Video Article

Application and Methodology of the Non-destructive ^{19}F Time-domain NMR Technique to Measure the Content in Fluorine-containing Drug Products

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Abstract

Here, we describe a protocol developed by our group that uses low-field fluorine-19 (^{19}F) time-domain (TD) nuclear magnetic resonance (NMR) to measure the average content of fluorinated drugs in their formulated drug product forms: tablets or capsules. This method is specific to fluorinated drugs because it detects only the content of fluorine, avoiding interference from the excipients that lack fluorine. The advantages of measuring the active content of fluorinated drugs using low-field ^{19}F TD-NMR versus high-field ^{19}F solid-state (SS) NMR are the simplicity of the method; the low cost; and the non-destructive nature of the technique, with all samples recoverable in intact forms (e.g., powders, tablets, and capsules), making this technique affordable for any laboratory.

We have tested the method with three fluorinated drug products available on the market - cinacalcet, lansoprazole, and ciprofloxacin - with doses ranging from 15 to 500 mg. The results of the analyses, measured by low-field ^{19}F TD-NMR, supported the reported label claims for the average drug content.

Based on the simplicity and reproducibility of the analysis, we envision this methodology being implemented in any laboratory, including manufacturing plants, as a process analytical technology (PAT) tool in the pharmaceutical industry.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55850/>

Introduction

In the pharmaceutical industry, the content of drug substances in their unit-of-dosage forms (e.g., tablets and capsules) must be within the range on the label claim to meet quality control and performance guidelines. High-performance liquid chromatography (HPLC) is the conventional analytical technique used to measure the content uniformity of drugs in their formulated forms. However, the method is lengthy and destructive, requiring the dissolution of the drug into its formulated form using the appropriate solvents prior to analysis. Even with automation, the process can take 1 - 3 h per sample, depending on the development of the appropriate analytical method^{1,2,3}. In addition to HPLC, near-infrared (NIR) has been used for the same purpose and as a PAT tool, with the caveat of requiring the chemometric analysis of the data, making its implementation lengthy and more complex⁴. Both techniques are destructive, with the formulated drug discarded after analysis.

In this manuscript, we describe the step-by-step protocol of a methodology previously published by our group⁵ to measure the average content of fluorinated drugs in their dosage forms (e.g., tablets and capsules) using low-field ^{19}F NMR. Over the years, the percentage of fluorinated drugs on the market approved by worldwide regulatory agencies as formulated drug products has increased from 2% in 1970 to 25% in 2013⁶. Therefore, we believe that there is a demand to develop a simpler but more specific method than the currently available methods for the quality control of the content of fluorinated drugs in their formulated forms.

Drugs containing fluorine-19 (^{19}F) in their structures have the advantage of being easily detected by ^{19}F NMR due to its 100% abundance and 83% sensitivity compared to protons⁷. Direct measurement by ^1H TD-NMR is not useful because both excipients and API have protons, and the total proton signal comes from all the components of the drug product, making it impractical to measure the API content in drug products using ^1H TD-NMR. Therefore, measuring the drug content of fluorinated drug products using ^{19}F TD-NMR has the advantage of no interference with excipients due to the lack of fluorine. To demonstrate our methodology of measuring the average drug content of fluorinated drug products, we selected three commercially formulated drug products containing fluorine in different dose ranges. **Figure 1** depicts the structures of the drugs

selected, where two of them - cinacalcet HCl⁸ and lansoprazole⁹-have a trifluoromethyl (CF₃) group in their structures, with doses ranging from 15 to 90 mg, and the third one - ciprofloxacin HCl monohydrate¹⁰ - contains one fluorine atom attached to an aromatic ring, with a 500-mg dose.

Here, we demonstrate the methodology we have developed⁵ to quantitate the average drug content of fluorinated drug products using a low-field benchtop TD-NMR instrument (23.4 MHz for ¹H and 22.0 MHz for ¹⁹F). We also compare two software packages (RI Calibration and Mnova software) that can be used to report the results.

