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Stability of Broad-Spectrum Antibiotic Ciprofloxacin at Elevated Temperatures via Liquid Chromatography-Mass Spectrometry

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Abstract

Ciprofloxacin (Cipro) is a commonly prescribed antibiotic, with a storage temperature of 35.6°F- 59°F (2°C-15°C). Recommended storage temperatures are assigned to antibiotics to maintain their integrity in treating bacterial infections. Recent studies have shown evidence for degradation of cipro and cephalexin when exposed to relatively high temperatures. Thermal degradation of the antibiotic can render it ineffective in treating infections. Therefore, it is important to evaluate the integrity of cipro at elevated temperature conditions.
Liquid chromatography-mass spectrometry is a well-established analytical technique for detecting a variety of antibiotics. Cipro is detected in this study using LC-MS and a caffeine internal standard after exposure to temperatures ranging from 93°C-135°C, simulating conditions in which antibiotics may be incorrectly stored. The established temperature range for cipro will be used to further investigate specific conditions at which the antibiotics degrade. A particular temperature will be evaluated at varying exposure times to establish a threshold exposure time. The threshold indicates the time at which the antibiotic shows evidence for degradation. Multiple temperature conditions and exposure times will be examined for this study. The data will be used to determine the conditions in which cipro is at risk for degradation, potentially hindering its ability to treat bacterial infections.

Keywords: Thermostability, Ciprofloxacin, Liquid Chromatography-Mass Spectrometry

1. Introduction

Antibiotics are typically assigned storage temperatures and conditions so that they maintain integrity in treating bacterial infections. A commonly prescribed broad-spectrum antibiotic, Ciprofloxacin (M.W. 331.4 g/mol), has a recommended storage temperature range of 35.6°F- 59°F (2°C-15°C)[1-2, 6]. Cipro (Figure 1) is used to combat a variety of bacterial infections, such as bone, skin, lungs, and urinary tract infections[1,6]. A unique advantage of cipro is that it can also be used to treat patients exposed to anthrax[1,6]. As part of the fluoroquinolone class of antibiotics, cipro works to target and inhibit the bacterial enzyme DNA gyrase. DNA gyrase is critical in the process of unwinding DNA during replication. Fluoroquinolones like cipro prevent bacterial cells from replicating DNA and therefore prevent bacterial cell proliferation.

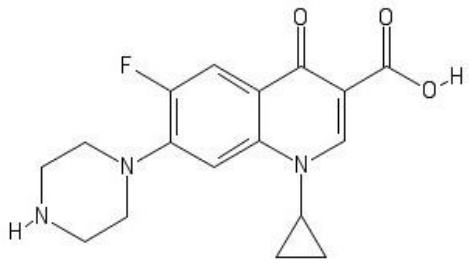

Figure 1. Chemical structure of broad-spectrum antibiotic ciprofloxacin

Antibiotics can often be unintentionally stored in temperatures outside of the recommended range. For example, a car's interior can reach temperatures 50 °F (10 °C) higher than the outdoor temperature. In some cases, that can be as high as 150 °F (65 °C)[5,9]. Heated stovetops are another common area where elevated temperatures can be found. Stovetop temperatures can range anywhere from 140 °F to 500 °F (60 °C to 260 °C)[12]. At a medium setting, for example, temperatures can easily reach 200 °F (93 °C) at a minimum. Previous studies suggest that when an antibiotic is exposed to these elevated temperatures, it can become ineffective in treating bacterial infections[2-4,7-8,10]. This investigation seeks to further explore, this claim.

Data for this determination will be obtained via liquid chromatography-mass spectrometry (LC-MS). LC-MS is an established analytical technique used for detecting a wide array of antibiotics[3-4,8,10]. LC-MS is used to determine the presence of a particular antibiotic, as well as the amount, within a sample. This technique has been used for the detection of antibiotics in sources such as soil, water, blood, and urine[3-4].

2. Methodology

2.1. Preparation Of Internal Standard Solutions

Caffeine powder, obtained from Aldrich Chemical Company (Milwaukee, WI, USA), was used to prepare an internal standard solution of 10 ppm caffeine in deionized water. Internal standards help account for signal fluctuations from the MS detector, thus increasing the precision of the results.

