}$ - ^1H H4QR (**IIId**) spectra were recorded. The acquisition time was 13 h for **IIa**, 10 h 40 min for **IIb** and **IIc**, and 6 h 20 min for **IIId**. The quantum order was selected using the phase cycling [11] and PFG techniques. No significant differences in spectrum quality were observed. The times given refer to the phase cycling selection experiments.
- (iii) For a 10 wt % 1,2-difluorobenzene (Aldrich) solution in ZLI1132, the H4Q (**IIIa**), H3Q (**IIIb**), ($n^S = 0, n^I = 3$) ^1H spin-echo HMQ (**IIIc**) and the $\{^{19}\text{F}\}$ - ^1H H3QR (**IIIId**) spectra were acquired. The acquisition time was 5 h for **IIIa**, 4 h for **IIIb** and **IIIc**, and 3 h 15 min for **IIIId**, using the phase cycling technique to select the desired quantum order. For this sample the ^{19}F SQ (**IIIe**) and H2Q (**IIIIf**) spectra were also recorded.
- (iv) For a 10 wt % α,α,α -trifluoromethoxybenzene (Aldrich) solution in ZLI1132, the H5Q (**IVa**), H4Q (**IVb**), ($n^S = 0, n^I = 4$) ^1H spin-echo HMQ (**IVc**) and the $\{^{19}\text{F}\}$ - ^1H H4QR (**IVd**) spectra were acquired. For this sample the ^{19}F SQ (**IVe**) and H3Q (**IVf**) spectra were also recorded.
- (v) For a 10 wt % α,α,α -trifluoromethylbenzene (Aldrich) solution in ZLI1132, the H5Q (**Va**), H4Q (**Vb**), ($n^S = 0, n^I = 4$) ^1H spin-echo HMQ (**Vc**) and the $\{^{19}\text{F}\}$ - ^1H H4QR (**Vd**) spectra were acquired. For the last two cases the same instrumental time as in case (ii) was needed. Only the

phase cycling technique was used. For this sample the ^{19}F SQ (**Ve**) spectrum was also recorded.

SQ spectra of each solution were acquired between HMQ experiments to be sure that experimental conditions did not change and that spectral parameters could be safely transferred amongst different spectra of the same sample.

Spectra at different values of τ were recorded routinely in order to obtain higher S/N ratios and to identify and discard artifacts due to hardware imperfections. The HMQ spectra were obtained as skyline projections on the F1 dimension of the 2D data set. All the spectra (**a–c**) were recorded on a 7.04 T Bruker AC300 spectrometer. Spectra (**d–f**) were recorded on a 7.04 T Bruker DRX using a $^1\text{H}/^{19}\text{F}/^{13}\text{C}$ three channel probe. Spectra **d** were obtained using the WALTZ-16, DIPSI and GARP multipulse sequences as low power broadband heteronuclear decoupling sequences to decouple the ^{19}F nuclei; no high power pulses were needed, avoiding the complications due to thermal dishomogeneities in the sample.

4. Results and discussions

4.1. Sample I

As predicted by the theory, heteronuclear interactions are effectively decoupled for sample **I** and the spin echo spectrum **Ic**, figure 3 (b), contains two lines whose frequencies depend only on the homonuclear T_{HH} coupling. As expected, the H2Q spectrum **Ia**, figure 3 (a), and H0Q spectrum **Ib**, show two lines whose frequencies depend on linear combinations of heteronuclear couplings which may be easily verified knowing the spectral parameters from the analysis of the SQ spectrum ($T_{\text{AB}} = |108.7| \text{ Hz}$, $T_{\text{AX}} = -1949.9 \text{ Hz}$, $T_{\text{BX}} = -58.1 \text{ Hz}$; A and B are the ^1H nuclei, X the ^{19}F nucleus, the sign of T_{AX} is assumed by analogy with similar cases; the sign of T_{BX} is determined by the intensity pattern in spectrum **Ia**; the SQ spectrum is not sensitive to the sign of T_{AB}). **Ia**, **Ic** and the SQ spectra are shown in figure 3.

