Coronaviruses have long been circulating in human and animal populations. Common colds in humans are caused by coronaviruses 15% to 30% of the time and until recently were generally not considered life threatening. The COVID-19 pandemic fueled by the SARS-CoV-2 virus has brought attention to coronaviruses in humans and animals. Domestic, caged, and wild felines can be infected with the SARS-CoV-2 virus, but a feline coronavirus (FCoV) has long been circulating among felines.

OBJECTIVE

Feline infectious peritonitis is fatal, and due to lack of approved treatments, unregulated antiviral drugs are used to treat this disease. This study set out to determine the purity of various batches of these drugs from several companies, characterize them, and note any impurities or other unusual characteristics. We also developed a method to qualitatively assess the primary components before administration.

SAMPLES

We tested 30 vials from 17 brands of GS-441524 and 5 vials from 1 brand of GC376. We compared the GS-441524 to a control standard from Ambeed and the GC376 to a standard from Cayman Chemical.

METHODS

We recorded physical appearance, pH, absorbance, HPLC retention times, and thin-layer chromatography retention factors for all of the samples. Some samples were used for nuclear magnetic resonance and mass spectrometric analysis.

RESULTS

Some of the GS-441524 vials were 10% to 25% more concentrated than advertised, but most of the GS-441524 samples tested were similar in purity and composition, both between batches and between brands. We also tested 5 vials of GC376 and found that 1 of the 5 vials contained GS-441524 rather than GC376 and the other 4 vials contained molnupiravir.

CLINICAL RELEVANCE

While all of the GS-441524 vials contained GS-441524, none of the GC376 vials tested contained GC376. GC376 is used in cats that are unresponsive to GS-441524, and use of the wrong antiviral can cause serious side effects. We provide suggested methods for distinguishing one drug from the other in new batches.

Keywords: coronavirus, FIP, GS-441524, GC376, molnupiravir
which develops into FIP. Once a cat develops symptoms of FIP, it is fatal and until recently was considered incurable. Recent advances in antiviral therapies led to the design of new, effective nucleoside antimetabolites. One such compound, GS-441524, showed efficacy against feline coronavirus and low toxicity in cat cells (Figure 1).

GS-441524 is the active metabolite of remdesivir, a drug approved by the FDA for use in humans hospitalized for COVID-19. Remdesivir and GS-441524 act as antiviral antimetabolites that are designed to inhibit RNA viruses by preventing RNA-dependent RNA polymerase from replicating the viral genome. RNA-dependent RNA polymerases are more promiscuous than human polymerases, so these polymerases utilize triphosphorylated GS-441524 in place of adenosine triphosphate. This causes the polymerase to stall 3 nucleotides after incorporation of GS-441524 due to steric hindrance preventing the RNA-dependent RNA polymerase from translocating further.

In addition, other antivirals have been shown to be effective in treating FIP such as GC376 and molnupiravir (Figure 1). It was discovered that GC376 in combination with GS-441524 shortened treatment duration compared to either drug alone. GC376 is a protease inhibitor and acts through a different mechanism than GS-441524. GC376 binds to and inhibits the main protease of several RNA viruses including coronaviruses such as SARS, Middle Eastern respiratory syndrome, and FCoV and also other RNA viruses such as norovirus. Molnupiravir acts on the RNA-dependent RNA polymerase similarly to remdesivir and GS-441524.

Access to GS-441524 varies across the globe and has changed over time. In Australia and the United Kingdom, GS-441524 tablets are legal for use in cats with FIP. In the US, remdesivir has been

