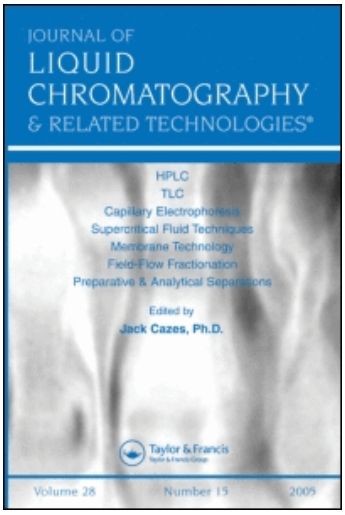

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New and Simple HPLC Method for the Determination of Lactic Acid Content in Ciprofloxacin Injection

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Abstract: A simple reversed-phase HPLC method was developed for routine analysis of lactic acid in Ciprofloxacin Injection. The analysis involves simple sample preparation to ensure complete hydrolysis of the oligomers of lactic acid. Using these conditions, recoveries ranging from 97.5 to 99.9% at three levels of the label claim were obtained. The standard solution and the sample solution were shown to be stable for at least 41 hours when stored at room temperature. Tests done have also demonstrated that the method is stable despite small changes in experimental conditions. Data supporting the development and validation of this method are presented.

Keywords: Ciprofloxacin, Lactic acid, Injection

INTRODUCTION

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) is a synthetic fluoroquinolone antibiotic with a high antimicrobial activity against all pathogens causing bacterial endophthalmitis.[1] Ciprofloxacin is a very potent fluoroquinolone against Gram-positive and Gram-negative and other microorganisms.[2] It is used for the treatment of urinary tract infections, bacterial gastroenteritis, enteric fever, osteomyelitis, infections of the bone, and respiratory tracts.[3]

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One of the inactive ingredients of the injectable dosage form of ciprofloxacin is lactic acid.[4] Lactic acid, 2-hydroxypropanoic acid, is a three carbon organic acid; one terminal carbon atom is part of an acid or carboxyl group, the other terminal carbon atom is part of a methyl or hydrocarbon group, and the central carbon atom is part of an alcohol carbon group. Lactic acid exists in two optically active isomeric forms and has many usages in beverages, foods, cosmetics, and pharmaceuticals as an acidifying agent and acidulant. Structural information of lactic acid is represented in Figure 1.

Typically analyses of samples of ciprofloxacin injection for the content of lactic acid had been performed using the method from the USP monograph.[5] This is an HPLC method, which uses a column with an L17 packing (cation exchange). The standard material that is used for this analysis is sodium L-lactate. The lactic acid peak in the sample is calculated versus the sodium L-lactate peak (the same retention time), and a conversion factor is applied to obtain the equivalent value of lactic acid in the sample. During initial studies of different lots of a ciprofloxacin injection finished product, the values obtained for the content of lactic acid using this method were lower than expected, below the specification of 90%–110% of claim ($\sim$3.72 mg/mL). Curiously, with time, the results for the content of lactic acid in ciprofloxacin injection increased; after about 6 months, the results were within the expected range. Further analyses of older samples showed that the lactic acid content subsequently stayed within specifications.

The objectives of this work, for the determination of the content of lactic acid in ciprofloxacin injection, were the following: 1) Develop a simple reversed-phase HPLC method for routine use, 2) Determine how to obtain 100% recovery of a sample of ciprofloxacin injection spiked with lactic acid, and 3) validate the method. Furthermore, reasons for the results that were previously obtained with the USP method were to be investigated.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade acetonitrile and potassium phosphate monobasic were purchased from Anachemia Canada, Inc. (Montréal, QC, Canada). HPLC grade o-phosphoric acid was purchased from American Chemicals Ltd. (Montréal,

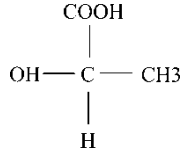

Figure 1. Lactic acid.

QC, Canada). DL-lactic acid, sodium L-lactate, lactide (3,6-Dimethyl-1,4-dioxane-2,5-dione), L-lactide ((3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione), and sodium hydroxide were purchased from Sigma-Aldrich (Oakville, ON, Canada). Distilled water, available in the laboratory, was filtered prior to use through a 0.2 μm filter.