4.2. Sample II

The spin-echo spectrum of the fluorobenzene sample **IIc**, figure 4 (c), contains three pairs of lines as expected

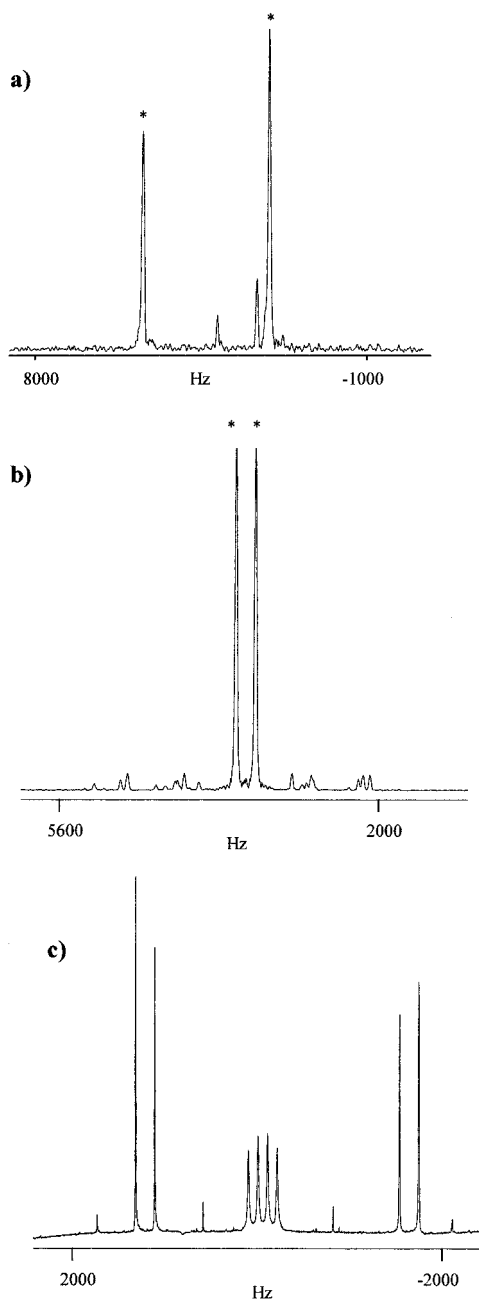


Figure 3. The 300 MHz ^1H spectra of a sample of 1,2-dichloro-4-fluoro-5-nitrobenzene (**I**) dissolved in the nematic solvent ZLI1132: (a) H2Q, (b) ($n^s = 0, n^l = 1$) ^1H spin-echo HMQ and (c) SQ spectra. The MQ spectra were acquired with 512 increments, 5 kHz in F_1 , $\tau = 10$ ms; 4 scans. The real lines in (a) and (b) are marked with * to distinguish them from extra lines arising from imperfect pulses.

from theory if the heteronuclear decoupling is effective. The presence of some additional lines, which we were unable to characterize unambiguously as artifacts, makes us aware that in this case the decoupling may be incomplete. Whereas two pairs of lines—marked by a star in figure 4(c)—are easily recognized as being real,

some doubts remain about the identification of the third pair, since the intensity behaviour at different τ does not allow a definite identification between the two pairs of lines, quite close in frequency and marked by + and – in figure 4(c).

This notwithstanding, we arbitrarily started spectral analysis using an automatic procedure already tested to analyse the spectra of rigid molecules [3(a)]. The procedure is based on a permutative approach which tests over all the distinct assignments of experimental to calculated lines of the MQ spectrum and uses, as iterative parameters, the elements of the Saupe order matrix to calculate direct dipolar couplings from an assumed geometry. Those parameters, and chemical shifts, are then used as starting parameter sets of a modified version of DANSOM [12(a)] for a fully automated analysis of the SQ spectrum. In our case, having assumed a regular hexagonal geometry for the aromatic ring, there are two order parameters versus three independent frequencies once the choice is made between the two pairs of lines. Since the difference in frequency is small, we do not expect a significant difference in the spectral parameter values; this hypothesis was verified using both possible choices in two parallel runs. The procedure took less than 5 min of CPU time for each run on a Digital Server 2000ALPHA_AXP computer to give just one solution for each run with a low RMS, table 1 (column a). The lower RMS was obtained when the frequencies marked + were used. Heteronuclear spin–spin couplings and chemical shifts were determined from the frequencies of H4Q and H3Q spectra, figures 4(a) and 4(b), using the heteronuclear direct coupling calculated from an assumed geometry and the isotropic chemical shifts as starting parameters. Assigning the calculated to the nearest experimental frequencies, the values reported in table 1 (column a) were calculated in a single iterative run. The spectral parameters which reproduce the SQ spectrum, obtained from DANSOM with the parameters in table 1 (column a) as starting values, are also reported in table 1 (column b).

