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RESEARCH PAPER

^{19}F Solid-State NMR Spectroscopic Investigation of Crystalline and Amorphous Forms of a Selective Muscarinic M_3 Receptor Antagonist, in Both Bulk and Pharmaceutical Dosage Form Samples

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ABSTRACT

The purpose of the following investigation was to display the utility of ^{19}*F solid-state nuclear magnetic resonance (NMR) in both distinguishing between solid forms of a selective muscarinic* M_3 *receptor antagonist and characterizing the active pharmaceutical ingredient in low-dose tablets. Ambient- and elevated-temperature solid-state* ^{19}*F fast (15 kHz) magic-angle spinning (MAS) NMR experiments were employed to obtain desired spectral resolution in this system. Ambient sample temperature combined with rotor frequencies of 15 kHz provided adequate* ^{19}*F peak resolution to successfully distinguish crystalline and amorphous forms in this system. Additionally, elevated-temperature* ^{19}*F MAS NMR further characterized solid forms through* ^{19}*F resonance narrowing brought about by the phenomenon of solvent escape. Similar solvent dynamics at elevated temperatures were utilized in combination with ambient-temperature* ^{19}*F MAS NMR analysis to provide excipient-free spectra to unambiguously identify the active pharmaceutical ingredient (API) conversion from crystalline Form I to the amorphous form in low-dose tablets. It is shown that* ^{19}*F solid-state NMR is exceptionally powerful in distinguishing amorphous and crystalline forms in both bulk and formulation samples.*

Key Words: ^{19}*F NMR; Fast MAS; Polymorphism; Solid-state NMR; Tablets*

INTRODUCTION

Compound I (Fig. 1) is a selective M_3 neuronal receptor antagonist which was discovered and developed at Merck Research Laboratories in Banyu, Japan. This specific selective antagonist is indicated for the treatment of chronic obstructive pulmonary disease (COPD) with the potential for once-a-day dosing. Advantages of selective anticholinergic drugs over non-specific antagonists include reduced side-effects and higher efficacy. The free base form of Compound I was found to exist in at least two crystalline, monotropic polymorphic forms. The stable polymorph was designated Form I while the metastable polymorph was designated Form II. Additionally, a sesquihydrate form of the free base Compound I is known to exist. The current investigation is aimed at both distinguishing between each polymorph in bulk samples as well as identification of active pharmaceutical ingredient (API) form in low-dose tablets through ^{19}F magic-angle spinning (MAS) nuclear magnetic resonance (NMR) analysis.

Solid-state NMR is becoming increasingly important in the characterization of pharmaceutical materials (1,2). Most work to date, however, has focused on ^{13}C investigations. Typically, the technique of MAS is combined with cross-polarization (CPMAS) and high-power proton decoupling (3) to yield adequate ^{13}C spectral resolution to provide unique ^{13}C NMR spectra for individual crystal forms in a polymorph system (4,5). This specific spectroscopic application has also been applied to identify the form of the API in formulation samples (6,7). When tablet doses become increasingly small, however, peaks from excipient components tend to dominate the overall ^{13}C spectrum. This phenomenon limits the utility of ^{13}C solid-state NMR in determining the API form in low-dose tablets. The current investigation utilizes the benefits of the ^{19}F nucleus for solid-state NMR investigations of API forms.

There are several advantages afforded by exploiting the ^{19}F nucleus for solid-state NMR investigations of API polymorphs. Primarily, ^{19}F possesses both a high gyromagnetic ratio and a natural abundance leading to high sensitivity for ^{19}F solid-state NMR. This high sensitivity renders ^{19}F an ideal nucleus for investigating low-drug-dose (1 to 2% weight API in formulation) pharmaceutical formulation samples where ^{13}C analysis would be significantly time-consuming. Additionally, few excipients used in preparing pharmaceutical formulations contain fluorine in their chemical structure. This leads to minimal ^{19}F excipient interference in spectra received from formulation samples.

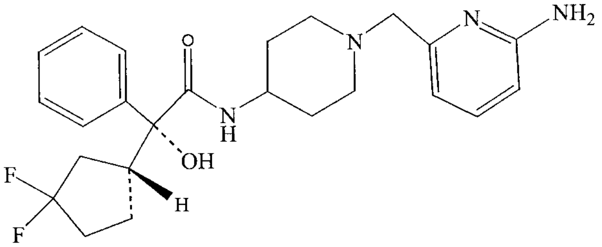

Figure 1. Structure of Compound I.

