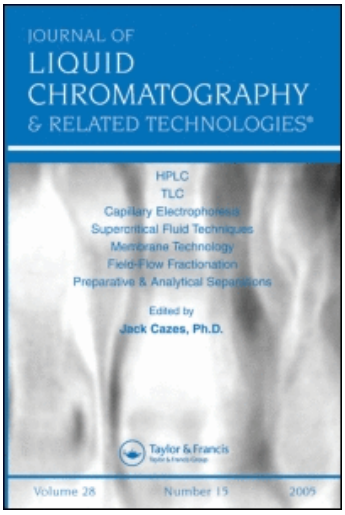

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FORMATION OF A VINYL ALCOHOL DERIVATIVE DURING SAMPLE PREPARATION OF 7-CHLORO-6,4'-DIFLUOROQUINOLONE FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

During analysis of 7-chloro-6,4'-difluoroquinolone (CDFQ) by high performance liquid chromatography (HPLC), a reaction product was formed during sample preparation. This compound was identified as a vinyl alcohol derivative of CDFQ, formed by loss of carbon monoxide from the carboxylate group of CDFQ during sonication in tetrahydrofuran (THF). The reaction to form the vinyl alcohol was shown to depend upon the presence of trace amounts of potassium acetate in the CDFQ, the peroxide content of the THF, and sonication variables of time and bath temperature. The sample preparation procedure was modified so that this reaction would not occur and meaningful HPLC analyses of CDFQ could be achieved. This study demonstrates that caution is required when quinolone compounds are prepared for HPLC analysis by heating or sonicating in THF solution.

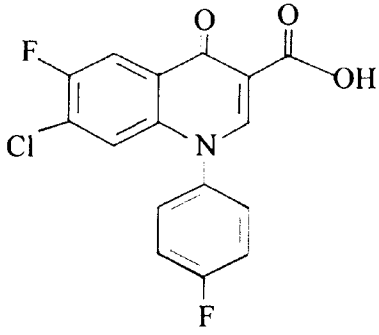


Figure 1. Chemical Structure of CDFQ.

INTRODUCTION

7-Chloro-6-fluoro-1-(4-fluorophenyl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid or, more commonly, 7-chloro-6,4'-difluoroquinolone (CDFQ) is an intermediate in the chemical synthesis of aryl-fluoroquinolone antibacterial agents difloxacin and sarafloxacin, intended for use in animal health products. Fluoroquinolones have been shown to be orally active antibacterial agents with activity against Gram-negative bacteria.[1] They act to inhibit DNA gyrase, a key enzyme in DNA replication.[2] The chemical structure for CDFQ is shown in Figure 1. Synthetic routes for CDFQ[3-5] and HPLC analyses of difloxacin,[6-7] sarafloxacin,[8-9] and other quinolones[10-11] have been reported.

Impurity analysis of CDFQ is carried out in our laboratories by reverse phase gradient HPLC on a C_{18} column with mobile phase consisting of tetrahydrofuran (THF), methanol, and potassium phosphate buffer at pH 5.3. During HPLC testing on three CDFQ lots, lots A and B showed the presence of a new peak which was not present in lot C. The magnitude of this peak was generally consistent within a set of samples, but varied greatly from one sample preparation set to the next. This observation led us to consider that this peak was being formed during the sample preparation process.

It was shown that this peak was indeed formed from CDFQ during sample preparation. The new component was identified as a vinyl alcohol analog of CDFQ, hereafter referred to as the vinyl alcohol. Critical parameters were evaluated and the sample preparation process altered to prevent this reaction.