The correspondence between the two sets is remarkable but not as good as the agreement found for a similar case (bromobenzene) reported in [3(a)]. The observed differences could be due to incorrectly assumed geometries or indirect spin–spin couplings, but in our opinion the most likely cause is incomplete decoupling. To verify the latter hypothesis, the frequencies of the spin echo spectrum **Ic** were reproduced using the D_{HH} in table 1 (column b): the differences between the calculated and experimental frequencies range from 140 Hz (outer lines) to 40 Hz (inner lines). Since we have recorded SQ spectra before and after each MQ spectrum, and observed differences in frequencies only within the linewidth (≈ 3 Hz), this clearly shows that the decoupling

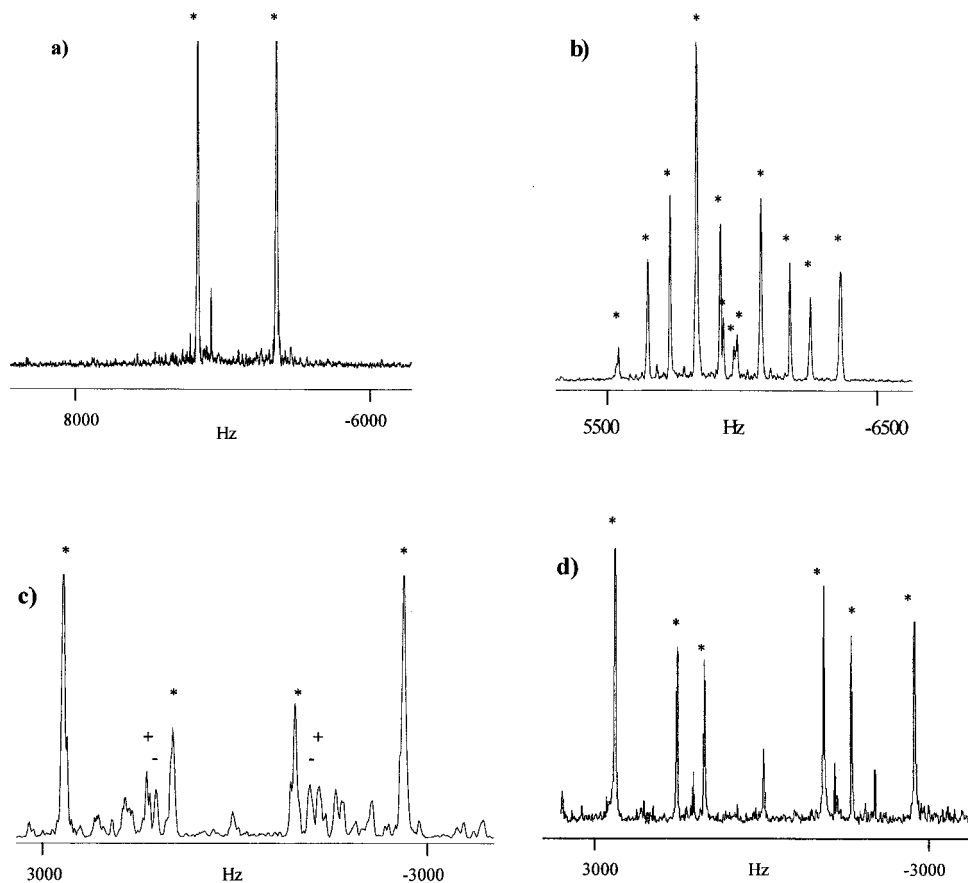


Figure 4. The 300 MHz ^1H spectra of a sample of fluorobenzene (**II**) dissolved in the nematic solvent ZLI1132: (a) H5Q, (b) H4Q, (c) ($n^s = 0, n^l = 4$) ^1H spin-echo HMQ, (d) $\{^{19}\text{F}\}$ - ^1H H4QR spectra. All the spectra were acquired with 1024 increments, 10 kHz in F_1 , $\tau = 20$ ms; 20 scans for (a), 16 scans for (b), (c) and (d). Spectrum (d) was obtained using WALTZ16 with $\tau = 13.4$ ms. The real lines in (a), (b) and (d) are marked with * to distinguish them from extra lines arising from imperfect pulses. See the text for explanations of - and + in (c).

is incomplete. However, from the spin-echo spectrum it is still possible to obtain a reasonably good set of spectral parameters to start the analysis of the SQ spectrum, as shown by the relatively mild smoothing conditions used in DANSOM. The features of DANSOM are described elsewhere [12(a-d)]; here it is sufficient to say that when the starting parameter set differs in a significant way from the correct final values, heavy smoothing functions have to be used in the hope that the algorithm will find the absolute minimum. Thus the reproduction of the SQ spectrum within a few iterative cycles, and using a light smoothing function, is strong proof of our assertion.

Further experimental evidence that in the ^1H spin-echo spectrum the decoupling is effective, comes from the $\{^{19}\text{F}\}$ - ^1H H4QR spectrum **IId**, figure 4(d). Only three pairs of lines are present and their frequencies correspond to the calculated ones.