The difficulty in utilizing the ^{19}F nucleus in solid-state NMR for the characterization of pharmaceutical compounds arises from the extremely strong homonuclear (^{19}F–^{19}F) and heteronuclear (^{1}H–^{19}F) dipolar interactions combined with ^{19}F chemical shift anisotropy (CSA). These strong interactions produce significant line-broadening in ^{19}F solid-state NMR spectra (8) and are the main reason for the lack of investigations using ^{19}F solid-state NMR for the characterization of pharmaceutical solids.

Recent technological advancements such as fast MAS (>12.0 kHz) (8), possibly combined with elevated sample temperatures (>100°C) (9) or high-power proton decoupling during ^{19}F detection (10), have dramatically improved resolution of ^{19}F solid-state NMR spectra. The current investigation utilized fast MAS (15.0 kHz) at both ambient- and elevated-temperature sample conditions to provide ^{19}F peak resolution. These conditions provided ample resolution to distinguish and characterize Compound I polymorph forms in bulk samples, as well as to characterize the solid form of API in low-dose Compound I tablets.

EXPERIMENTAL

Materials

Crystalline forms of Compound I were prepared by the Process Research Division of Merck & Co. Amorphous Compound I was prepared through lyophilization. Tablets were prepared by the Pharmaceutical Research and Development Division of Merck & Co. (West Point, PA). Tablet sample preparation for ^{19}F NMR experiments consisted of crushing tablets in a mortar and pestle before packing into the NMR sample rotor.

Solid-State NMR Methods

All solid-state NMR spectra were performed on a Bruker DSX-400 NMR spectrometer (9.4 T magnetic field strength) using a Bruker CRAMPS probe, 4-mm zirconia rotors with ZrO endcaps, and a standard quadrupole echo pulse sequence (with 20 μsec echo time). The ^{19}F resonance frequency was 376.5 MHz with $\pi/2$ pulse length of 2 μsec, 4 k of data points were acquired in 21 msec and then zero-filled to 8 k before transformation using 100 Hz of line broadening. Recycle delays for the ^{19}F echo NMR experiments were 3.0 sec. Rotor frequencies were 15.0 kHz, and all ^{19}F spectra are referenced to poly(tetrafluoroethylene) (Teflon®) which was assigned a chemical shift of −122 ppm. Between 32 and 19,000 acquisitions were necessary (depending on the sample) to obtain an adequate S/N ratio in the ^{19}F spectra. Elevated-temperature experiments were performed at 110°C.

RESULTS AND DISCUSSION

Ambient-Temperature ^{19}F MAS NMR

The ambient-temperature ^{19}F MAS NMR spectra for crystalline free base and amorphous Compound I forms are displayed in Fig. 2. The spectrum for Form I (Fig. 2a) consists of ^{19}F peaks at −88.0 and −93.4 ppm, while the spectrum for Form II (Fig. 2b) contains ^{19}F peaks at −85.0, −90.2, and −98.3 ppm. Additionally, the ^{19}F spectrum received for sesquihydrate Compound I (Fig. 2c) yields ^{19}F peaks at −84.3 and −94.0 ppm. The amorphous Compound I ^{19}F MAS NMR spectrum (Fig. 2d) displays a single, broad ^{19}F peak centered at −89.0 ppm. The significant full-width at half-maximum (FWHM) of the amorphous ^{19}F peak (~7 kHz) compared to the crystalline Compound I forms (~4.5 kHz) is a result of the inherent disorder associated with amorphous materials (11).

The ^{19}F NMR spectra for all three crystalline forms consist of ^{19}F peaks which are still significantly broadened due to the incomplete averaging of dipolar interactions afforded by the current experimental conditions. This residual broadening is