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**Figure 1**—Comparison of brands of GS-441524. A—Structures of remdesivir, GS-441524, GC376, and molnupiravir. B—Line graph showing the absorbance spectra of 3 brands: Oscar, Panda, and Shire with the GS-441524-positive control that are all advertised to be 15 mg/mL. C—Sample HPLC traces of Oscar, Panda, and Shire brands of GS-441524 with the positive control. D—A bar graph showing the advertised concentrations of all 17 GS-441524 brands tested in pink and the measured concentration in yellow. When multiple vials were tested, the measured concentration for each individual vial is shown as a dot, the average concentration is shown in the bar graph, and error bars are provided.
approved for use in humans and that allows for off-label use, but cost and accessibility have hindered widespread implementation. To bridge this gap, crowd-sourced social media groups orchestrate the shipping and distribution of unregulated antivirals for at-home treatment of FIP. In this study, we shed light on the purity and composition of these unregulated antiviral drugs. The results herein are invaluable for practitioners who may encounter cats treated by pet owners with these drugs. Researchers investigating the efficacy of GS-441524 in cats have reported a cat enrolled in a study was treated with a course of unregulated GS-441524 tablets. Understanding the potential variations in drug content and methods for distinguishing between different antivirals is essential for ensuring the safety and efficacy of subsequent veterinary interventions in cases where cats have been exposed to these substances.

Below we examine 17 brands of GS-441524 and 5 of GC376 sourced and provided by a social media group, FIP Global CATS. We tested for purity and concentration and characterized the components of these drugs.

## Methods

### Materials

MilliQ water was used throughout the experimental preparation and for dilutions. HPLC-grade acetonitrile was obtained from Oakwood Chemical. Trifluoroacetic acid was purchased from Millipore Sigma. Western Family 70% isopropanol was obtained from Smith’s Pharmacy. DMSO-d6 was obtained from Cambridge Isotope Laboratories, Inc. Optima-grade methanol for mass spectrometry (MS) samples was obtained from Fisher Chemical. UV cuvettes were obtained from GMBH. Antivirals were a convenience sampling of vials sourced and provided by members of FIP Global CATS apart from the “Panda” brand, which was donated by a pet owner who sourced them from MaxPaw. Vials were examined for evidence of tampering before use (Supplementary Figure S1).

The pharmaceuticals evaluated in this study, namely, GS-441524, molnupiravir, and GC376, are not legally available for use in the US for the treatment of FIP. GS-441524 is legally available for use in Australia and the United Kingdom, while molnupiravir is authorized for use in Cyprus. It is important to note that these drugs are neither approved by the FDA nor commercially available in the US for the specified purpose. Researchers and practitioners should exercise caution and adhere to applicable regulations when considering the use of these pharmaceuticals in the US or other jurisdictions where they are not approved for veterinary use.

### pH measurements

pH measurements were measured both using pH paper and a pH probe. For the GS-441524 vials, which were larger, we used a Mettler Toledo LE410 probe with a Mettler Toledo 5 easy plus meter. The GC376 vials were measured using an STmidroS probe from Ohaus and a Starter 3100 meter from Ohaus.

### High-performance liquid chromatography

The HPLC system is an Agilent Series 1260 infinity II (model G7157A; Agilent Technologies) with a variable wavelength detector (model G7114A; Agilent Technologies) and detected using a 1260 infinity II fraction collector (model G1364E; Agilent Technologies). All the analyses of data were done using Agilent OpenLab CDS ChemStation Edition Ver. Rev. C. 01.10[201]. The separation protocol was 45 minutes long, using an Xbridge prep C18 5-µM OBD column starting at 5% buffer B and going to 100% buffer B in the first 35 minutes and then staying at 100% B for 10 minutes. Buffer A was 99.9% MilliQ water with 0.1% trifluoroacetic acid, and buffer B was 90% acetonitrile, 9.3% MilliQ water, and 0.7% trifluoroacetic acid.

### Thin-layer chromatography

Thin-layer chromatography (TLC) is described in detail (Supplementary Material S1). Briefly, TLC silica gel 60 F254 from Millipore was used. Samples were diluted 1:100 in water before application. The GS-441524 plates were developed in 100% isopropanol from Fisher Scientific, and the GC376-containing plates were developed in pure ethyl acetate.

### Spectroscopy

Absorbance spectra and fluorescence were measured using a Molecular Devices SpectraMax MS spectrophotometer and were recorded and analyzed using SoftMax Pro 7.1 from Molecular Devices. Samples were diluted 1:1,000 in MilliQ water and pipetted into a disposable UV-cuvette semi-micro from GMBH.