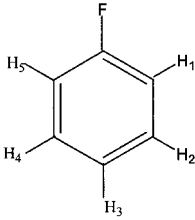
In the meantime, it is important to stress that using the spin-echo HMQ spectrum, or the broadband decoupled

HMQ spectrum only $P = 2^{k/2}/2$ ($k/2$)! = 12 permutations (k is the number of lines in the spectrum) are needed, whereas, if all twelve lines in the H4Q spectrum of **II** had to be used, a total of $p_k = 12! \approx 479\,001\,600$ permutations would have been performed!

4.3. Sample III

The ^1H spin-echo spectrum of **III** (there are two symmetrically equivalent ^{19}F whose coupling value is expected to be quite high) contains more than seven pairs of symmetric lines against the two expected for an $AA'BB'XX'$ spin system, figure 5(c). The spin-echo decoupling is clearly ineffective. As a result, the ^{19}F decoupled H3Q spectrum **IIIId**, figure 5(d), has been acquired and used to determine the homonuclear couplings. Applying the permutative approach described previously to the frequencies of spectrum **IIc** (two independent values versus two order parameters) and using the H4Q, figure 5(a), and H3Q figure 5(b) spectra, the full set of spectral parameters has been determined

Table 1. Chemical shifts, Δv_i , and dipolar couplings, D_{ij} , obtained from the analysis of the H5Q (**IIa**), H4Q (**IIb**), ($n^S = 0, n^I = 4$) ^1H spin echo HMQ (**IIc**) spectra (column a) and the SQ (column b) 300 MHz spectrum of a sample of fluorobenzene (**II**) dissolved in the nematic solvent ZLI1132.



i, j	D_{ij}/Hz		J_{ij}/Hz^a
	a ^b	b	
1,2	-1192.8	-1147.88 ± 0.06	8.36
1,3	-208.8	-207.20 ± 0.11	1.07
1,4	-108.9	-112.34 ± 0.06	0.43
1,5	-147.1	-155.28 ± 0.11	2.74
2,3	-871.3	-883.39 ± 0.07	7.47
2,4	-147.1	-153.75 ± 0.11	1.82
1,F	-747.5	-764.42 ± 0.10	8.98
2,F	-177.1	-173.95 ± 0.11	5.69
3,F	-125.98	-121.98 ± 0.12	0.22
$(v_1 - v_3)/\text{Hz}$	43.18	-39.72 ± 0.09	
$(v_2 - v_3)/\text{Hz}$	40.80	30.25 ± 0.09	

^a Taken from the analysis of fluorobenzene in isotropic solution and kept fixed.

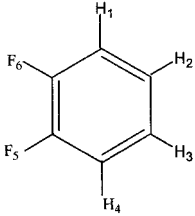
^b Values obtained from MQ spectra when the pair of frequencies marked ⁺ in figure 4(c) was used as described in the text.

with the exception of the D_{FF} coupling whose value was estimated by scaling D_{23} by the factor $(\gamma_{\text{F}}/\gamma_{\text{H}})^2$ and reducing this value by 10% to take into account the longer CF bond. The ^1H and ^{19}F SQ spectra were analysed easily by DANSOM iterating on all the chemical shifts and direct couplings. Table 2 shows the spectral parameters obtained from the analysis.

4.4. Samples IV and V

In the ^1H spin-echo spectrum of sample **IV**, figure 6(c), more than the six lines expected for an $AA'BB'C$ spin system are present; the spin-echo sequence is clearly ineffective as a decoupling technique. Also in the ^{19}F decoupled H4QR spectrum, **IVd**, there are more lines than the three pairs expected; in particular we were unable to identify unambiguously which of the three lines indicated by \ominus is the real one since their relative intensities do not change with τ , figure 6(d). In contrast, the pair marked by \oplus is readily identified since at slightly different τ values their relative intensities invert, figure 6(d). Thereafter, using the frequency of the central

Table 2. Chemical shifts, Δv_i , and dipolar couplings, D_{ij} , obtained from the analysis of the 300 MHz ^1H SQ and 282 MHz ^{19}F SQ spectra of a sample of 1,2-difluorobenzene (**III**) dissolved in the nematic solvent ZLI1132.



i, j	J_{ij}/Hz^a	D_{ij}/Hz
12	8.32	-972.79 ± 0.07
13	1.62	-163.26 ± 0.09
14	0.26	-97.68 ± 0.14
15	5.80	-136.98 ± 0.09
16	9.00	-834.39 ± 0.09
23	7.62	-767.26 ± 0.17
25	0.22	-104.37 ± 0.08
26	5.8	-170.51 ± 0.11
56	-20.22	-547.89 ± 0.07
$(v_1 - v_2)/\text{Hz}$	18.03 ± 0.12	

^a Taken from the analysis of 1,2-difluorobenzene in isotropic solution and kept fixed.