Protocol

Caution: Please consult all relevant material safety data sheets (MSDS) before using any chemicals. Drug substances in general are toxic and should be handled with the appropriate personal protective equipment (PPE; e.g., gloves, safety glasses, lab coat, full-length pants, and closed-toe shoes), with special caution when weighing powder materials. It is recommended to use a balance placed in a hood with good air flow to minimize the powder from solid materials disseminating out of the area surrounding the balance.

NOTE: The software instructions are specific for RINMR, RI Calibration, and Mnova. For other TD-NMR vendor instruments and software packages, the instructions will vary.

1. Preparation of Samples Prior to the ¹⁹F TD-NMR Measurement

1. Weigh the samples for calibration (powder drug substance or active pharmaceutical ingredient (API)) in the appropriate NMR tubes (2.5-, 3-, 5-, 10-, 18-, and 25-mm OD NMR tubes) until the marking line (height of the coil for the NMR probe), or around 30 mm in height. Notice that the height may depend on the probe.
NOTE: Cinacalcet HCl is an example of an API to use for calibration to determine the drug content in cinacalcet commercial tablets. The weight will depend on the density of the powder. In the case of cinacalcet HCl, the weights of the API are around 7.2, 3.5, 1.0, 0.3, 0.1, and 0.07 g when filling the height of the coil for the 25-, 18-, 10-, 5-, 3-, and 2.5-mm NMR tubes, respectively.
2. Weigh the formulated drug product samples (tablets or capsules) in the appropriate NMR tubes until the marking line (height of the coil for the NMR probe), or around 30 mm in height, as indicated above.
NOTE: 25- or 18-mm tubes are appropriate for large-sized tablets/capsules or when enough formulated drug product is available. For smaller tablets/capsule or when not enough formulated drug product is available but the signal-to-noise ratio may still be sufficient for measurement, use 10-mm tubes. Measure the tablets directly, or crush if desired. No observation of variations in their measurements have been observed here, as mentioned in the **Discussion**.
 1. When preparing commercial tablets of cinacalcet, weigh around fifteen 90-mg tablets in the 25-mm NMR tube (around 9.2 g), fifty 30-mg tablets in the 25-mm NMR tube (around 9.6 g), twenty-two 30-mg tablets in the 18-mm NMR tube (around 4.2 g), and twenty-three 60-mg tablets in the 25-mm NMR tube (around 8.8 g).

2. Preparation of the NMR Instrument for Measurement

1. **Tuning the NMR instrument to ¹⁹F**
 1. Carry out all measurements at the temperature of the permanent magnet in the NMR instrument. Here, use 40 °C; no temperature control is available for the 26-mm ¹⁹F NMR probe used here. Carry out all measurements at a frequency of 22.0 MHz for ¹⁹F using the 23-MHz (for ¹H) NMR instrument.
 2. Place the Teflon standard sample inside a 25- or 26-mm NMR tube and place it into the probe inside the magnet. Allow for equilibration for around 5-10 min and tune the instrument to ¹⁹F. Select "Auto O1" under the "Commands" menu in the RINMR software. Repeat the measurement at least 3 times and take the last value of the O₁ parameter (frequency offset).
2. **Calibration of the ¹⁹F 90-degree pulse**
 1. Calibrate the 90-degree pulse for F-19 with the standard Teflon sample using the standard automated calibration sequence available in the instrument. Select "Auto P90" under the "Commands" menu in the RINMR software.
NOTE: Normally, the 90-degree pulse does not need to be measured frequently unless the instrument is not performing or the probe has not been used for some time. The 90-degree value obtained for Teflon with the 26-mm ¹⁹F NMR probe used here was 7.05 μs.