### Mass spectrometry

Mass spectrometry was performed at either the University of Utah Department of Chemistry Mass Spectrometry Facility with a Waters Xevo G2S Q-ToF w/Acquity I Class UPLC Tandem Mass Spec, or on a Waters Acquity QDA detector. Mass spectra are recorded in positive ion mode. In the MS facility, MassLink Workstation software, including Qualitative Analysis (version B.07.00; Agilent), was used for processing both raw MS and MS-MS data, including molecular feature extraction, background subtraction, data filtering, and molecular formula estimation. The raw data were processed using the Find by Molecular Feature (MF) algorithm called Molecular Feature Extractor (MFE) within MassHunter Qualitative Analysis software. Extracted molecular features were processed to create a list of compounds. Data recorded on the QDA detector were analyzed using Mass Lynx V4.2 (Waters).

### Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectra were recorded at the University of Utah Nuclear Magnetic Resonance Facility using a Varian Inova 400 MHz or a Bruker Avance III 600 MHz spectrometer. The spectra were recorded with 2D correlation algorithms such as TOCSY and HSQC. The spectra were processed using MestReNova software, and the chemical structures were assigned using the Varian NMRSolver Software.
Resonance core. $^1$H and $^{13}$C NMR spectra were on a Varian Mercury 400-MHz spectrometer. 2-D NMR spectra were recorded on a Varian Inova 500-MHz spectrometer. $^1$H-NMR spectra were acquired at 400 MHz, and the chemical shifts (δ) of proton resonances were reported relative to the residual solvent peak (2.50 ppm for DMSO-d6).

Results

Samples

For GS-441524, we obtained samples with 17 different labels. For Oscar, Panda, Shire, and Trusted, we tested multiple vials. We measured absorbance and pH on 4 vials of Oscar, 8 vials of Panda, 8 vials of Shire, and 2 vials of Trusted. In addition, we received and analyzed 5 vials of GC376 all from the same manufacturer, which we refer to as JJ. Photos were taken, and an inventory of the tested vials was made (Supplementary Figure S1).

Comparison of GS-441524 between manufacturers and vials

We measured the pH of each sample using both pH paper and a pH probe, and all of the vials measured similarly in low pH, all are under 2 in agreement with the original safety and efficacy study of GS-441524, which used a pH of 1.5. We also ran TLC plates and calculated retention factors for most of the brands (Table 1). Raw data are shown (Supplementary Figure S2).

We next calculated the concentrations of each vial of GS-441524 vial. We used the positive control purchased from Ambeed to make GS-441524 standard curves at 3 different wavelengths (Supplementary Figure S3). We determined that the extinction coefficient of GS-441524 in MilliQ water at 240 nm is 31,710 M$^{-1}$·cm$^{-1}$ and at 245 nm is 25,336 M$^{-1}$·cm$^{-1}$, within 5% of the published extinction coefficient of remdesivir triphosphate at 245 nm of 24,100 M$^{-1}$·cm$^{-1}$. We used this extinction coefficient to calculate the concentration of each vial of GS-441524. We did not receive duplicates of every brand, but we did receive multiple vials of Oscar, Panda, Shire, and Trusted, and we found that most of the concentrations did not vary dramatically between vials; the SDs were all less than 1 mg/mL. The highest difference was seen in Panda with an SD of 0.71 mg/mL. Shire had an SD of 0.71 mg/mL, and the variation was lowest with Oscar at 0.55 mg/mL.

For 14 out of 17 of the brands tested, the measured concentrations were within 10% of the concentration claimed by the company. For 3 of the brands, Oscar, Panda, and Karma, the concentrations were over 10% higher than advertised: Karma was 12% higher, Oscar was 18% higher, and Panda was 22%. We compared the observed concentration to the manufacturer’s claimed concentrations (Figure 1).

In contrast, the Shire vials were dramatically under concentrated. We received over 50 vials and chose 8 vials to be tested at random. We found the Shire vials to be consistently under concentrated, averaging nearly 50% below the marketed value.