2.2. Preparation Of Laboratory Standards

A stock solution was prepared by dissolving cipro, obtained from MP Biomedicals (Solon, OH, USA), at a concentration of 100 ppm in deionized water. Fresh stock solution was prepared before each experiment and was kept refrigerated at 4 °C in the dark. Standards of cipro (1, 5, 10, 25, and 40 ppm) were prepared by a serial dilution of the stock with deionized water. These standards were also used as the control samples within the experiment, as they were not exposed to elevated temperature conditions.

2.3. Preparation Of Samples For Thermostability Experiments

Powdered samples of cipro (about 1.0 g) were exposed to elevated temperatures of 93°C, 100°C, and 135°C for time periods ranging from 1 hour to 16 hours. Experimental samples of cipro were prepared at concentrations of 5, 10, 25, and 40 ppm using the cipro exposed to each temperature condition. The same procedure as described in Methodology 2.2 was used for the experimental samples.

2.4. Liquid Chromatography

The LC system used was a Thermo Finnigan Surveyor system (San Jose, CA, USA) equipped with an autosampler. LC separations were obtained with a Restek Raptor C18 reversed phase column (Bellefonte, PA, USA) having dimensions of 2.1 mm x 50 mm and a particle size of 2.7 µm. The sample injection volume was 5 µL. An isocratic

mobile phase consisting of 75% deionized water with 0.1% formic acid and 25% methanol with 0.1% formic acid was used at a flow rate of 250 µL/min. Total analysis time for each sample was 3 minutes. All laboratory standards and samples were run in sets of five.

2.5. Mass Spectrometry

The MS system used was a Finnigan MAT LCQ ion trap equipped with a heated capillary interface and an electrospray ionization source. The MS was operated in positive ion mode. Thermo Scientific Xcalibur software was used to control all experimental conditions as well as analyze the quantitative data.

3. Data

3.1. Analysis Of Standards

The detection of cipro and caffeine internal standard was accomplished in 3 minutes using an isocratic LC method and electrospray ionization source. Selected ion monitoring has been used as an analytical technique to study many antibiotics[10]. The XCalibur software can reduce background using programs that detect signal from only the masses of interest. Each ion, specifically m/z = 195 (caffeine) and m/z = 332 (cipro), was monitored as a function of time, resulting in a selected ion mass chromatogram. The area of each chromatographic peak (Figure 2) was analyzed using the XCalibur software.

A calibration curve was created using the standards made using Methodology 2.2. The curve was established over a range of 1-40 ppm for cipro, and a constant concentration of 10 ppm for caffeine (Figure 3). All standard solutions were run in sets of five. The chromatographic peak areas of the analyte and internal standard were obtained for each concentration. The ratio of chromatographic peak areas for cipro to caffeine was calculated and plotted against cipro concentration. A linear relationship with a correlation coefficient R of 0.9725 was established.

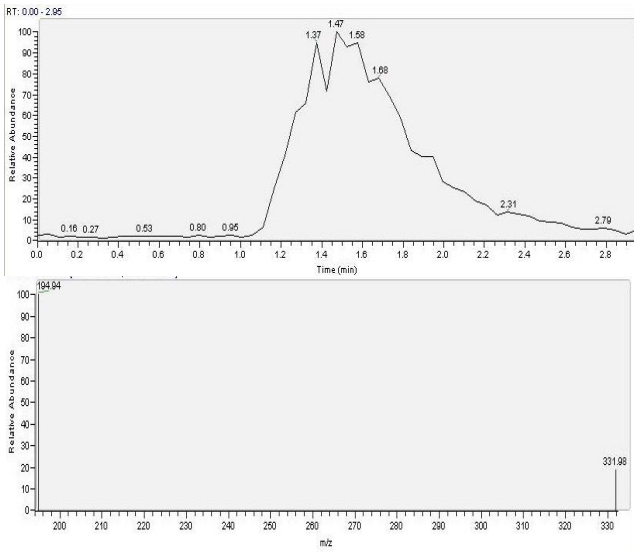

Figure 2. Selected ion mass chromatogram of ciprofloxacin with caffeine internal standard