Chromatographic System and Conditions

Analysis was carried out using a Surveyor HPLC system, as well as a TSP HPLC system (both from Thermo Electron Corporation, Canada). Validation of the method was also performed using the Agilent 1100 HPLC system (Agilent Technology, Canada). The HPLC systems are composed of the following units: a solvent delivery module, an automatic sample injector, a column oven, and a UV detector programmed at 210 nm. The analysis was performed using a YMC-ODS-Aq column, 250 mm × 4.6 mm I.D., 5 μm particle size, 120 Å (Waters Corporation, Milford, MA, USA), maintained at 30°C. The filtered and degassed mobile phase was pumped at 1.2 mL/minute. Standard and sample solutions of 10 μL was injected into the HPLC system for analysis.

RESULTS AND DISCUSSION

Method Development for the Content of Lactic Acid in Ciprofloxacin Injection

Many methods are available for the analysis of organic acids by reversed-phase HPLC.[6–11] Of these methods, there are several that utilize a C_{-18} column;[10,11] a mobile phase consisting of a phosphate buffer with a pH of 2.5 and a small percentage of acetonitrile ($\leq$3%) is often used. These conditions were used as the starting point for method development. As the mobile phase is very highly aqueous, columns that are stable under these conditions were investigated. Three different columns were tested, using a standard preparation of sodium L-lactate for development: 1) Synergi 4 μ Fusion RP-80, 150 × 4.6 mm, 2) Zorbax SB-Aq, 5 μm, 150 × 4.6 mm, and 3) YMC ODS-AQ (Waters), 5 μm, 250 × 4.6 mm.

The Synergi and Zorbax columns did not provide sufficient retention of the sodium lactate, with the peak just beyond the void volume of the column, even with a mobile phase of 100% buffer. The YMC ODS-AQ column, however, provided acceptable retention of the sodium L-lactate (retention time = 5.9 minutes, flow 1.0 mL/min with 100% buffer as mobile phase); however the peak, while symmetric, was very broad. An amount of 3% of acetonitrile was added to the mobile phase, which resulted in excellent peak shape and still acceptable retention. The method using this

Table 1. Final proposed method for content of lactic acid in ciprofloxacin injection

Method parameter	Proposed conditions
Buffer	25 mM potassium phosphate monobasic adjust pH = 2.5 with phosphoric acid
Mobile phase composition	ACN : Buffer (3 : 97)
Column	YMC ODS-AQ (Waters), 5 μm, 250 mm × 4.6 mm I.D. 120 Å
Column temperature	30°C
Flow	1.2 mL/min
Detector	210 nm
Injection volume	10 μL
Test concentration	~0.72 mg/mL as lactic acid
Retention time sodium lactate	3.6 minutes

column was then optimized: the flow rate was increased to reduce run time and column temperature was also increased to 30°C to reduce back pressure. The final conditions for analysis are presented in Table 1.

The column that is used is the same as is used for the determination of assay and impurities of ciprofloxacin (internal method). Under these conditions, however, the ciprofloxacin peak will not elute and will, therefore, not cause any interference. Refer to Figure 2 for a chromatogram of a standard preparation of sodium L-lactate injected using the conditions in Table 1.