We used HPLC to purify the GS-441524 from the diluents and to determine purity. Each run of the crowdsource vials contained a major peak consisting of over 80% of the AUC at 220 nm. The masses of the major peaks from Shire, Panda, Oscar, and the positive control had an m/z of 292.105, which matched the expected protonated mass of 292.10 (Supplementary Figure S4). To validate the MS data, we also ran NMRs of the Shire, Panda, and positive control samples. The samples were prepared differently, so the proton peaks were shifted between samples (Supplementary Figure S5). However, the heteronuclear single quantum coherence results (Supplementary Figure S6) show similar patterns in

Table 1—Results of testing of 10 brands of unregulated GS-441524 from lowest to highest calculated concentration and a positive control from Ambeed.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Advertised concentration</th>
<th>Calculated concentration</th>
<th>pH</th>
<th>240-nm purity (%)</th>
<th>Retention time (min)</th>
<th>Retention factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shire</td>
<td>15 mg/mL</td>
<td>8.73 mg/mL</td>
<td>1.31</td>
<td>84.9</td>
<td>6.281</td>
<td>0.5</td>
</tr>
<tr>
<td>Seka</td>
<td>15 mg/mL</td>
<td>14.5 mg/mL</td>
<td>1.99</td>
<td>90.0</td>
<td>6.334</td>
<td>0.5</td>
</tr>
<tr>
<td>Trusted</td>
<td>15 mg/mL</td>
<td>14.7 mg/mL</td>
<td>0.61</td>
<td>85.0</td>
<td>6.345</td>
<td>0.5</td>
</tr>
<tr>
<td>Lucky 15</td>
<td>15 mg/mL</td>
<td>16.0 mg/mL</td>
<td>1.60</td>
<td>92.8</td>
<td>6.402</td>
<td>0.5</td>
</tr>
<tr>
<td>Oscar</td>
<td>15 mg/mL</td>
<td>17.7 mg/mL</td>
<td>1.82</td>
<td>88.4</td>
<td>6.426</td>
<td>0.5</td>
</tr>
<tr>
<td>Valor</td>
<td>17 mg/mL</td>
<td>17.8 mg/mL</td>
<td>1.58</td>
<td>86.3</td>
<td>6.263</td>
<td>0.5</td>
</tr>
<tr>
<td>Panda</td>
<td>15 mg/mL</td>
<td>18.3 mg/mL</td>
<td>1.50</td>
<td>88.7</td>
<td>6.346</td>
<td>0.5</td>
</tr>
<tr>
<td>Rainbow</td>
<td>20 mg/mL</td>
<td>19.9 mg/mL</td>
<td>1.64</td>
<td>89.3</td>
<td>6.403</td>
<td>0.5</td>
</tr>
<tr>
<td>Lanzi</td>
<td>20 mg/mL</td>
<td>19.9 mg/mL</td>
<td>1.53</td>
<td>91.9</td>
<td>6.364</td>
<td>0.5</td>
</tr>
<tr>
<td>Karma</td>
<td>18 mg/mL</td>
<td>20.1 mg/mL</td>
<td>1.62</td>
<td>88.5</td>
<td>6.318</td>
<td>0.5</td>
</tr>
<tr>
<td>Ambeed</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>91.9</td>
<td>6.297</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Concentration was measured using absorbance at 240 nm. pH was measured using a pH probe. Retention time and purity were measured using HPLC. The retention factors were calculated using thin-layer chromatography plates developed in pure isopropanol.

N/A = Not available.
the proton $^{13}$C correlations between samples indicating to us that these were likely all GS-441524 in different buffer conditions.

We also found a minor peak that consisted of less than 5% of the total area. The minor peak had a retention time of around 8 minutes compared to the average of 6.34 minutes for GS-441524 (Table 2). This impurity absorbs at 240 nm like GS-441524, so the concentration measurements (Table 1) were slightly inflated. The concentrations were also shown corrected for percent purity at 240 nm. The fractions containing the impurity were found to have a larger m/z of 328 g/mol and are shown for Shire, Panda, and Oscar (Supplementary Figure S7). This impurity was contaminated with polyethylene glycol (PEG) even after being HPLC purified. HPLC purified and lyophilized peak 2 was a transparent liquid as if PEG was still present, and the results found in NMR analyses of the liquid were indicative of PEG. The percentage of the impurity measured at 240 nM ranged from 0.7% in Trusted to 4.7% in Panda. These impurities were not visible by TLC but were measurable with HPLC. We hypothesize that these larger impurities were oxidized GS-441524 possibly due to the cyanide group oxidized to an amide and oxidation of nitrogen 1 of the purine ring. These products have been published to occur as remdesivir degradation products and would correspond to the 328 g/mol mass.30

