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Thermostability Analyses of Broad Spectrum Antibiotics Cephalexin and Ciprofloxacin via Liquid Chromatography-Mass Spectrometry

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Abstract

Cephalexin and ciprofloxacin are broad spectrum antibiotics that are used to treat common bacterial infections in humans. Cephalexin is considered a beta-lactam antibiotic, specifically part of the cephalosporin class of antibiotics. The antibiotic targets the bacterial cell wall and inhibits its growth. It is most effective in treating Gram-positive cocci but is somewhat active in treating Gram-negative bacilli. Cephalexin is commonly used to treat infections of the skin, ears, and urinary tract, as well as pneumonia and other respiratory tract infections. Cephalexin can also be used for patients who are allergic to penicillin. Ciprofloxacin is part of the fluoroquinolone class of antibiotics. It targets and inhibits the bacterial enzyme DNA gyrase, preventing DNA replication. Unlike cephalexin, it is more effective in treating Gram-negative bacteria than Gram-positive bacteria. Ciprofloxacin is used to treat bacterial infections of the skin, lungs, bones, urinary tract, and also patients exposed to anthrax. Antibiotics have recommended storage temperatures in order to maintain their reliability in treating bacterial infections. Cephalexin has a prescribed storage temperature of 35.6°F-46.4°F (2°- 8°C). Ciprofloxacin is recommended to be stored at a temperature of 35.6°F- 77°F (2°C-25°C). Higher temperatures can lead to chemical degradation of the antibiotic, rendering it ineffective. When used routinely, antibiotics may be inadvertently stored at temperatures higher than the suggested range. Therefore it is necessary to determine the thermostability of these antibiotics when exposed to elevated temperatures. Thermostability data will be obtained via liquid chromatography-mass spectrometry, an established analytical technique for detecting a wide array of antibiotics. The data will be analyzed for temperatures ranging from 90°F-200°F (32°C-90°C), simulating temperature conditions in which antibiotics may be incorrectly stored. The data will be used to determine if the prescribed storage conditions for cephalexin and ciprofloxacin are valid.

Keywords: Thermostability, Cehpalexin, Liquid Chromatography-Mass Spectrometry

1. Introduction

Cephalexin (M.W. of 347.4 g/mol) and ciprofloxacin (M.W. of 331.4 g/mol) are commonly prescribed antibiotics used to treat an array of bacterial infections in humans. Cephalexin (Figure 1) is a semi-synthetic antibiotic and part of the beta-lactam family[1-2]. The four-membered, nitrogen-containing ring at its core is characteristic of beta-lactam antibiotics[3]. This beta-lactam ring is essential to the mode of action in killing bacteria. Cephalexin is more effective in treating Gram-positive bacteria but still has an effect on Gram-negative bacteria[2,4]. Cephalexin functions by inhibiting cell wall synthesis. This is achieved when cephalexin binds to penicillin binding proteins (PBPs). The binding prevents the peptidoglycan layers in bacterial cell walls from crosslinking[3]. Because peptidoglycan layers are essential to the cell wall's structural integrity, bacterial cells eventually undergo osmotic lysis or autolysis due to the enzyme inhibition by cephalexin. Cephalexin is most commonly used to treat infections of the skin, ears, and urinary

tract[1,2]. Respiratory tract infections such as pneumonia can also be treated with cephalexin. One unique advantage of cephalexin is that it can be prescribed for patients who are allergic to penicillin[1].

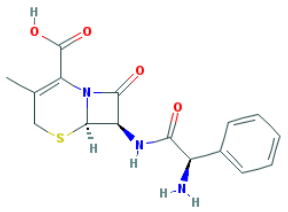

Figure 1. Chemical structure of broad-spectrum antibiotic cephalexin

Ciprofloxacin (Figure 2) is part of the fluoroquinolone class of antibiotics[5]. These antibiotics target and inhibit the bacterial enzyme DNA gyrase[6]. This prevents bacterial cells from carrying out DNA replication. Ciprofloxacin has more of an effect on Gram-negative bacteria but can still be used on Gram-positive bacteria[6]. Ciprofloxacin treats similar infections such as bacterial infections of the skin, lungs, bones, and urinary tract. In addition, patients exposed to anthrax can be prescribed ciprofloxacin for treatment[5-6].