line, and following the steps described for case **II**, the ^1H and ^{19}F SQ spectra of trifluoromethoxybenzene were analysed readily. Since we are dealing with a flexible molecule, the procedure to calculate the T_{HF} couplings from spectra **IVa** and **IVb** (respectively the 5 and 4 quantum spectra) is more complex, and an approach derived from that described by Castiglione *et al.* [3(b)] for the automatic analysis of flexible molecules has to be followed. No conformational equilibrium is considered and the molecule is frozen in a single conformation, in our case the one in which the oxygen atom lies in the plane of the aromatic ring. From the order parameters calculated from the homonuclear dipolar couplings, assuming a regular hexagonal geometry for the phenyl moiety, the D_{HF} couplings were estimated and the frequencies of the H4Q spectrum calculated. In this case, the simple criterion of assigning calculated to the nearest experimental frequencies could result in false minima; on the other hand, and as previously stressed, the full permutative approach applied to the 18 frequencies identifiable as real lines in spectrum **IVb**, figure 6(b), is clearly unrealistic. However, frequencies can be assigned following two different strategies: (a) by assigning to the nearest experimental frequency if no other calculated or experimental frequency is found within a fixed interval (arbitrarily chosen to be 100 Hz), or (b) by computing all the possible permutations P_S of the calculated

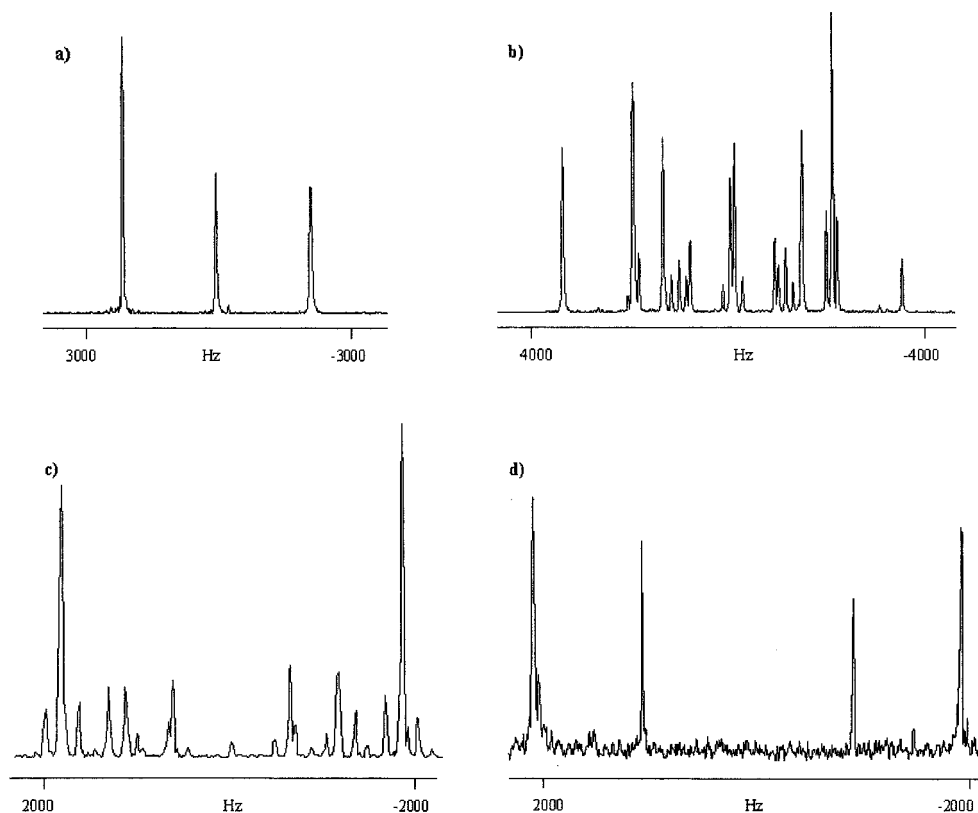


Figure 5. The 300 MHz ^1H spectra of a sample of 1,2-difluorobenzene (**III**) dissolved in the nematic solvent ZLI1132: (a) H4Q, (b) H3Q, (c) ($n^s = 0, n^l = 3$) ^1H spin-echo HMQ, (d) $\{^{19}\text{F}\}$ - ^1H H3QR spectra. All the spectra were acquired with 512 increments, 7 kHz in F_1 , $\tau = 21.2$ ms; 16 scans for (a), 12 scans for (b), (c) and (d). Spectrum (d) was obtained using WALTZ16 with $\tau = 8.4$ ms.

versus the experimental values. If $S = R$ (S and R are the numbers of experimental and calculated frequencies within a given interval, respectively) then $P_S = S!$ permutations will be calculated; if $S < R$ then $P_{R,S} = R(R-1)(R-2)\dots(R-S+1)$. Solutions with high RMS and/or poor assignments obtained by iterating on the spectral parameters can be rejected. If no solution is found, a larger interval value can be fixed and the calculations repeated. In our case, iterating on the D_{HF} couplings and chemical shifts, a solution with a low RMS and good assignments was found immediately. The HMQ spectra recorded do not depend on the D_{FF} coupling: a rough estimate of the D_{FF} coupling from the ^{19}F SQ spectrum was used as starting value for the analysis of the ^1H spectrum taking advantage of the fact that ^1H frequencies show only a small dependence on this coupling. The final values obtained from the analysis of the SQ ^1H and ^{19}F spectra are reported in table 3 (column a).