Comparison of GC376 vials

We received 5 GC376 vials, which we labeled JJ1 through JJ5. JJ1 and JJ2 were reportedly older while JJ3, JJ4, and JJ5 were all from the same newer batch. We first completed an inventory and inspection of these vials (Supplementary Figure S8). We compared the 5 vials using absorbance, TLC, and HPLC (Figure 2). We measured the absorbance spectra of all 5 vials. Of the 5 curves none matched the GC376 standard, and 1 did not match the other 4. The one that differed from the others, JJ1, looked similar to GS. While examining JJ1, we applied aliquots of JJ1 to Kim wipes and used a UV lamp to measure fluorescence and JJ1 fluoresced while JJ2 did not (Supplementary Figure S9). The fluorescence spectrum of JJ1 is comparable to the fluorescence spectra of our GS-441524-positive control with a fluorescence maximum at 420 nm when excited at 250 nm (Supplementary Figure S10). In addition, the pH of JJ1 was under 2 like the GS-441524 samples. We ran TLC plates on JJ1 and JJ2 along with the GS-441524-positive control and samples of Panda and Shire (Supplementary Figure S11).

JJ1 fluoresced like the GS-441524 and had a comparable retention factor of 0.025 whereas JJ2 had a larger retention factor of 0.186 and was not fluorescent. We ran HPLC and the traces of JJ1 alone, JJ2 alone, and JJ1 and JJ2 combined. JJ1 has a retention time of 6.43 minutes comparable to that of the GS-441524 average of 6.34 minutes, and the major peak of JJ2 has a retention time of 10.3. Finally, the m/z of JJ1 was 291.104, which matched the positive control for GS-441524 (Supplementary Figure S12). The concentration of GS-441524 in JJ1 was calculated to be 10.7 mg/mL.

We recorded the pH, retention times, and retention factors of all 5 vials (Table 3). We obtained a standard for GC376 from Cayman Chemical to use as a standard. The identity of the standard was confirmed by the mass of the positive control with an expected m/z of 404 (Supplementary Figure S13). Using HPLC, we observed the GC376 standard displays 2 major peaks caused by the spontaneous loss of the sulfate moiety as is expected in aqueous solutions. The formation of the aldehyde creates a mixture of stereoisomers due to epimerization.31 The HPLC traces of the GC376 standard compared to all the JJ vials were markedly different (Figure 3). We also recorded the absorbance spectra of all samples and GC376. The published $\lambda_{max}$ for GC376 is 206 nm in NaCl, which is similar to the $\lambda_{max}$ of the major peak of our GC376 standard at 215 nm in MilliQ water.31 However, the absorption spectra for JJ2 to JJ5 showed 2 peaks, which do not align with any published GC376 spectra. Another antiviral drug, molnupiravir (EIDD-2801), does have a similar bimodal fluorescence maximum at 420 nm when excited at 250 nm (Supplementary Figure S10). In addition, the pH of JJ1 was under 2 like the GS-441524 samples. We ran TLC plates on JJ1 and JJ2 along with the GS-441524-positive control and samples of Panda and Shire (Supplementary Figure S11).