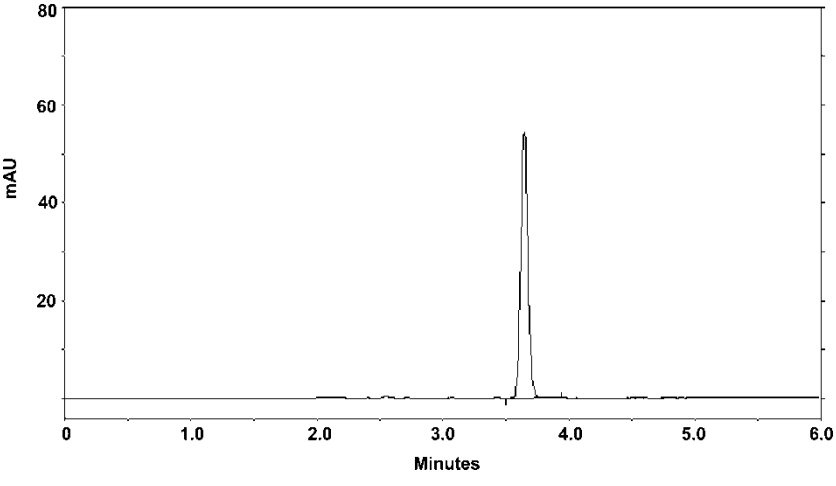

Figure 2. Standard solution containing sodium L-lactate equivalent to approximately 0.6 mg/mL as lactic acid.

Recovery Studies for Lactic Acid in Ciprofloxacin Injection

Initial Recovery Studies of Lactic Acid

Studies were carried out to determine whether 100% recovery of samples containing lactic acid could be obtained when calculated against a standard of sodium L-lactate. A sample of ciprofloxacin injection was spiked to contain lactic acid at 100% of the label claim (~3.72 mg/mL). Standards solutions in water of lactic acid were also prepared. Finally, samples of ciprofloxacin injection after 38 months storage at 25°C/60%RH were also analyzed. Analysis of the 38 month old samples gave results of about 96%–97% of the label claim. However, analysis of the freshly spiked sample and standards solutions of lactic acid produced only about 72% recovery in all cases (when calculated versus the sodium L-lactate standard preparation). Upon further investigation, it was noticed that there were late eluting peaks at 16.6 minutes and 17.9 minutes, or ~RRT 4.5 and ~RRT 4.9 (refer to Figure 3) present in not only the spiked sample of ciprofloxacin injection, but in the standard of lactic acid as well. These two peaks were not observed in the 38 month stability samples, however. This seems to indicate something about the properties of the lactic acid standard used. It was noted, that if the sum of the areas of these two peaks were added to the area of the lactic acid peak, the recovery was around 102% of the theoretical concentration.

Investigation Into Late Eluting Peaks in Lactic Acid Standard

Investigation of the properties of lactic acid indicated that in concentrated form especially, lactic acid contains a mixture of related compounds. It was

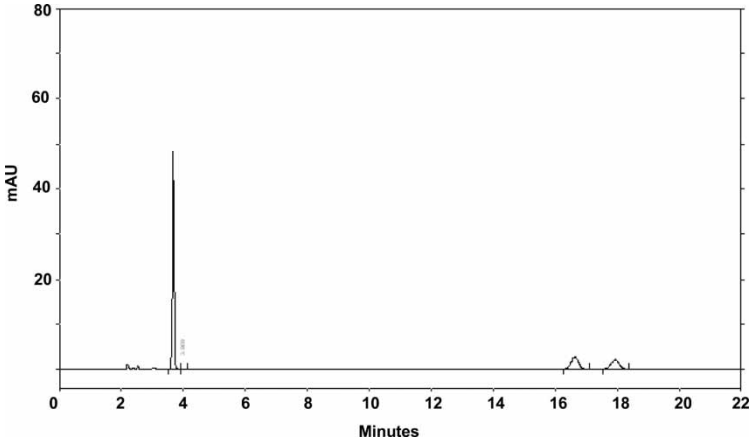

Figure 3. Sample of ciprofloxacin injection, diluted 5 mL/25 mL with mobile phase (final concentration ~0.72 mg/mL lactic acid).

first thought that the two peaks in question could be due to lactide isomers. Upon injecting these materials, however, a third peak was observed (~RRT 7). Interestingly, this third peak quickly degraded and one of the original peaks at RRT 4.5 was observed to increase. It appears that the lactide standard quickly hydrolyzes into this peak. Following these observations, further research was performed. It is often suggested that lactic acid in concentrated form will contain oligomers (dimers, trimers, etc.) of lactic acid, which would result in a lower than expected concentration of monomeric lactic acid.[12] This may explain the presence of the two extra peaks in the freshly prepared solutions of lactic acid. If these peaks can then be hydrolyzed into lactic acid, it may be possible to obtain 100% recovery of lactic acid directly, rather than taking the sum of the three peaks. This could also explain the results obtained in previous analyses for stability samples of ciprofloxacin injection. If the samples initially contain oligomers, the result of lactic acid would be low calculated versus sodium L-lactate. With time, as the oligomers hydrolyze in the aqueous solution to monomeric lactic acid, the concentration would appear to increase. Following these observations, sample preparation tests were undertaken to see whether these unknown peaks at RRT 4.5 and RRT 4.9 could be transformed into lactic acid, and if 100% recovery of the spiked sample could be obtained.