3. Measurement of the T₁ or Longitudinal Relaxation Time for Standard Pure API Samples

1. For a better signal-to-noise ratio, place the API sample prepared in the 25-mm NMR tube inside the F-19 probe installed in the magnet and let it equilibrate for at least 10 min.
2. Measure the T₁ relaxation time, following the instructions of the instrument manual, for each API sample using a standard experiment for inversion-recovery from the RINMR software.
NOTE: The recommended experimental parameters are 16 scans collected with a 0.1-μs dwell time; 8 data points; a 90-degree pulse, as mentioned above; and a dead time of 10 μs for the F-19 26-mm probe. An array of delays from 100 μs to 8 s (e.g., 100 μs, 500 μs, 10 ms, 100 ms, 300 ms, 700 ms, 1 s, 2 s, 5 s, and 8 s) for drugs with CF₃ and delays from 100 μs to 20 s (e.g., 100 μs, 500 μs, 1 ms, 100 ms, 500 ms, 1 s, 5 s, 10 s, 15 s, and 20 s) for drugs with one fluorine group attached to the aromatic ring are recommended.
 1. In the RINMR software, load the "invrec.exe" pulse sequence by selecting "Load" under "Sequence" and clicking "Open." Check that the basic parameters have the correct values by clicking "A" or selecting "Acquisition" under the "Parameters" menu.
 2. Select "Scripts" under the "Tools" menu; a window will pop up. Select the "T1.ris" tab and click the green arrow; a window will pop up. Select the delay list file with appropriate delays or create the list after clicking "Open." Once the delay list is satisfactory, click "OK."

NOTE: A window will display a prompt for the creation of a file for all spectra that will be collected in order to calculate the T_1 value. The location and names of the folders and files are up to the user.

3. Click on "Save" for the instrument to start acquiring the data and automatically saving the files for the delays in the selected folder.

NOTE: The RIMNR software will create the T_1 relaxation curves and calculate the T_1 relaxation times for each case. A window will pop up with the T_1 relaxation curve and the time constant or T_1 value. Here, the value of T_1 for cinacalcet HCl was 2.4 s.

4. Measuring Calibration Samples with Pure API

1. Measuring the API samples to build the calibration curve

1. Place one calibration sample in the magnet for a particular API. Equilibrate the samples for a minimum of 5 min for the smaller tubes and 10 min for the larger tubes.
2. Acquire a free induction decay (FID) or SOLID (90x-90y FID) experiment with the appropriate parameters following the instructions in the instrument manual. In the RIMNR software, load the "solid.exe" (or "fid.exe") pulse sequence by selecting "Load" under "Sequence" and clicking "Open."

NOTE: The recommended experiment is SOLID for solid materials that are fast-relaxing with short T_2 s (transversal relaxation time). The recommended experimental parameters are 128 scans collected, with a 0.1 μ s dwell time, 1,024 data points, a 90-degree pulse (7.05 μ s in this case, calibrated using Teflon), and a dead time of 10 μ s for the ^{19}F 26-mm probe. A recycle delay of 7.5 s for drugs with the CF_3 group and 20 s for drugs with the F group attached to an aromatic (due to longer T_1 relaxation times) have been used for the cases presented here, based on their T_1 measurements.

3. Measure all samples prepared for each API after they are equilibrated to the temperature of the magnet for 5 - 10 min to generate the calibration curves of every API sample. Save the data in a folder, giving a distinct name to every experimental run.
4. Calculate the weight percentage of the samples based on the amount in each tube and considering the purity of the API sample in the largest tube.

5. Measurement of the drug product samples as tablets

1. Place the already prepared formulated drug product samples (tablets or capsules) in the magnet to measure them one at a time. Equilibrate the sample for 5 min for small NMR tubes or for 10 min for the samples in the larger NMR tubes.
2. Acquire the same NMR experiment (*i.e.*, SOLID) with the same conditions as for the calibrated samples for each particular API.