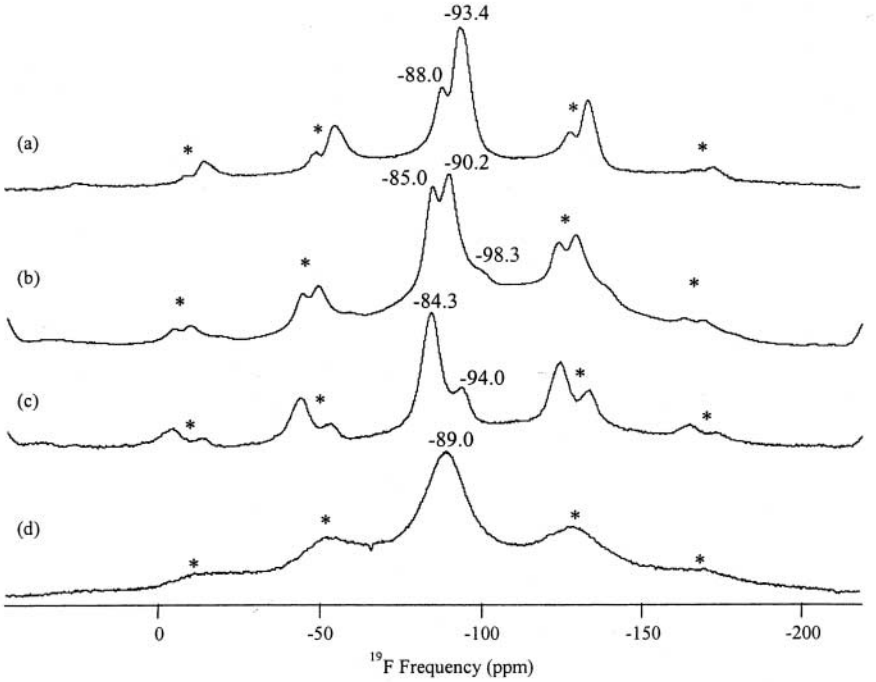

Figure 2. Ambient-temperature ^{19}F MAS NMR of (a) Form I, (b) Form II, (c) sesquihydrate, and (d) amorphous form of Compound I. Spinning sidebands are labeled with asterisks.

evident in the substantial FWHM of the ^{19}F peaks in the crystalline materials leading to the lack of baseline resolved ^{19}F resonances. Due to this lack of complete resolution, specific correlations of isotropic ^{19}F peaks to crystallographic structure will not be attempted. However, the line-narrowing afforded by ambient-temperature ^{19}F MAS NMR with rotor frequencies of 15.0 kHz provides adequate ^{19}F resolution to clearly distinguish between all three crystalline forms of free base Compound I.

As a test for the ability of ^{19}F MAS NMR to identify the solid form of Compound I in tablets, placebo (directly from Compound I formulation process) was externally spiked with approximately 2% w/w of Form I Compound I. The resulting ^{19}F MAS NMR spectrum is displayed in Fig. 3a. The spectrum clearly displays the characteristic Form I ^{19}F NMR peaks at −88.0 and −93.4 ppm without any noticeable excipient interference. Additionally, the FWHM and relative peak intensity for the ^{19}F spectrum of spiked placebo remain identical to those determined for pure Form I.

Compound I tablets prepared with Form I API were analyzed using ^{19}F MAS NMR. The tablets contain Compound I at a 2% w/w concentration. Ambient-temperature ^{19}F MAS NMR analysis (Fig. 3b) yields a ^{19}F spectrum displaying a rather broad isotropic resonance with distinctions at −88.0 and −93.4 ppm. Comparing this ^{19}F spectrum to that of pure Form I Compound I (Fig. 2a), it is evident that the API in the tablets is not exclusively Form I. A substantial conversion of Form I into either another crystalline form of Compound I or amorphous Compound I has occurred during some step of the formulation process for these tablets. Significant conversion of Form I to Form II or sesquihydrate Compound I is an unlikely explanation due to the lack of intensity in the region of the ^{19}F spectrum where those forms have ^{19}F resonances (−85.0 ppm for Form II and −84.3 ppm for sesquihydrate). The broad ^{19}F peak measured for the tablet does contain minimal intensity in this particular region of the spectrum, and therefore some conversion to these forms cannot be ruled out, but that contribution to the overall spectrum, if any, is small. The ^{19}F spectrum of the tablets does appear quite similar to the ^{19}F spectrum received for amorphous Compound I (broad peak centered at