Sample Preparation Tests (Hydrolysis Tests)

Various sample preparation tests were performed to try to successfully transform the peaks at RRT 4.5 and RRT 4.9 into monomeric lactic acid. The sample used is a laboratory made lot of ciprofloxacin injection, 10 mg/mL, spiked to contain lactic acid at 3.617 mg/mL (about 100% of claim). The percentage recovery of the lactic acid peak was calculated versus a standard solution of sodium L-lactate prepared in mobile phase. The sample preparation tests performed are presented in Table 2, and results obtained are presented in Table 3. Acceptable percentage recoveries were found in

Table 2. Sample preparation conditions for determination of lactic acid in ciprofloxacin injection

Sample #	Sample preparation conditions
1	Dilute ciprofloxacin injection 5/25 with mobile phase
2	Pipette 5.0 mL of ciprofloxacin injection into a 25 mL volumetric flask. Add 1 mL of 10 N NaOH and allow to react at RT 10 minutes. Complete to volume with mobile phase and mix.
3	As per #2, using 1 mL 1 N NaOH (precipitates; filter)
4	As per #2, using 1 mL 5 N HCl
5	As per #2, using 0.5 mL 1 N NaOH
6	As per #2, using 0.5 mL 1 N NaOH for 30 minutes

Table 3. Spike recoveries of sample preparation study

Sample #	Peak area lactic acid (RRT 1.0)	Peak area RRT 4.5	Peak area RRT 4.9	% Recovery lactic acid[a]
1	185945	44430	37304	72.2
2	14034	0	0	5.5
3	260896	0	0	101.3
4	187510	44098	36541	72.8
5	248023	7526	7973	96.3
6	259745	0	0	100.9

[a]% Recovery calculated using only the lactic acid peak area versus the sodium L-Lactate standard.

both Sample #3 and Sample #6. For Sample #3, however, undesirable fronting of the lactic acid peak was observed, likely due to the higher concentration of NaOH in the sample. Therefore, Sample #6 was chosen as the appropriate condition, as 100% recovery and good peak shape were both observed for lactic acid. Observations have demonstrated that this sample preparation would precipitate with time. Therefore, it was found to be necessary to neutralize the reaction mixture after 30 minutes by addition of 0.5 mL of 1 N HCl before completing to volume with mobile phase. This solved the problem of sample precipitation without affecting the lactic acid recovery. Refer to Figure 4 for a chromatogram of a sample preparation of ciprofloxacin injection treated with 1 N NaOH prior to analysis for lactic acid.

Validation

The method developed for the determination of Lactic acid in ciprofloxacin injection was then validated. The results obtained are presented in the following sections. All data were generated using an HPLC system from Agilent (1100 Series). The intermediate precision study was performed using a HPLC system from Thermo Electron.

The specificity of the method was tested by injecting a mobile phase blank and a synthetic finished product solution containing all ingredients but lactic acid. No peak was observed at the retention time of the lactic acid peak. A standard solution of sodium L-lactate was injected. The lactic acid peak was observed at the retention time of 3.53 minutes. A synthetic sample preparation of ciprofloxacin injection was injected. The lactic acid peak was observed at the retention time of 3.50 minutes.