6. Generation of the calibration curves with pure API samples and adding the data from drug product samples as tablets in the calibration curves

1. In these cases, select the region of the SOLID experiment from 5 to 300 points to create the calibration curves. The data can be analyzed in three different ways, as outlined below.
2. Open the RI Calibration software. Recall all the SOLID data by clicking on the clip symbol to open the FID or SOLID files from a particular API. For all the samples measured, enter the weight percentages under the "Conc" column and the weights under the "Mass" column. Select the appropriate region (5-300 points) using the average method to build the calibration curve (open the icon of "Display NMR data" to select the range of points for the calculation). Save the calibration curve.
Note: The Cal column will provide the percentage of drug in the samples measured.
3. In the RI Calibration software with the same selected region (5-300 points), use the fit method to build the calibration curve. Save the calibration curve. Follow the same instructions as in step 4.2.2.
4. Open the software (*e.g.*, Mnova). Drag all the SOLID data from a particular API and enter the weight percentages of the standard samples; manual instructions can be found by clicking "Content" under the "Help" menu. In the "Advanced" menu, select "Time Domain" and then "Quantitation;" a window will pop up. Click on the blue plus sign to select the integration area. Integrate the appropriate region (5-300 points) using the magnitude mode to build the calibration curve.
5. Save the calibration curve. In the table, enter the percentages of drugs per sample only for the standards under the "Concentration (x)" column and the weights for all the samples under the "Mass (m)" column; calibration samples should be in red (checked). Make sure that the "Signal Function" is "y=s/m" (to normalize to signal/mass).
6. For reproducibility and repeatability, measure each sample 3 times on different days and build the calibration curve in both software packages. Save the calibration curve files.

7. Calculation of the Fluorinated API Drug Weight in the Formulated Drug Product Using the Calibration Curves

1. **Calculation of the weight percentage and average dosage amount per tablet/capsule of fluorinated API drug in the formulated drug product**
 1. Use the file for the calibration curve from the RI Calibration software and the average method. Read the NMR data from the measured tablets (or capsules) and enter their weights to determine the weight percentage of fluorinated API in the formulated tablets or capsules from the calibration curve.
Note: The "Cal" column will provide the percentage of drug in the measured samples (calibration and commercial tablets or unknowns). Notice that the O1 parameter must be the same for the calibration samples and the tablets or capsules for the RI Calibration software.
 2. Use the file for the calibration curve from the RI Calibration software with the fit method. Read the NMR data from the tablets (or capsules) measured and enter their weights to determine the weight percentage of fluorinated API in the formulated tablets or capsules

from the calibration curve. Follow the same instructions as in step 5.2.1. Notice that the O_1 parameter must be the same for the calibration samples and the tablets or capsules for the RI Calibration software.

- Use the Mnova file and drag the acquired NMR data for the formulated API (or measure them all on the same day). Follow the instructions in step 4.2.4. Enter the weights to determine the weight percentage of the fluorinated API in the formulated tablets or capsules from the calibration curve.

Note: In the table, the calibration samples will be in red (checked) and the unknown or commercial tablets/capsules in blue (unchecked). The "Concentration (x)" column will show the calculated values for the commercial tablets of unknown samples in blue based on the values of the calibration samples in red. Notice that the software does not require the use of the same O_1 parameter to calculate the weight percentage.

- Calculate the amount of fluorinated drug per tablet or capsule as the average value from the equation below (D = dose of API in mg, W_{NMR} = weight percentage of API obtained from the calibration curve, w = total weight of formulated product from the weight of the formulated drug coming from the measured tablets or capsules, and N = number of formulated tablets or capsules used for the measurement) and compare with the values calculated by the software based on the calibration curves:

$$D = \frac{W_{NMR} * w}{N}$$

Representative Results

We have tested low-field ^{19}F TD-NMR with three commercial drug products (cinacalcet, lansoprazole, and ciprofloxacin) to measure the average drug content of the fluorinated drug products. **Figure 1** shows the structures of the tested drugs, displaying the locations of the fluorine atoms.

We tested several lots and dosage forms (*i.e.*, 30, 60, and 90 mg) of cinacalcet tablets, one dosage form (15 mg) of brand-name and generic lots of lansoprazole capsules, and one dosage form (500 mg) and one lot of ciprofloxacin tablets. NMR signal decays for cinacalcet (**Figure 2**), as an example, and calibration curves for the three drugs (**Figures 3 - 5**) were obtained from the RI Calibration software using the average and fit methods and from Mnova using magnitude mode. Data from those curves were also plotted to check their reproducibility across the triplicates measurements (**Figures 3 - 5**), showing excellent linearity and good reproducibility. The results of the measurements for the three drugs are tabulated (**Tables 1 - 3**) and show good agreements with the label claims for the three drugs.