It is important to point out that, whereas for cases **II** and **III** no difference in decoupling efficiency was observed among the multipulse sequences used, the ^1H 4QR spectrum of trifluoromethoxybenzene (**IVd**) was

obtained using only DIPSI and, as discussed previously with a low decoupling efficiency. What is more, we never succeeded in obtaining the ^{19}F decoupled H4QR spectrum of trifluoromethylbenzene whatever the multipulse sequence used. Since cases **IV** and **V** share the same spin system, the observed behaviour is quite surprising and can be attributed only to a different magnitude of the dipolar couplings. Only after several attempts, mainly due to a lack of first order features in the ^1H spectrum usually present in spin systems containing nuclei with different γ values (the 1:3:3:1 quadruplets expected in the ^1H SQ spectrum shown in figure 7(a) are barely recognizable), the ^1H and ^{19}F SQ spectra of **V** were analysed using the standard Castellano Bothner-By approach [2(a)] and the values of the spectral parameters are reported in table 3 (column b). The D_{FF} for **V** proves to be nearly an order of magnitude greater than the intrafluorine coupling in **IV** and twice as much as the D_{FF} coupling in **III**. In our experience the high values of the D_{FF} coupling contributes strongly to the absence of first order features in the ^1H SQ spectra, and also produces, as an obvious consequence, very wide ^{19}F SQ spectra (\cong nearly 9000 Hz for **Ve** versus