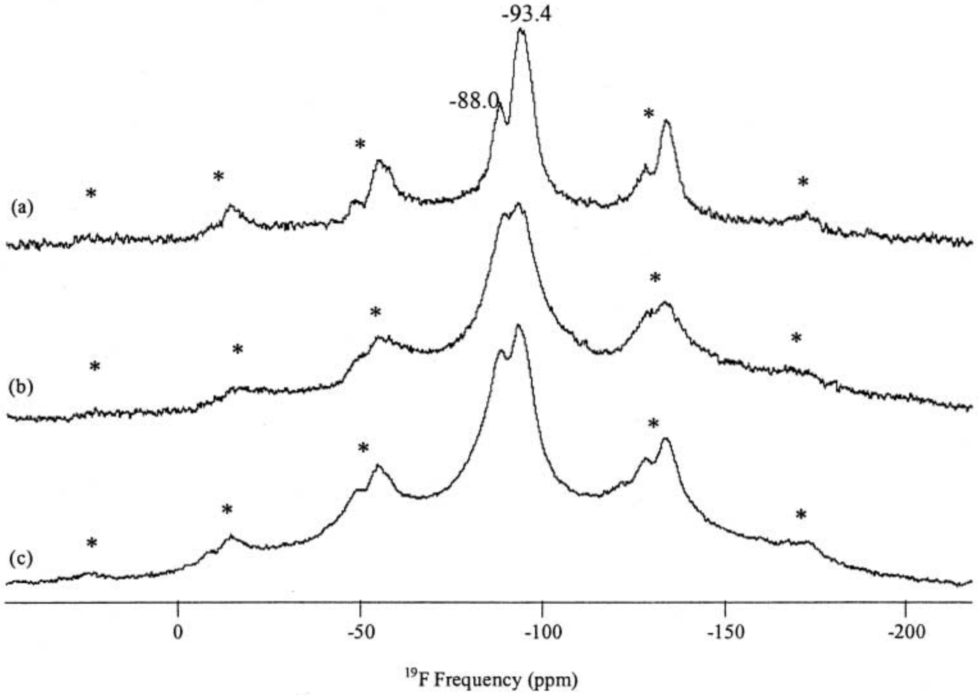

Figure 3. Ambient-temperature ^{19}F MAS NMR of (a) placebo spiked with ~2% w/w Form I, (b) tablets dosed with 2% w/w Form I, and (c) 50/50 mixture of Form I and amorphous Compound I. Spinning sidebands are labeled with asterisks.

~-89.0 ppm with $\sim$7 kHz FWHM). However, total conversion to amorphous state has not occurred as evidenced by the distinct Form I Compound I features remaining at −88.0 and −93.4 ppm on top of both the broad isotropic peak and the spinning sidebands. To confirm the spectroscopic features of Form I/amorphous Compound I form mixtures, ^{19}F MAS NMR was performed on a 50/50 mixture of Form I/amorphous Compound I. The resulting spectrum (Fig. 3c) appears quite similar to that received from the low-dose tablet (Fig. 3b). Therefore, ambient-temperature ^{19}F MAS NMR analysis indicates that significant conversion of Form I Compound I to the amorphous form has occurred during the tablet formulation process.

High-Temperature ^{19}F MAS NMR

In an attempt to obtain higher fluorine peak resolution for both samples of pure Compound I forms as well as formulation samples, fast-spinning ^{19}F MAS NMR was combined with elevated sample temperature of 110°C (well below the melting temperature for Form I which is 172°C). Figure 4 displays the elevated-temperature ^{19}F MAS spectra of the monotropic polymorphs of Compound I (Form I and Form II). From Fig. 4, it is clear that the elevated-temperature ^{19}F MAS NMR spectra for both forms yield no significant increase in peak resolution compared to the ^{19}F MAS NMR spectra received at ambient temperature (Fig. 2). In fact, the elevated-temperature ^{19}F MAS NMR spectrum for Form I (Fig. 4a) yields a single, broad peak compared to the resolved peaks received from ambient-temperature ^{19}F MAS NMR (Fig. 2a). This high-temperature effect is believed due to increased motion of the ^{19}F nuclei at the elevated temperature. For Form I Compound I, this increase in motion at this temperature slightly decreases the effect of line-broadening interactions on the ^{19}F spectrum at the expense of fluorine crystallographic site identity. Therefore, the overall peak FWHM decreases at the expense of peak resolution.