To test the effect on sample preparation, half of the tablets of ciprofloxacin were crushed. **Table 3** shows that the results of the average drug content from the intact and the crushed tablets agree well with the label claim, indicating that there is no need to destroy the tablets to perform the measurement. In addition, it demonstrates that the method is non-destructive and that the samples can be recovered in their intact forms.

To determine the significance on the measurement of the physical and chemical forms, we used ciprofloxacin free base and HCl monohydrate as calibration standards to determine the average content of the drug in intact and crushed ciprofloxacin tablets. **Figure 6** shows those results. **Figure 6A** depicts the results when ciprofloxacin HCl monohydrate was used as the calibration standard (red dots), and **Figure 6B** shows ciprofloxacin free base (red dots). The blue dots in **Figure 6** correspond to the intact and crushed ciprofloxacin tablets. Only in **Figure 6A** does the average content of ciprofloxacin in the tablets and crushed tablets agree well with the label claim, indicating that the correct calibration standard is ciprofloxacin HCl monohydrate. X-ray powder diffraction (XRPD) data on cipro tablets, ciprofloxacin free base, and ciprofloxacin HCl monohydrate were recorded to confirm their different physical forms based on the XRPD patterns (**Figure 7**).

Figure 1. Chemical Structures of lansoprazole, Ciprofloxacin Hydrochloric Acid Monohydrate, and Cinacalcet Hydrochloric Acid. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

Figure 2. Decay of the NMR Signal, Measured for Cinacalcet HCl, Over Time, Showing the Different Data Treatments.

(A) RI Calibration software average method, (B) RI Calibration software fit method, and (C) Mnova software area method. The region from 5 - 300 points was used for the analysis for all data treatments. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

Figure 3. Calibration Curves (NMR Signal versus Percentage of Drug) for Cinacalcet HCl, Calculated as Free Base, Generated from Measurements Performed in Triplicate.

(A) RI Calibration software in the average mode, (B) RI Calibration software in the fit mode, and (C) Mnova software area method. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

(A) RI Calibration (average method)

(B) RI Calibration (fit method)

(C) Mnova (area/integral method)

Figure 4. Calibration Curves (NMR Signal versus Percentage of Drug) for lansoprazole, Generated from Measurements Performed in Triplicate.

(A) RI Calibration software in the average mode, (B) RI Calibration software in the fit mode, and (C) Mnova software area method. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

(A) RI Calibration (average method)

(B) RI Calibration (fit method)

(C) Mnova (area/integral method)

Figure 5. Calibration Curves (NMR Signal versus Percentage of Drug) for Ciprofloxacin HCl H₂O, Calculated as the Free Base, Generated from Measurements Performed in Triplicate.

(A) RI Calibration software in the average mode, (B) RI Calibration software in the fit mode, and (C) Mnova software area method. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

(A) Calibration curve with ciprofloxacin HCl monohydrate

(B) Calibration curve using ciprofloxacin free base

Figure 6. NMR Data.

NMR signal as a function of percentage of drug substance as free base for intact and crushed tablets of ciprofloxacin (blue dots) when using (A) ciprofloxacin HCl monohydrate (red dots) and (B) ciprofloxacin free base (red dots) as reference standards. The data was generated using Mnova software. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

Figure 7. X-ray Power Diffraction Data.