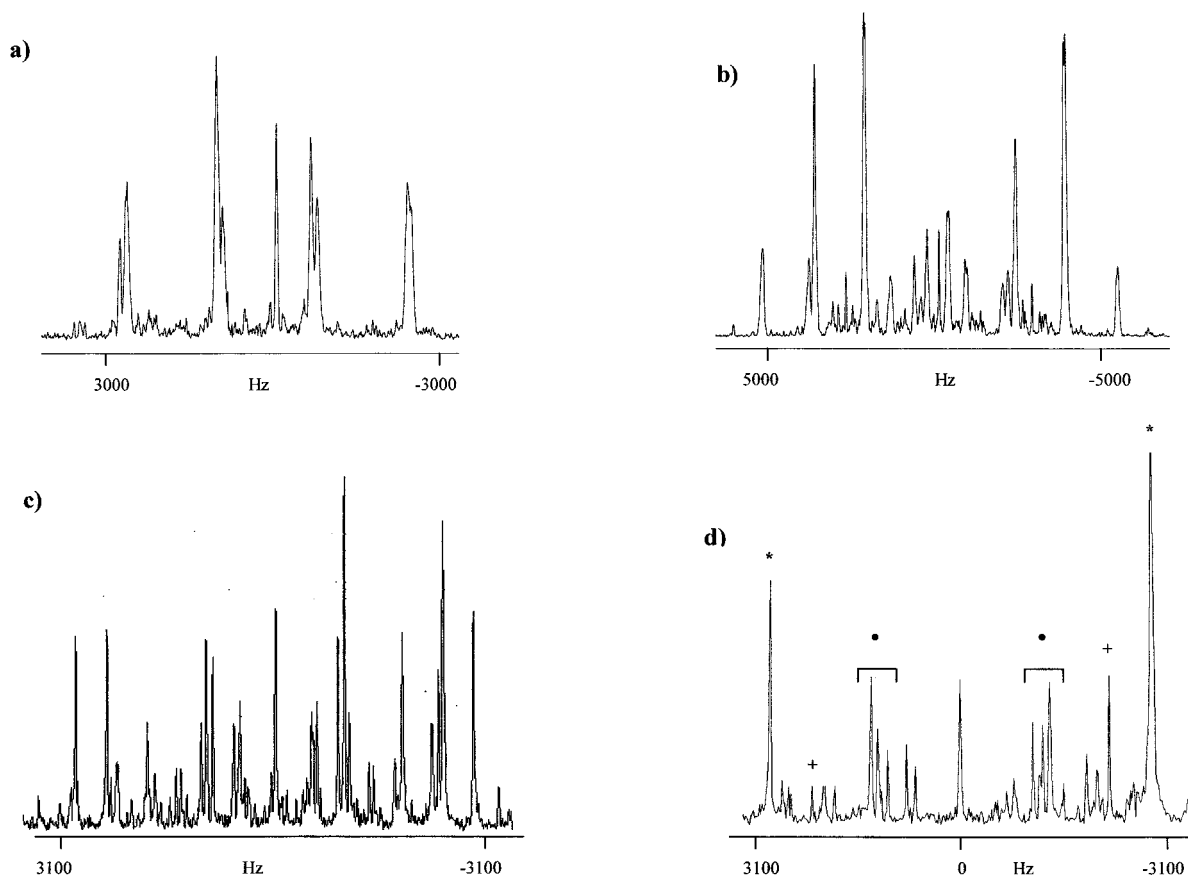


Figure 6. The 300 MHz ^1H spectra of a sample of α,α,α -trifluoromethoxybenzene (**IV**) dissolved in the nematic solvent ZLI1132: (a) H5Q, (b) H4Q, (c) ($n^s = 0, n^l = 4$) ^1H spin-echo HMQ, (d) $\{^{19}\text{F}\}$ - ^1H H4QR spectra. All the spectra were acquired with 1024 increments, 10 kHz in F_1 , $\tau = 20$ ms; 20 scans for (a), 16 scans for (b), (c) and (d). Spectrum (d) was obtained only using DIPSI and $\tau = 15.7$ ms. See text for explanation of the peak labels in (d).

$\cong 3100$ Hz and $\cong 4500$ Hz for the SQ spectra of **VI** and **III**, respectively). Given such a wide frequency range, it is not surprising that the multipulse sequences failed to decouple the ^1H MQ spectra of **V** since they were developed mainly for isotropic cases for which the frequency dispersion of the spectra is significantly smaller than 9000 Hz, but other factors related to the relative magnitude of the couplings involved could also play an important role.

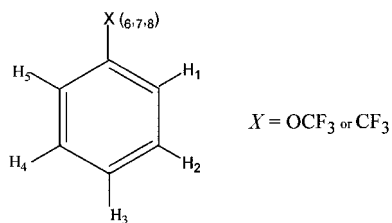
As previously stated, in the ^1H spin-echo spectrum of sample **IV** there are far more lines than expected and so it was not possible to use that spectrum as a starting point for the spectral analysis procedure. On closer inspection, figures 6(c) and 6(d), line patterns common to spectra **IVc** and **IVd** can be identified: the partially ineffective decoupling produces clusters of lines in the spin echo spectrum of **IV** centred at the frequency at which a transition is found in its ^{19}F decoupled H4QR spectrum **IVd**. This is clearly evident for the pair indicated by + but, at different τ values, it can also be observed for the outer pair; on the other hand the pair

marked by * is not fully decoupled even in **IVd**. Unfortunately such a consideration is of no practical use since 'real' lines in **IVc** can be identified as such only *a posteriori*.

5. Conclusions

The presence in the spin system of nuclei with different values of γ plays a very important role in analysing complex anisotropic spectra using a fully automated procedure based on MQ spectra, since it is possible to reduce the coherence order (which implies shorter instrumental times and higher S/N ratios) whilst still obtaining spectra with a number of experimental lines compatible with the permutative approach that the procedure requires. A proper choice of the MQ allows the full set of spectral parameters to be used in the final analysis of the SQ spectra to be obtained. However, the critical point in the procedure is the possibility to obtain the hetero decoupled $(N^l - 1)\text{QR}$ spectrum; this condition can pose severe limitations on the generalization of the procedure since the reliability of the decoupling

Table 3. Chemical shifts, $\Delta\nu_i$, and dipolar couplings, D_{ij} , obtained from the analysis of the 300 MHz ^1H SQ and 282 MHz ^{19}F SQ spectra of samples of α,α,α -trifluoromethoxybenzene (IV) (column a) and α,α,α -trifluoromethylbenzene (V) (column b) dissolved in the nematic solvent ZLI1132.



ij	D_{ij}/Hz	
	a	b
12	-1689.83 ± 0.09	-1619.66 ± 0.05
13	-246.52 ± 0.26	-239.70 ± 0.11
14	-54.17 ± 0.10	-61.98 ± 0.06
15	-0.55 ± 0.39	-22.26 ± 0.18
16	-317.68 ± 0.20	-468.71 ± 0.09
23	-422.26 ± 0.28	-480.97 ± 0.13
24	-1.27 ± 0.39	-22.59 ± 0.18
26	-120.97 ± 0.19	-133.22 ± 0.08
36	-94.31 ± 0.09	-102.63 ± 0.07
67	194.58 ± 0.07	1060.52 ± 0.04
$(\nu_1 - \nu_3)/\text{Hz}$	-160.72 ± 0.25	-138.51 ± 0.14
$(\nu_2 - \nu_3)/\text{Hz}$	-82.19 ± 0.26	-130.98 ± 0.14