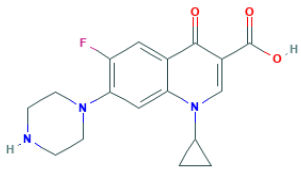

Figure 2. Chemical structure of broad-spectrum antibiotic ciprofloxacin

In order to remain reliable in treating bacterial infections, many antibiotics are given recommended storage temperatures and conditions. Cephalexin has a prescribed storage temperature of 35.6°F-46.4°F (2°- 8°C), while ciprofloxacin is recommended to be stored at temperatures of 35.6°F- 77°F (2°C-25°C)[2,4,6-7]. Antibiotics have the potential to undergo chemical degradation when exposed to relatively high temperatures[8-9]. This could render the antibiotic ineffective in treating bacterial infections. Antibiotics can often be inadvertently stored at temperatures higher than the suggested range. For example, the inside of a vehicle on a summer day can quite easily reach temperatures of 50°C. Therefore it is necessary to determine the thermostability of these antibiotics when exposed to elevated temperatures.

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 compounds. LC-MS is used to determine the presence of a particular compound, as well as the amount, within a sample. This technique has been used for the detection of compounds in sources such as soil, water, blood, and urine[8-9]. LC-MS has many potential analytical applications due to its versatility and specificity.

2. Methodology

2.1. Preparation Of Solvent With Internal Standard

Caffeine powder was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). An internal standard solvent solution was prepared by dissolving caffeine in deionized water at a concentration of 10 ppm.

2.2. Preparation Of Laboratory Standards

Cephalexin monohydrate powder was obtained from MP Biomedicals (Solon, OH, USA). A stock solution was prepared by dissolving cephalexin at a concentration of 100 ppm in the prepared internal standard solvent. The stock solution was refrigerated at 4 °C in the dark. Fresh stock solution was prepared prior to each sequence of experiments. Laboratory standards (1, 5, 10, 25, and 40 ppm) of cephalexin were prepared by diluting the stock solution with the prepared internal standard solvent.

2.3. Preparation Of Samples For Thermostability Experiments

Small amounts (about 0.5 g) of cephalexin monohydrate were subjected to two different elevated temperatures (93°C and 190 °C) for 1 hour and 16 hour time periods. Following the exposure to elevated temperatures, 5, 25, and 40 ppm samples were prepared in the same manner described above for the laboratory standards.

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 7 minutes. All laboratory standards and samples were run in triplicate.

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

The isocratic LC method developed for this study allowed for the detection of the caffeine internal standard and cephalexin in less than four minutes. Using an electrospray ionization source, both the analyte and internal standard were primarily detected as molecular ions. Specifically, the method was designed to monitor m/z = 195 (caffeine) and m/z = 347 (cephalexin). Each specific ion was monitored as a function of time, resulting in a selected ion mass chromatogram. Selected ion monitoring is a well-established analytical technique for a wide variety of antibiotics[10]. The XCalibur software was programmed to only detect signal from the masses of interest, thus reducing background. The data was then analyzed to calculate the area of each chromatographic peak. Figure 3 displays a selected ion mass chromatogram of cephalexin with the caffeine internal standard.

A calibration curve was developed for cephalexin over a range of 1-40 ppm. Specifically, cephalexin standards having concentrations of 1, 5, 10, 25, and 40 ppm were analyzed (each containing the 10 ppm caffeine internal standard). For each standard, the chromatographic peak areas of both the analyte and internal standard were obtained. All standard solutions were run in triplicate.

For each standard, the ratio of chromatographic peak areas for cephalexin/caffeine was obtained and plotted versus cephalexin concentration. A linear relationship was obtained with a correlation coefficient R of 0.9989. The use of the internal standard in this calibration helps account for signal fluctuations from the MS detector, thus increasing the precision of the results. Figure 4 displays the calibration curve for cephalexin with the caffeine internal standard.