X-ray power diffraction data for ciprofloxacin free base (top), ciprofloxacin drug product as cipro from the tablets (middle), and ciprofloxacin HCl monohydrate (bottom). Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons. [Please click here to view a larger version of this figure.](#)

Lot no.	Weight (gr)	% Weight free base	No. of Tablets	Label claim (mg)	RI Calibration average method			Mnova			RI Calibration fit method		
					Average (mg)	SD	%RSD	Average (mg)	SD	%RSD	Average (mg)	SD	%RSD
100213	9.19	14.69	15	90	94.08	0.26	0.27	94.09	0.26	0.28	94.68	0.46	0.49
D064074	8.62	15.67	15	90	90.97	0.24	0.26	90.99	0.21	0.23	90.11	0.35	0.39
1026356	8.58	15.73	15	90	89.15	0.68	0.77	89.15	0.68	0.76	88.25	0.03	0.04
D061829	8.83	15.64	23	60	58.94	0.19	0.32	58.94	0.19	0.32	59.22	0.73	1.23
10021308	9.62	15.59	50	30	29.79	0.23	0.78	29.79	0.23	0.78	29.68	0.15	0.49
D1026396	4.22	15.64	22	30	29.5	0.15	0.49	29.5	0.14	0.48	29.41	0.65	2.2
D118714	4.22	15.65	22	30	29.63	0.02	0.06	29.63	0.02	0.07	28.98	0.57	1.97

Table 1. Calculated Doses of Cinacalcet Free Base in Commercial Tablets, with their Statistics Calculated by the RI Calibration Software Package using the Average and Fit Methods and by the Mnova Software. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons.

Lot no.	Weight (gr)	% Weight	No. of Capsules	Label claim (mg)	Mnova			RI Calibration fit method		
					Average (mg)	SD	%RSD	Average (mg)	SD	%RSD
DV1891 (PREVACID) sample 1	4.71	6.38	20	15	16.36	0.21	1.31	16.04	0.63	3.95
DV1891 (PREVACID) sample 2	4.7	6.39	20	15	16.19	0.44	2.7	14.75	0.1	0.65
2GE2027 (generic) sample 1	4.61	6.51	20	15	16.17	0.18	1.13	15.14	0.69	4.59
2GE2027 (generic) sample 2	4.58	6.55	20	15	16.22	0.28	1.74	14.61	0.33	2.25

Table 2. Calculated Doses of lansoprazole in Brand-name and Generic Capsules, with their Statistics Calculated by the RI Calibration Software Package using the Average and Fit Methods and by the Mnova Software. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons.

Lot no.	Weight (gr)	% Weight free base (no water)	No. of Tablets	Label claim (mg)	Mnova			RI Calibration fit method		
					Average (mg)	SD	%RSD	Average (mg)	SD	%RSD
PUB 3030 (generic) tablets	7.72	64.76	10	500	515.26	5.39	1.05	509.48	8.53	1.67
PUB 3030 (generic) crushed tablets	5.53	64.7	7.15	500	511.17	3.64	0.71	500.1	11.42	2.28

Table 3. Calculated Doses of Ciprofloxacin Generic Cipro Tablets and Crushed Tablets, with their Statistics Calculated by the RI Calibration Software Package using the Average and Fit Methods and by the Mnova Software. Reprinted from⁵, copyright 2015, with permission from John Wiley & Sons.

Discussion

As more fluorinated drugs are becoming available in the market, we have developed a specific and simple methodology to measure the average drug content of fluorinated drug products using low-field ^{19}F TD-NMR⁵. We tested this method on three commercial drug products: tablets containing cinacalcet HCl, capsules containing lansoprazole, and tablets containing ciprofloxacin HCl monohydrate. The advantages of this method are its specificity due to the lack of fluorine in the excipients and its simplicity. We tested the measurement capabilities on intact formulations (either capsules or tablets) for cinacalcet and lansoprazole formulated drugs. However, in the case of ciprofloxacin formulated drug, we tested intact and crushed tablets to evaluate sample preparation. Part of the development of the method focused on the comparison of two software packages, RI Calibration (common software for the quantitation of TD-NMR data) and a recent commercially available version of Mnova software that can process TD-NMR data (Mnova is one of the common software packages for processing high-field NMR data). We evaluated both software packages on their ability to report the results and on their compatibility with standard commercial office software packages⁵.