Elevated-temperature ^{19}F MAS NMR was also performed on sesquihydrate and amorphous Compound I forms. For both forms, the sample temperature was taken to 110°C and a ^{19}F MAS NMR spectrum was immediately acquired. Remarkably, the resulting ^{19}F MAS NMR analysis at this temperature yielded a ^{19}F spectrum for sesquihydrate (Fig. 5a) and amorphous Compound I (Fig. 5b) which contain multiple, sharp,

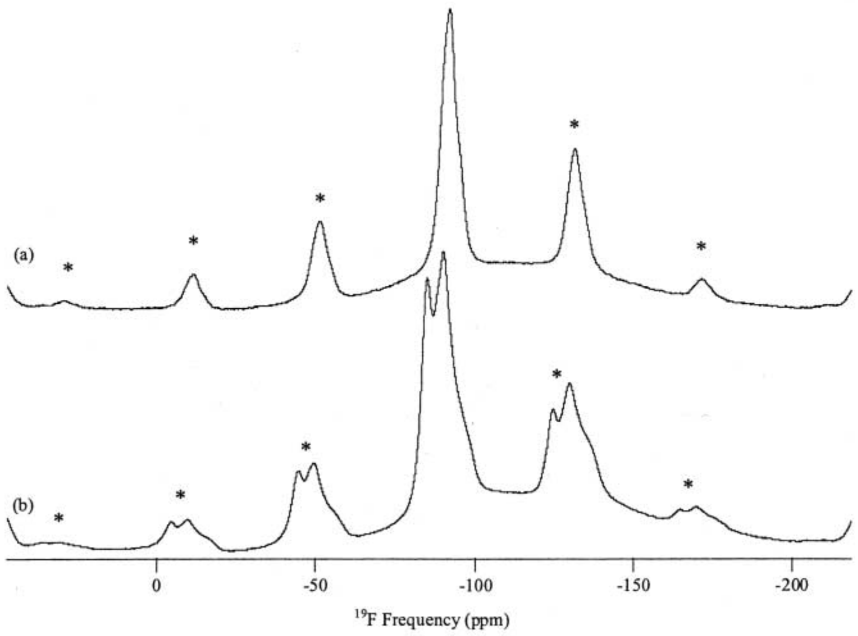

Figure 4. Elevated-temperature (110°C) ^{19}F MAS NMR of (a) Form I and (b) Form II Compound I polymorphs. Spinning sidebands are labeled with asterisks.

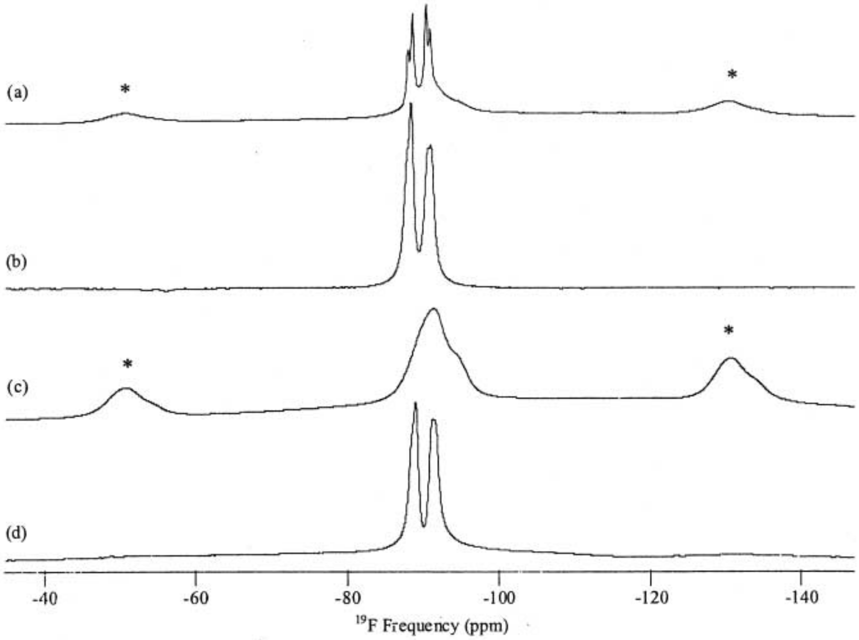

Figure 5. Elevated-temperature (110°C) ^{19}F MAS NMR of (a) sesquihydrate run immediately after sample temperature reached 110°C, (b) amorphous Compound I run immediately after sample temperature reached 110°C, (c) sesquihydrate run 1 hr after sample sitting at 110°C, and (d) tablets dosed with 2% w/w Form I run immediately after sample temperature reached 110°C. Spinning sidebands are labeled with asterisks.