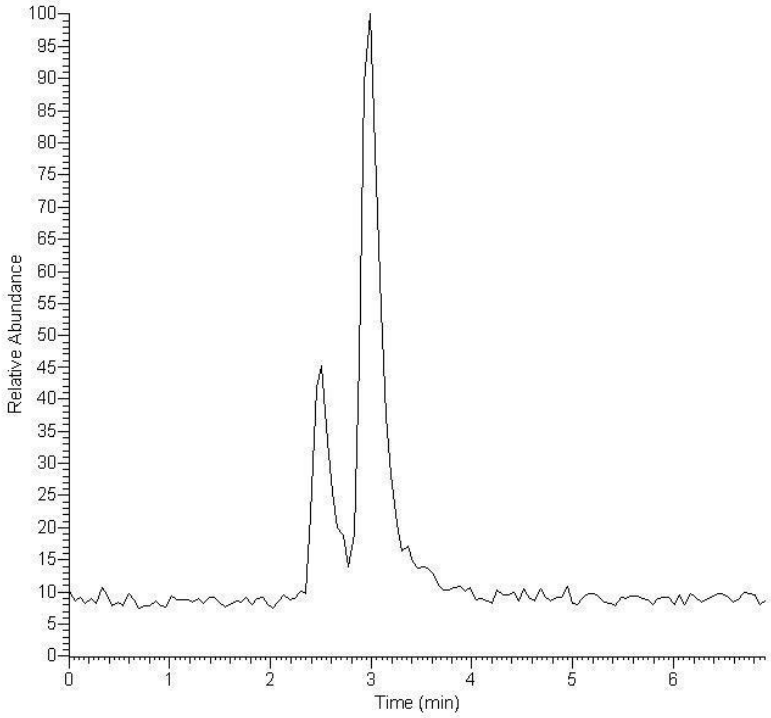

Figure 3. Selected ion mass chromatogram of cephalexin (second peak) with caffeine (first peak) internal standard

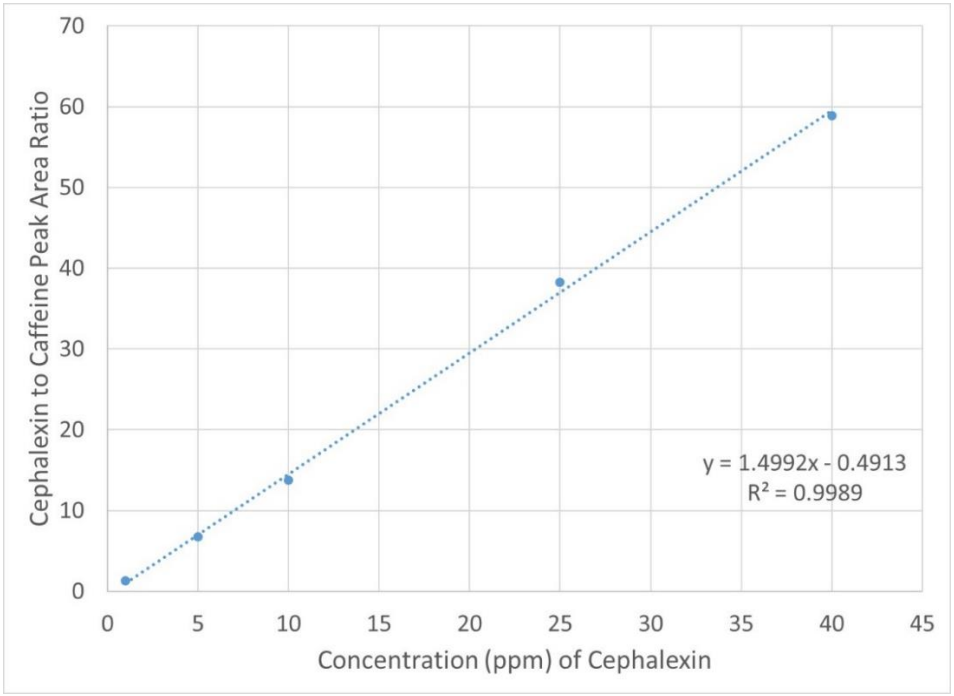

Figure 4. Internal standard calibration curve for cephalexin with caffeine standard