<table>
<thead>
<tr>
<th>Brand</th>
<th>Advertised concentration</th>
<th>240-nm Purity (%)</th>
<th>Calculated concentration corrected for 240-nm purity</th>
<th>Percent major impurity at 220 nm (%)</th>
<th>Percent major impurity at 240 nm (%)</th>
<th>Impurity peak retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shire</td>
<td>15 mg/mL</td>
<td>95.3</td>
<td>15.7 mg/mL</td>
<td>1.1</td>
<td>1.0</td>
<td>7.717</td>
</tr>
<tr>
<td>Seka</td>
<td>15 mg/mL</td>
<td>95.5</td>
<td>13.8 mg/mL</td>
<td>2.0</td>
<td>2.0</td>
<td>7.557</td>
</tr>
<tr>
<td>Trusted</td>
<td>15 mg/mL</td>
<td>95.5</td>
<td>13.5 mg/mL</td>
<td>4.3</td>
<td>4.2</td>
<td>7.613</td>
</tr>
<tr>
<td>Lucky 5</td>
<td>15 mg/mL</td>
<td>98.1</td>
<td>15.7 mg/mL</td>
<td>1.1</td>
<td>1.0</td>
<td>7.717</td>
</tr>
<tr>
<td>Oscar</td>
<td>15 mg/mL</td>
<td>95.3</td>
<td>16.9 mg/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>7.740</td>
</tr>
<tr>
<td>Valor</td>
<td>17 mg/mL</td>
<td>97.3</td>
<td>17.3 mg/mL</td>
<td>1.2</td>
<td>1.2</td>
<td>7.631</td>
</tr>
<tr>
<td>Panda</td>
<td>15 mg/mL</td>
<td>94.0</td>
<td>17.2 mg/mL</td>
<td>4.2</td>
<td>4.1</td>
<td>7.648</td>
</tr>
<tr>
<td>Rainbow</td>
<td>20 mg/mL</td>
<td>97.4</td>
<td>19.4 mg/mL</td>
<td>0.6</td>
<td>0.7</td>
<td>7.700</td>
</tr>
<tr>
<td>Lansi</td>
<td>20 mg/mL</td>
<td>97.4</td>
<td>19.4 mg/mL</td>
<td>0.9</td>
<td>0.9</td>
<td>7.686</td>
</tr>
<tr>
<td>Karma</td>
<td>18 mg/mL</td>
<td>96.8</td>
<td>19.5 mg/mL</td>
<td>1.0</td>
<td>1.1</td>
<td>7.622</td>
</tr>
<tr>
<td>Ambeed</td>
<td>N/A</td>
<td>95.3</td>
<td>N/A</td>
<td>0.9</td>
<td>1.0</td>
<td>7.740</td>
</tr>
</tbody>
</table>

The percent purity at 240 nm was used to calculate the corrected and more accurate concentrations. Next is shown the percentage of the major impurity recorded at either 220 nm or 240 nm, and finally the impurity retention time.
absorption spectrum. The m/z for JJ2 was 330.14, which corresponds to the mass of molnupiravir + H+ confirming that the drugs in vials JJ2 to JJ5 were indeed molnupiravir rather than GC376. Molnupiravir has shown efficacy against COVID-19 and has also been used in felines with FIP but has demonstrated characteristic side effects including, “folded ears, losing whiskers, and severe leukopenia.” The fact that the “GC376” vials were actually mislabeled samples of molnupiravir explains the observation of these side effects in cats treated with the vials (personal communication).

To differentiate between antiviral compounds, specifically GS-441524, GC376, and Molnupiravir, we investigated several chemical techniques aiming for accessibility and reliability. Among the methods investigated, we found that pH measurements proved informative, particularly in discerning GS-441524, which is generally stored at a pH under 2. pH measurements can be helpful. However, we found that using TLC was the most reliable, versatile, and straightforward method for qualitative differentiation of the 3 drugs. We accomplished this by using a fine needle to drop a small spot of each drug on a TLC plate with fluorescent backing. When visualized under a high-energy UV light (254 nm), each drug is visibly different (Figure 3). The fluorescence of GS-441524 is visible as a blue glow. In contrast, the efficient light absorption of Molnupiravir resulted in a dark spot. GC376 is the least absorbent resulting in a faint spot when compared to the other 2. These distinctions can be further accentuated by developing the TLC plates using 95% ethanol are described (Supplementary Material S1). We acknowledge that the best practices require more advanced analytical methods and result in a Certificate of Analysis with FDA approval and adherence to the associated.

Table 3—Results of testing 5 vials of unregulated GC376.