"liquid-like" peaks. The ^{19}F spectrum for sesquihydrate additionally contains a broad, featureless peak with associated sets of spinning sidebands. The sharp ^{19}F peaks in both amorphous and sesquihydrate Compound I ^{19}F spectra are not accompanied by spinning sidebands. The lack of sidebands in addition to the exceptional sharpness of ^{19}F peaks is indicative of ^{19}F nuclei virtually free of broadening interactions. Sesquihydrate Compound I was held at 110°C for approximately 1 hr and then a ^{19}F MAS NMR spectrum was re-acquired. The resulting spectrum (Fig. 5c) contains a single, broad isotropic ^{19}F resonance with multiple sets of spinning sidebands. Amorphous Compound I also behaved similarly when held at high temperature for an extended period of time (not shown). The appearance and disappearance of "liquid-like" ^{19}F peaks from high-temperature ^{19}F MAS NMR spectra for sesquihydrate and amorphous Compound I forms is attributed to solvent escape. As solvent (water) begins to escape from a crystal lattice, increased atomic motion will occur in regions of the molecule from which the solvent is escaping. The added motion of the ^{19}F nuclei results in substantial averaging of broadening interactions. When all solvent has escaped, the added motion decreases and the effect of normal broadening interactions of ^{19}F nuclei in the solid-state returns.

The presence of incorporated water in the unit cell of the sesquihydrate accounts for the solvent escape effect on the ^{19}F MAS NMR spectrum for this form. The solvent involved in the amorphous form, however, is less intuitive. Amorphous pharmaceutical compounds are, in general, more hygroscopic than their crystalline counterparts. If the water sublimation step in the lyophilization procedure utilized to create the amorphous Compound I failed to completely remove water from the solid, then remaining water would be incorporated randomly into the amorphous structure. Although amorphous materials do not possess a repeating unit cell, a network still exists for the incorporation of water (12). This incorporated water is believed to be the solvent responsible for the solvent escape effect on the elevated-temperature ^{19}F MAS NMR spectrum of amorphous Compound I. This hygroscopic nature of amorphous Compound I, and the corresponding effect on the elevated-temperature

^{19}F MAS NMR spectra, aid in unambiguous identification of the amorphous form in formulation samples.

Confirmation of the presence of amorphous Compound I in the Compound I tablets (same tablets analyzed in Fig. 3b) is received through elevated-temperature ^{19}F MAS NMR analysis. Performing ^{19}F MAS NMR at sample temperature of 110°C yields the spectrum displayed in Fig. 5d. The sharp, liquid-like ^{19}F peaks indicating solvent escape from the Compound I lattice are clearly present in the high-temperature spectrum of the tablet. These sharp peaks are a result of solvent escape from amorphous API. The tablet was held at 110°C for approximately 1 hr and then a ^{19}F MAS NMR spectrum was re-acquired. The resulting spectrum (not shown) still displayed the sharp ^{19}F peaks but with decreased peak intensity. The ^{19}F sharp peak intensity continued to drop as the tablets were held longer at 110°C. This prolonged solvent escape phenomenon for the tablets is most likely due to excipients slowing down the escape of water from amorphous API. Combined with ambient-temperature ^{19}F MAS NMR analysis, the solvent escape occurrence witnessed in the spectrum of elevated-temperature ^{19}F MAS NMR unambiguously identifies the presence of amorphous Compound I in the 2% w/w Form I Compound I tablets.

Ambient- and high-temperature ^{19}F MAS NMR were utilized to distinguish between crystalline and amorphous forms of Compound I. Spectra received from ambient-temperature ^{19}F solid-state NMR analysis with rotor frequency of 15.0 kHz displayed ample ^{19}F peak resolution to distinguish between Form I, Form II, sesquihydrate, and amorphous Compound I forms. Additionally, high-temperature (110°C) ^{19}F solid-state NMR analysis was shown to facilitate fluorine resonance narrowing in water-containing samples. Similar ambient- and high-temperature investigations were performed on 2% w/w API tablets to display the utility of ^{19}F solid-state NMR towards characterizing the form of active ingredient in formulation samples. No attempt at form quantitation was made in the current investigation, and this is the subject of further research.

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