After a linear calibration was obtained, cephalexin samples that had been exposed to elevated temperatures were analyzed using the same LC-MS method. Samples were prepared at 5, 25, and 40 ppm cephalexin (with 10 ppm caffeine internal standard) and run in triplicate. The peak area ratios were obtained and interpolated with the calibration curve. Both the standards and heated samples were run sequentially in the same analysis session. A t-test was conducted at the 90% confidence interval to determine if there was a significant difference in concentration between the treated and untreated cephalexin. The results are summarized in Table 1.

Table 1. Observed concentrations of cephalexin solutions after exposure to elevated temperatures

Cephalexin	**40ppm**	**25ppm**	**5ppm**	**Significant?**
1 hour at 93°C	41.64	27.86	4.485	No
16 hours at 93°C	35.66	23.46	3.127	Yes
1 hour at 190°C	0.8859	--	--	Yes

For samples heated at 93°C for just one hour, no significant degradation in cephalexin concentration was observed for any of the samples. However, after 16 hours, a significant decrease in concentration was observed. An increase in exposure temperature to 190°C did result in an almost complete degradation of the cephalexin after just one hour. A detectable signal was obtained only for the sample prepared at 40 ppm and that signal was the equivalent of less than 1 ppm cephalexin.

4. Conclusion

Thermal degradation of cephalexin has been observed following exposure to elevated temperatures. The level of degradation clearly corresponds to the temperature level and the length of time to which the antibiotic has been exposed. Significant decreases in the apparent concentration of cephalexin were observed after a 16 hour exposure at 93°C, while nearly complete degradation was observed after only one hour exposure at 190°C. Because this LC-MS method monitors the signal of the molecular ion of the antibiotic, it should be readily applicable to the analysis of other antibiotics, provided the molar mass of the analyte is known.

5. Future Work

Future studies will aim to provide more detail into the thermal degradation patterns of cephalexin, including analyzing the effects of a larger number of temperature variations and exposure times. Also planned is the application of this method to other antibiotics. A study of the thermostabilty of ciprofloxacin, another widely used broad spectrum antibiotic, is underway. The analytical method developed for cephalexin has already shown to be amenable for the detection of ciprofloxacin, as shown in Figure 5.

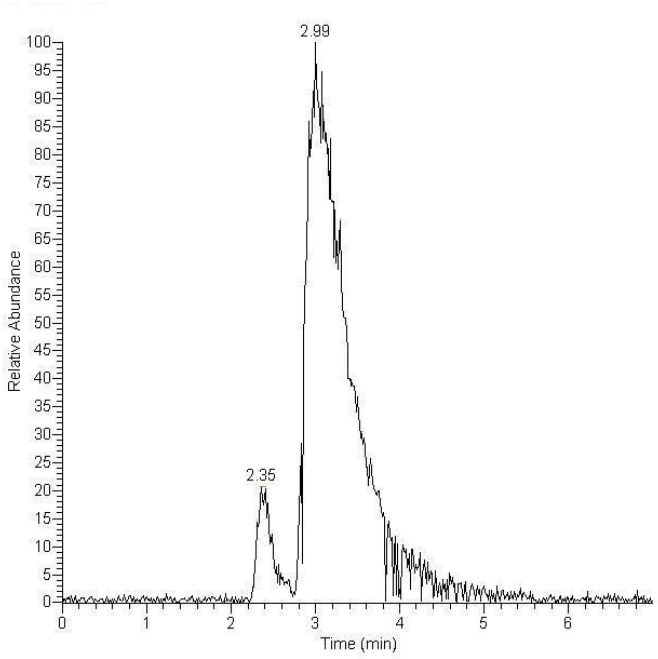

Figure 5. Selected ion mass chromatogram of ciprofloxacin (2nd peak) with caffeine (first peak) internal standard

6. Acknowledgements

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