MATERIALS AND METHODS

The HPLC instrumentation used most frequently in this study was from Spectra-Physics (Thermo Separation Products, Division of Thermo Instrument Systems, Inc., Riviera Beach, FL, USA), which included a P2000 HPLC pump, an AS3000 autosampler, and UV1000 detector. Data aquisition and processing were accomplished by a PE Nelson ACCESS*CHROM GC/LC Data System (Perkin-Elmer Systems, Inc., Cupertino, CA, USA). The HPLC column was either a YMC-Pack ODS-A, A-302-5 (5 μm, 150 x 4.6 mm) (YMC, Inc., Morris Plains, NJ, USA) or Zorbax RX-C18 column of the same dimensions (Mac-Mod Analytical, Inc., Chadds Ford, PA). The injection volume was 20 μL and the flow rate of the mobile phase was 1.0 mL/min. The wavelength employed for UV detection was 325 nm.

A gradient system was employed for HPLC impurity analysis. Mobile phase A consisted of 68% 0.02 M aqueous potassium phosphate (pH 5.3-5.4), 27% tetrahydrofuran (THF), and 5% methanol. Mobile phase B was 35% buffer, 60% THF, and 5% methanol. The gradient program consisted of 100% mobile phase A for 30 min, linear change to 100% mobile phase B from 30 to 60 min, linear return to mobile phase A from 60-62 min, and mobile phase A continued from 62-80 minutes. HPLC grade THF, acetonitrile, and methanol were obtained from EM Science (Gibbstown, NJ), reagent grade monobasic potassium phosphate was from J. T. Baker, (Phillipsburg, NJ) and water was from a Millipore Milli-Q system (Millipore, Milford, MA).

Sample dissolutions for HPLC were performed with a Branson 2200 Ultrasonic Cleaner (Branson Ultrasonic Corp., Danbury, CT, USA). Quantofix peroxide test sticks obtained from Aldrich Chemical Company (Milwaukee, WI) were used to measure peroxide concentration in THF.

Inductively Coupled Plasma (ICP) analyses were performed with a Thermo Jarrell Ash Atomscan 25 instrument (Division of Thermo Instrument Systems, Inc., Franklin, MA, USA). Samples were prepared for analysis by dissolution in 20% ammonium hydroxide.

Gas chromatography was performed with a Hewlett-Packard Model 5890 instrument (Hewlett-Packard Company, Palo Alto, CA, USA). Acetate was determined as acetic acid using a AT-1000 megabore column and flame ionization detector.

Proton nmr, C13 nmr, and mass spectral data for identification of the vinyl alcohol were provided by the Structural Chemistry Group, Pharmaceutical Products Division, Abbott Laboratories. The vinyl alcohol sample for spectral

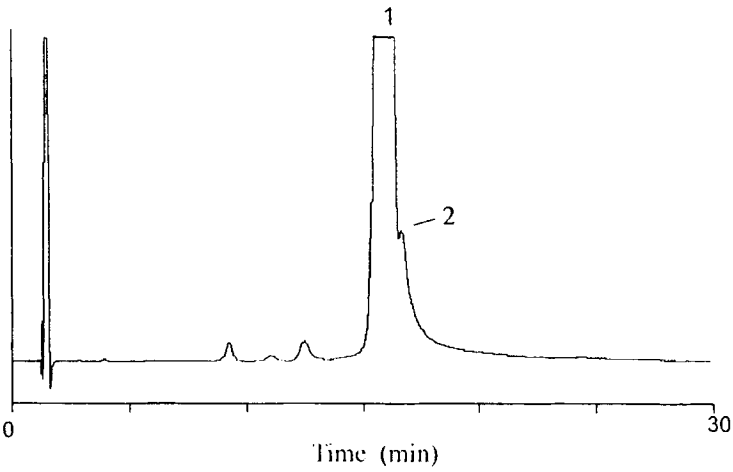


Figure 2. Chromatogram for CDFQ, lot A on Zorbax C18. Principal peaks: 1: CDFQ; 2: vinyl alcohol..