Six (6) standard solutions containing sodium L-lactate at concentrations equivalent to 0.15 mg/mL to 1.50 mg/mL of lactic acid representing

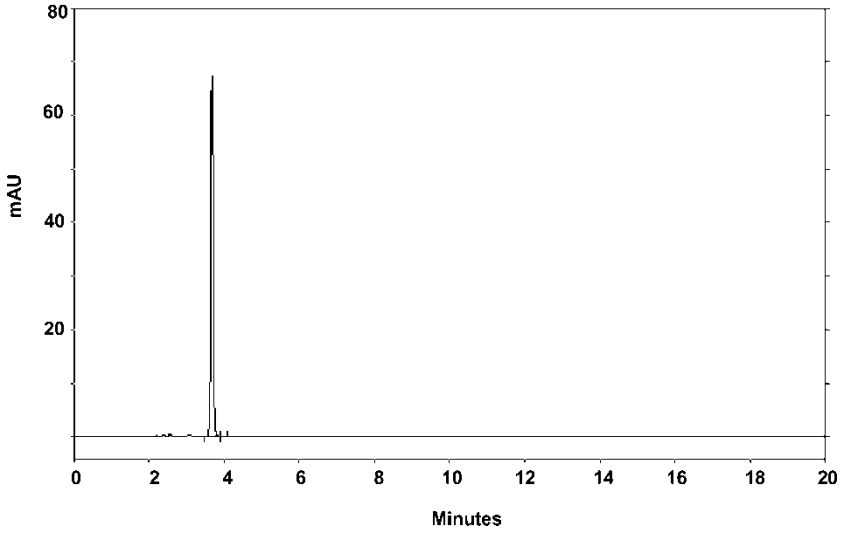

Figure 4. Chromatogram of a ciprofloxacin injection sample prepared using the new preparation method (Sample #6).

20% – 200% of the standard solution were prepared and injected. Details of the calibration curve results are presented in Table 4.

The method was found to be precise, accurate, and linear at concentrations from 1.86 mg/mL to 4.46 mg/mL for lactic acid. This represents approximately 50% to 120% of the set limit for lactic acid (3.72 mg/mL). The precision of the system was evaluated from five (5) replicate injections of the sodium L-lactate standard solution (equivalent to approximately 0.72 mg/mL lactic acid). Typical results are as follows: Tailing factor ($T \leq 2$) = 0.99 with a RSD of 0.06%.

The synthetic finished product was prepared at 50%, 100%, and 120% of the Lactic acid label claim, representing approximately 1.86 mg/mL, 3.72 mg/mL, and 4.46 mg/mL. Results are reported in Table 5. The overall average recovery for Lactic acid is 98.9% with a recovery range of 97.5% to 99.9%.

Table 4. Coefficient of determination, slope and intercept of lactic acid

Compound	Coefficient of determination (R^2)	Slope	Intercept
Lactic acid	1.0000	364.7793	−0.3043

Table 5. Synthetic ciprofloxacin injection containing lactic acid at 50%, 100% and 120% of the label claim

Accuracy	Nominal conc. (mg/mL)	Actual conc. (mg/mL)	Recovery (%)	Average (%)	RSD (%)
~50%	1.91	1.89171	99.0	99.0	0.4
		1.89234	99.1		
		1.89710	99.3		
		1.88387	98.6		
		1.90039	99.5		
		1.88287	98.6		
~100%	3.72	3.71452	99.9	99.8	0.1
		3.71381	99.8		
		3.70882	99.7		
		3.70935	99.7		
		3.70890	99.7		
		3.70978	99.7		
~120%	4.44	4.33301	97.6	97.8	0.3
		4.32846	97.5		
		4.35504	98.1		
		4.35518	98.1		
		4.33602	97.7		
		4.33916	97.7		

On a different day, sample preparations of ciprofloxacin injection containing lactic acid at 100% of the label claim were analyzed using a different batch of mobile phase, a different HPLC system (Thermo Eelctron), as well as a different analyst performing the analysis. Results showed a recovery of 103% with a coefficient of variation of 0.6%.

A standard solution of sodium L-lactate was reanalyzed after approximately 24 hours and 41 hours and compared to a freshly prepared standard solution. The results showed no significant decrease of lactic acid after 41 hours (99.6% recovery) when stored at room temperature. In the same manner, a sample preparation of ciprofloxacin injection was found to be stable at least 43 hours when stored at room temperature (99.9% recovery).