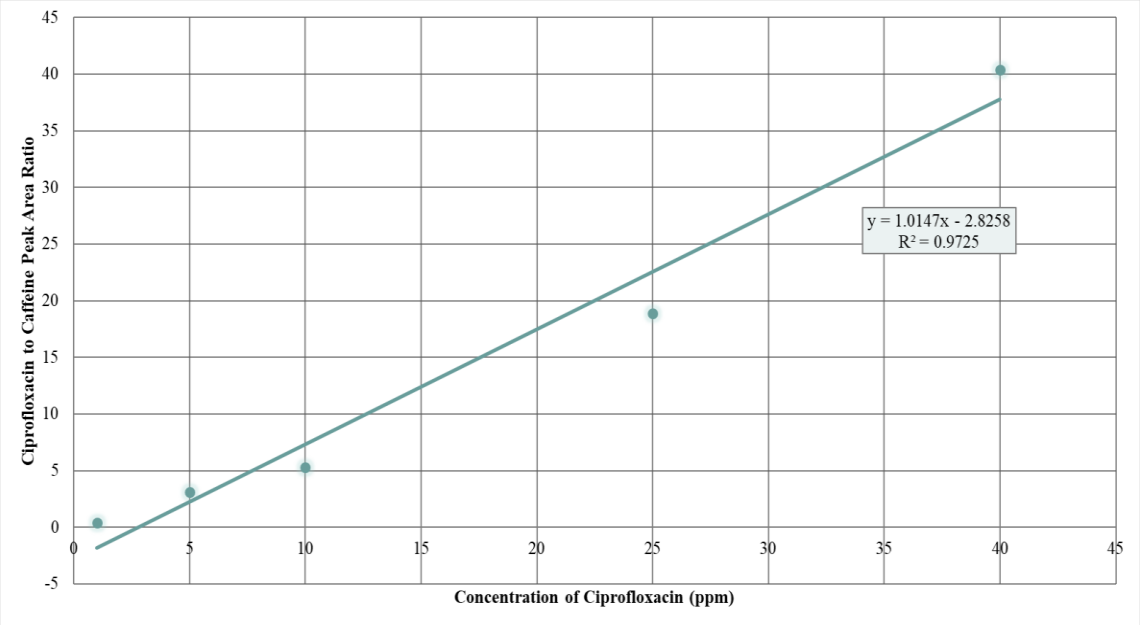

Figure 3. Internal standard calibration curve for ciprofloxacin (1-40 ppm) with caffeine standard (10 ppm)

3.2. Analysis Of Cipro At Various Temperatures

After solid cipro was exposed to elevated temperatures, as described in Methodology 2.3, samples were prepared in deionized water with a 10 ppm caffeine internal standard. Using the same LC-MS method, experimental cipro samples were analyzed in sets of five. Peak area ratios of cipro to caffeine were calculated at each concentration and condition. These values were then interpolated with the calibration curve to find the apparent concentration of cipro in the experimental samples.

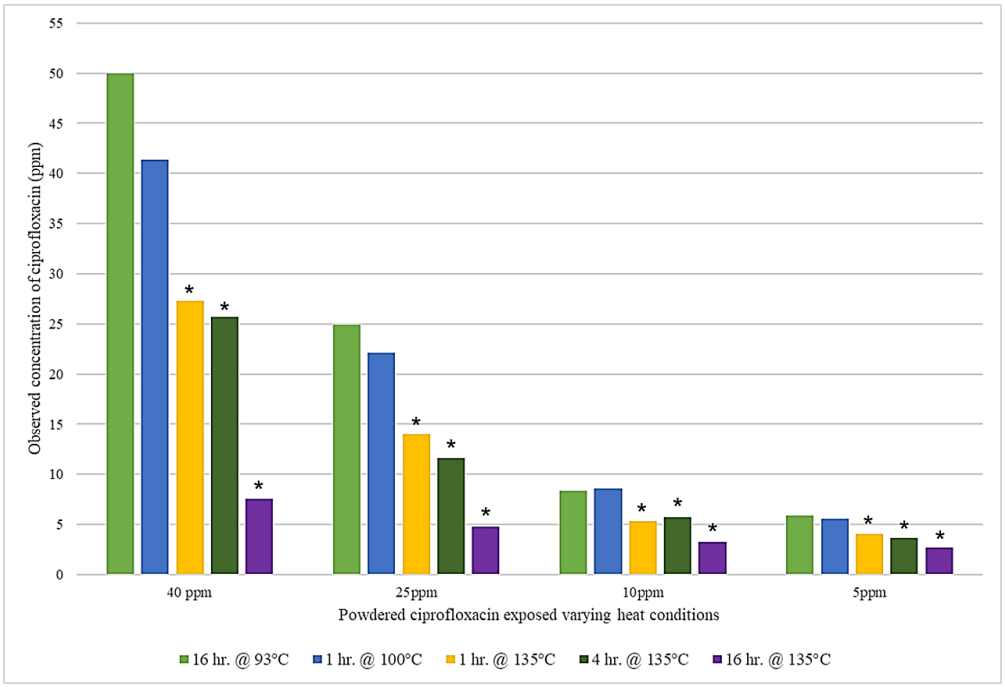

Figure 4. Observed concentrations of heat-treated ciprofloxacin in solution

A t-test was conducted at the 95% confidence interval to determine if there was a significant difference between the concentrations of standard and experimental cipro samples after exposure to elevated temperatures at increasing time intervals (Figure 4). Samples for which a significant difference was observed are marked in Figure 4 with a single asterisk. The percent concentration of cipro remaining after these exposure conditions was also calculated and results are presented in Table 1.