<table>
<thead>
<tr>
<th>Vial</th>
<th>pH</th>
<th>Retention time (min)</th>
<th>Retention factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>JJ1</td>
<td>1.87</td>
<td>6.436</td>
<td>0.03</td>
</tr>
<tr>
<td>JJ2</td>
<td>5.97</td>
<td>10.323</td>
<td>0.2</td>
</tr>
<tr>
<td>JJ3</td>
<td>7.10</td>
<td>10.095</td>
<td>0.2</td>
</tr>
<tr>
<td>JJ4</td>
<td>7.17</td>
<td>10.105</td>
<td>0.2</td>
</tr>
<tr>
<td>JJ5</td>
<td>7.25</td>
<td>10.164</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The retention factor was calculated using thin-layer chromatography plates developed in pure ethyl acetate.

Figure 2—Comparison vials of GC376. A—Line graph showing the absorbance spectra of all 5 vials of GC376, labeled JJ1 to JJ5, and the positive control GS-441524. B—Sample thin-layer chromatography (TLC) plate developed in ethyl acetate comparing JJ1 and JJ2 to the positive control, Panda (P or P1), and Shire (Sh or SH1). A bar graph showing retention factors (Rf) of how far the compound traveled over how far the solvent traveled on the plate. The stars represent a *P < .01. C—HPLC trace of milli-absorance units (mAU) measured at 240 nm showing JJ1 in blue and JJ2 in orange. D—An HPLC trace of milli-absorance units (mAU) measured at 240 nm showing the coinjection of 2:1 JJ1 to JJ2. JJ = Manufacturer 金俊, jīn jùn.
regulations; however, our proposed method serves as a practical approach for preliminary differentiation of these 3 antivirals.

Discussion

Feline infectious peritonitis remains a significant concern in cats, particularly in cats that are young or with weakened immune systems. New antiviral drugs have been discovered that show efficacy against FIPV including GS-441524, GC376, and molnupiravir. Despite the effectiveness demonstrated by these antivirals, the lack of FDA approval for use of these drugs in cats in the US and the lack of access to approved remdesivir poses challenges for the treatment of FIP. Anivive Lifesciences is actively pursuing approval for GC376, but the lengthy approval process prompts pet owners to seek unregulated medications through crowdsource groups. These drugs can have limited characterization and quality control, so we sought to determine the components and purity of these medications.

Our study examined 30 vials with 17 different labels of GS-441524. We found that the properties of all the GS-441524 vials matched controls including absorbance, fluorescence, HPLC retention time, and retention factors on TLC. In addition, 13/17 brands closely matched the manufacturer concentration claims. Three vials were slightly overconcentrated (10% to 25%), and 1 was significantly under concentrated. An observed impurity in some brands correlated with perceived quality by crowdsource group administrators, suggesting their qualitative assessments align with our findings.

We also analyzed 5 vials advertised to contain 53 mg/mL of GC376, and contrary to expectation, we found that none of the tested vials contained GC376. One vial contained GS-441524, and 4 contained molnupiravir determined by HPLC retention time, fluorescence, absorbance, and MS. The vial containing GS-441524 had a concentration of 10.7 mg/mL. GC376 is commonly used as a second-line, add-on therapy for cats unresponsive to GS-441524 alone. GS-441524 is most used at a dosage of 10 mg/kg, but doses in cats unresponsive to lower doses GS-441524 have been treated at as high as 50 mg/kg. The addition of 6 to 10 mg/kg GS-441524 from the mislabeled GC376 could result in unnecessary toxicity. GS-441524 has limited solubility in water and has been shown at high concentrations to precipitate causing stones in cats. We propose a straightforward approach for assessing medication identity before use in cats. We acknowledge that the best practices require more advanced analytical methods and result in a Certificate of Analysis with FDA approval and adherence to the associated regulations; however, our proposed method serves as a practical approach for preliminary differentiation of these 3 antivirals.

This study provides an overview of several brands of antivirals and is restricted to a convenience sample acquired during 2021 and 2022. In addition,
the results were constrained because 13 of the labels analyzed were limited to only 1 vial, and only liquid formulations were tested. More research is needed for similar analysis on pill formulations of these antivirals. It is unclear if these results are applicable to other brands or batches. Given these results, veterinary practitioners are encouraged to inform pet owners that the use of unregulated antivirals in cats may lead to the administration of unpredictable antiviral identities and concentrations.

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**Disclosures**

The authors have nothing to disclose.

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**References**


**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org