Cinacalcet⁸ HCl (**Figure 1**) is formulated as tablets in three doses (30, 60, and 90 mg, accounted as free base). We tested several lots from those three commercial dose strengths using our ^{19}F TD-NMR methodology to calculate the average amounts of cinacalcet as free base in the tablets. Calibration curves for cinacalcet HCl were generated in triplicate, as indicated in the **Protocol**, using two software packages that provided linear calibration curves with good reproducibility (**Figure 3**). **Table 1** shows the results that agree well with the label claim for the doses tested, with good standard deviation and percent relative standard deviation (RSD) values.

Lansoprazole⁹ (**Figure 1**) is sold in a 15-mg formulated dose, which is a model system to test our methodology for low-dose capsule formulations. We tested our method, as described above, and used samples as-received. As in the case of the cinacalcet HCl tablets, the calibration curves, as triplicates, were linear and had good reproducibility (**Figure 4**). The results of the analysis show that the dose tested agrees well with the label claim, with good standard deviation and percent RSD values (**Table 2**). Ciprofloxacin¹⁰ monohydrate hydrochloride (**Figure 1**) is formulated in tablets containing ciprofloxacin HCl monohydrate, with a 500-mg dose as free base. As in previous cases, the calibration curves in triplicate were linear, with good reproducibility (**Figure 5**). The results of the analysis show that the dose tested agrees well with the label claim, with good standard deviation and percent RSD values (**Table 3**). In this case, we also evaluated the method using intact and crushed tablets to determine the effects of sample preparation. In both cases, the results were in good agreement with the label claim (**Table 3**), demonstrating that the average drug content results are barely affected by the preparation of the sample.

Our method using ^{19}F TD-NMR measures the average fluorinated drug content in formulated drug products, directly from their solid states as powders, tablets, or capsules, without the need of any other manipulation (e.g., dissolution, as in liquid chromatography (LC) methods). Therefore, the right chemical and the correct physical forms of the reference standards must be evaluated to determine their impact on the results of the analysis. To demonstrate this point, we built the calibration curves of ciprofloxacin with ciprofloxacin HCl monohydrate and its free base as the calibration standards. When the standard used for the calibration curve was the free base, the measurement of the formulated cipro tablets indicated 137.8% and 138.8% of drug substance in the formulated whole and crushed tablets, respectively (data represented in graph B

of **Figure 6** as blue dots). Obviously, these results are far from the label claim of the ciprofloxacin free base around 64.7% (**Table 3**). Graph A of **Figure 6** shows the results agreeing with the label claim when the calibration curve is done with ciprofloxacin HCl monohydrate (red dots) to measure the cipro and crushed tablets (blue dots). The differences between the results are due to incorrect physical and chemical forms used as the free base for the reference standard samples. This assessment was confirmed by comparing the XRPD patterns of ciprofloxacin free base, ciprofloxacin HCl monohydrate, and generic cipro tablets. Ciprofloxacin free base has a different crystal form than ciprofloxacin HCl monohydrate and cipro tablets due to the different XRPD patterns (**Figure 7**).

When comparing the software packages for calibration, both RI Calibration and Mnova produce satisfactory results. Mnova has the advantage of being more compatible with other commercial office software packages for statistical and scientific graphing and data analysis, which is useful for reporting. Mnova software is revised and updated every year, while the RI Calibration software is not. Regarding the two data analysis methods used in RI Calibration, the "fit" method provides reasonable results but is poorer than the "average" method because the "fit" method relies on extrapolation to the zero of the measured data, adding more uncertainty to the final results.

In conclusion, we have developed a simple methodology based on low-field ^{19}F TD-NMR to measure the average drug content of fluorine-containing drug products. This simple protocol provides results in good agreement with label claims in less time than classic methodologies (e.g., LC). The technique is non-destructive and does not require any sample manipulation, with complete sample recovery using intact forms during analysis (e.g., powder, tablets, and capsules). The standards used to build the calibration curve must have the same chemical and physical forms as the drug in the formulation form. Regarding the software packages used for the analysis (RI Calibration and Mnova), both are adequate, but Mnova is up-to-date and fully compatible with standard office software packages. Based on these conclusions, we foresee this methodology being implemented in laboratories and manufacturing plants as a process PAT tool in the pharmaceutical industry.

Disclosures

The authors have nothing to disclose.

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