Table 1. Percent concentration remaining of heat-treated ciprofloxacin in solution after various exposure conditions

Percent (%) cipro remaining after exposure for:	**40 ppm**	**25 ppm**	**10 ppm**	**5 ppm**
16 hr. @ 93°C	125	99.6	83.1	118
1 hr. @ 100°C	104	88.4	85.9	111
1 hr. @ 135°C	68.3	56.0	52.9	81.6
4 hr. @ 135°C	64.3	46.4	57.0	72.8
16 hr. @ 135°C	18.9	18.9	32.3	54.2

Cipro samples heated at 93°C for 16 hours showed no significant degradation in concentration (Figure 4). However, for exposure conditions of 135°C for all time intervals of 16, 4, and 1 hours, there was a significant decrease in apparent cipro concentration. The data also shows that at a temperature of 135°C, there is an increase in cipro degradation as exposure time increases (Table 1). Intermediate exposure conditions of 100°C for 1 hour showed no significant decrease in apparent cipro concentration.

4. Conclusion

Elevated temperatures were shown to cause thermal degradation of the broad-spectrum antibiotic cipro. Exposure conditions of 135 °C for just one hour and beyond showed to have a significant impact on the thermal degradation of cipro. This temperature is comparable to that of a hot stovetop, which may be an unlikely location for antibiotic storage, but gives a starting point from which to determine a threshold temperature for degradation.

According to the data, an increase in exposure temperature is shown to directly affect the thermal degradation of cipro. As the temperature increases from 93 °C to 135 °C, there is an increase in the degradation measured by LC-MS (Table 1 and Figure 4). There is also a direct correlation between the level of antibiotic degradation and the amount of exposure time. At 135 °C, as the amount of exposure time increases, the apparent concentration of cipro decreases. This begs the possibility that cipro may also significantly degrade at lower temperatures than 135 °C, if the antibiotic is exposed for longer periods of time, e.g. 100 °C for 16 hours.

One area of improvement in this experiment is the way in which antibiotic samples were heat-treated and prepared for study. The communal oven that was used for creating a high temperature environment for cipro could have had fluctuations in humidity and other factors that could affect the stability of the antibiotic. This could ultimately influence the apparent concentration of cipro that was measured in each sample. Additionally, the data calculated in Table 1 shows an increase in percent cipro remaining after exposure to temperatures of 93°C and 100°C at concentrations of 40 ppm and 5 ppm. This indicates that there may have been issues with sample preparation or signal drift from the instrumentation.

5. Future Work

Further experiments of this kind would seek to determine the threshold temperature and exposure times at which cipro shows evidence for degradation. The temperatures studied in this experiment reflect conditions in which antibiotics are unlikely to be stored. An interesting study could investigate the effects of temperature and humidity on cipro degradation, which would simulate conditions more similar to that of real-world environments.

There are other multiple other methods that can be used to study this phenomenon. Further research on this topic may include other instrumentation, such as Nuclear Magnetic Resonance or Mass Spectrometry-Mass Spectrometry (MS-MS), to detect the antibiotic of interest and its subsequent components during degradation. Previous work completed by Maia et. al reveals degradation pathways and products of fluoroquinolone antibiotics. The researchers proposed six different products that result from the degradation or transformation of cipro[6]. Often these products include reduced or oxidized carboxylic acid groups, or destruction of the piperidine ring. Consistent among all proposed products, however, is the conservation of the quinolone group, central to cipro and other fluoroquinolone antibiotics. Tandem mass spectrometry can be used to analyze cipro and its chemical fragments by their mass-to-charge ratios. These fragments can be further to potentially determine the degradation pattern of cipro. Future studies can also include a biological approach by employing the Kirby-Bauer Method or a broth dilution to determine the antibacterial efficacy of the antibiotic before and after exposure to elevated temperatures.

6. Acknowledgements

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7. References

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