Robustness was determined by varying the column temperature by $\pm 2^{\circ}$C, the percentage of organic in the mobile phase by $\pm 1\%$, and the buffer pH by ± 0.2 units. Results are summarised in Table 6. As illustrated, small shifts in the retention times of peaks are observed when the above mentioned parameters are modified. The peak shape was not affected by any of the changes tested. Therefore, the proposed method is found to be robust over variations of $\pm 2^{\circ}$C of the column temperature, variations of $\pm 1\%$ organic in the mobile phase composition, and over ± 0.2 units of the buffer solution.

Table 6. Effects of different parameters on the finished product preparation

Conditions	Standard solution RT (min)/tailing	Sample solution RT (min)/tailing
Initial conditions		
Buffer pH:2.5/ACN:buffer (3 : 97)	3.505	3.509
Column temp. 30°C	1.016	1.012
Buffer pH:2.3/ACN : buffer (3 : 97)	3.523	3.527
Column temp. 30°C	1.019	1.015
Buffer pH:2.7/ACN : buffer (3 : 97)	3.477	3.481
Column temp. 30°C	0.992	0.999
Buffer pH:2.5/ACN : buffer (2 : 98)	3.732	3.737
Column temp. 30°C	1.026	1.029
Buffer pH:2.5/ACN : buffer (4 : 96)	3.338	3.336
Column temp. 30°C	0.987	0.988
Buffer pH:2.5/ACN : buffer (3 : 97)	3.521	3.521
Column temp. 28°C	1.010	1.003
Buffer pH:2.5/ACN : buffer (3 : 97)	3.484	3.489
Column temp. 32°C	1.003	0.999

CONCLUSION

A simple reversed-phase HPLC method was developed for routine analysis of lactic acid in ciprofloxacin injection. However, recovery of freshly spiked samples of ciprofloxacin injection and lactic acid standard preparations when diluted in mobile phase only produced low recovery (~70%) when calculated versus a standard of sodium L-lactate. In these samples, two additional unknown peaks were also observed. At the same time, 38 month old samples of ciprofloxacin injection diluted only in mobile phase, produced close to 100% of the label claim of lactic acid and the two aforementioned unknown peaks were not observed.

Further research indicated that concentrated lactic acid may contain oligomers (dimers, trimers, etc.) in addition to monomeric lactic acid. It may be possible to hydrolyze these oligomers back to lactic acid. Hydrolysis of the finished product under basic conditions (1 N NaOH) at room temperature eliminates the two unknown peaks mentioned above, and produces 100% recovery of the lactic acid peak. The fact that the two additional peaks were not observed in the 38 month samples of ciprofloxacin injection diluted in mobile phase, may indicate that with time, in the finished product, the lactic acid has slowly been fully hydrolyzed back to its monomeric form.

In order to ensure the full recovery of lactic acid, all sample preparations of ciprofloxacin injection should be treated as follows: Transfer 5 mL of

ciprofloxacin injection to a 25 mL volumetric flask; add 0.5 mL 1 N NaOH and allow to react at room temperature for 30 minutes, neutralize with 0.5 mL 1 N HCl, and then complete to volume with mobile phase and mix.

The proposed method was validated and exhibits the following performance characteristics. No significant peak interferes with the lactic acid peak. The analysis of lactic acid exhibits a RSD of 0.3%. The method gave acceptable results in terms of instrumental, column parameters, and reproducibility (0.6% RSD), when analyzed by a different analyst, on a different day, using a different HPLC system (Thermo Electron), as well as freshly prepared mobile phase. The recovery of Lactic acid is 97.5% to 99.9% from synthetic solutions at three different levels of the label claim (50%, 100%, and 120%). Lactic acid shows a linear response with a coefficient of determination (R^2) of 1.0000 in the range of 20% to 200% of the label claim concentration. The standard solution has shown to be sufficiently stable for 41 hours and the sample solution has shown to be sufficiently stable for 43 hours when stored at room temperature. The method is relatively insensitive to small changes in experimental conditions. In conclusion, the method developed for the analysis of lactic acid in ciprofloxacin injection has been validated, and is acceptable for its intended use.

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