method depends on the magnitude of the couplings and these can vary significantly depending on the heteronucleus, the molecule and the experimental conditions (temperature and mesophase used as orienting medium). When the heteronucleus is ^2H , due to its low γ value the simple spin-echo HMQ experiment can produce the decoupled $(N^1 - 1)\text{QR}$ spectrum since the condition $[H', H] \cong 0$ is usually satisfied; in contrast, for ^{19}F it has been found that even using broadband decoupling sequences such as WALTZ-16, DIPSI and GARP, specifically designed to optimize decoupling efficiency in isotropic solutions, it is not always possible to obtain $(N^1 - 1)\text{QR}$ spectra. It is not possible at this stage to make predictions about the possibility of obtaining this kind of spectra for different, more complex spin systems, since so few cases have been discussed. Nor is it possible, for the same reasons, to assess whether DIPSI performs better than WALTZ-16 and GARP, but as a rule of thumb, simply by checking the width of the ^{19}F SQ spectrum, it should be easy to evaluate the practicality of trying to follow the procedure described here.

Quite recently, new broadband decoupling sequences for liquid crystals, used to decouple ^1H [13] and ^{19}F [14] from ^{13}C , the observed nucleus, have been described

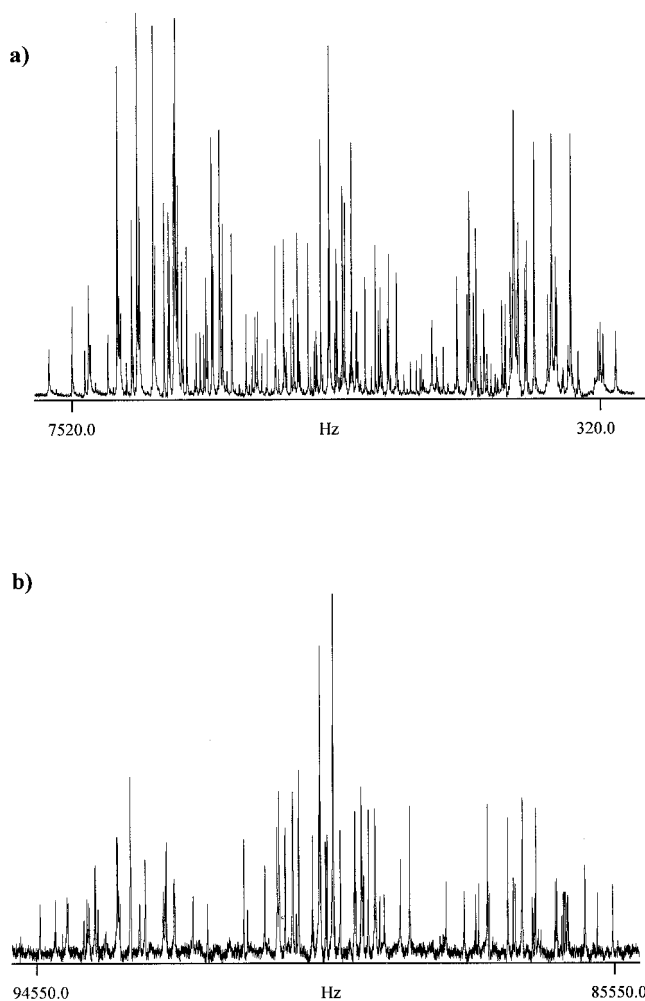


Figure 7. The 300 MHz ^1H SQ (a) and 282 MHz ^{19}F SQ (b) spectra of a sample of α,α,α -trifluoromethylbenzene (V) dissolved in the nematic solvent ZLI1132.

and their efficiency proved [13]; their use could enhance the possibility of obtaining good MQ $\{^{19}\text{F}\}$ - ^1H spectra of more complex molecules, the *sine qua non* condition to the applicability of the spectral automatic analysis procedure described here. Also, it should be possible at least in principle, to decouple the HMQ spectra by applying a high power continuous wave radiofrequency irradiation as used sometimes to decouple ^2H nuclei in order to avoid excessive linebroadening in the observed ^1H SQ spectrum (see [2(a)] and references therein). In our case the spectral width is relatively small compared with the tens of kHz usually found in ^2H spectra and, what is more, we have very few lines in the $(N^1 - 1)\text{QR}$ spectra with a typical linewidth of a few tens of Hz due to the 2D nature of the MQ experiment: thermal broadening should not pose further problems. Unfortunately in both cases the required hardware is not available to use and at present we are unable to verify these hypotheses, but further work in this direction is advisable.

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