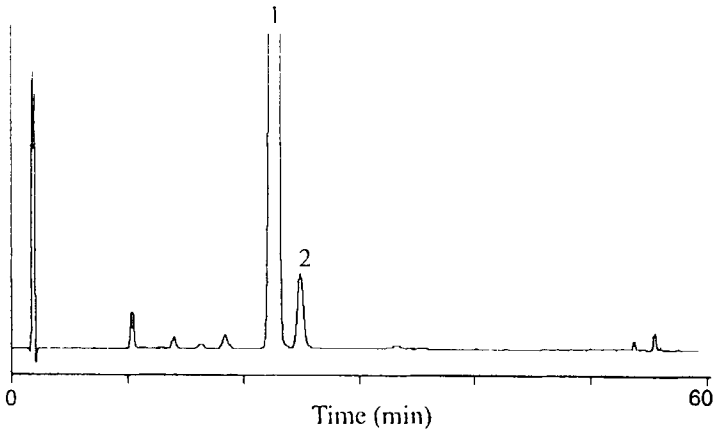


Figure 3. Chromatogram for CDFQ, lot A on YMC C18. Principal peaks: 1:CDFQ; 2:vinyl alcohol.

Table 1

Variation in Vinyl Alcohol PA (%) for Independent Sample Preparation of CDFQ, Lot A

Sample Prep No.	Peak Area Percent
1	1.3
2	3.6
3	1.6
4	7.4
5	0.6

analysis was obtained by sonication of CDFQ lot A (30 mg in 25 mL THF) for 85 minutes. HPLC analysis indicated near quantitative conversion of CDFQ to the vinyl alcohol.

Sample Preparation

The sample preparation procedure for CDFQ employed during most of this work consisted of dissolving ~30 mg sample in 25 mL THF in an ultrasonic bath followed by dilution to 100 mL with 1:1 (v/v) acetonitrile/water. This procedure was later modified to prevent formation of the vinyl alcohol as described in the Results and Discussion section.

RESULTS AND DISCUSSION

Release testing of CDFQ includes HPLC assay and HPLC impurity analyses. During HPLC assay analysis of three lots of CDFQ, designated A, B, and C, lots A and B showed the presence of a new peak. Inspection of a typical chromatogram of lot A (Figure 2) shows a shoulder on the main component when a Zorbax RX-C18 column was used. With a YMC ODS-A column and other conditions unchanged, a large peak was completely resolved from the main component (Figure 3). This peak was later shown to result from the vinyl alcohol.

Following the initial HPLC analyses of these CDFQ lots, many more sample preparations and assays were performed. The peak area percentage (PA%) of the new peak varied substantially from preparation to preparation (Table 1).

It was considered that the variations observed were due to sample non-uniformity. However, after the lots (A, B) were well blended the variability in PA% for this peak persisted.

After further study of sample preparation and HPLC analyses, the following experimental facts became clear: (1) the amount of vinyl alcohol formed from a given CDFQ sample often varied substantially from one sample preparation to the next and (2) the amount of vinyl alcohol present did not change after the sample preparation step. These observations led to the hypothesis that the vinyl alcohol was being formed *during sample preparation* and that variations in the amount formed for a given sample were mainly due to differences in duration and water temperature in the ultrasonic bath.

In order to test this, a sample of CDFQ lot A was dissolved in THF by vortexing at room temperature. The sample was then divided and half of the sample solution was further treated by sonication for 20 minutes. The sonicated sample preparation gave 2.0% of the vinyl alcohol while the sample solution which was only vortexed at room temperature yielded only a trace. These results provided strong evidence that the vinyl alcohol was being formed during sonication of CDFQ in THF.

In order to probe the effect of sonication time on the amount of vinyl alcohol formed, CDFQ lot A was sonicated in THF for 0.5 and 1.0, hour (Figure 4). In a related experiment, another sample from lot A was heated in THF at 60°C for 1, 2, and 3 hours (Figure 5). In both cases, a steady conversion of CDFQ into the vinyl alcohol was observed. As shown in Figure 5, the conversion of CDFQ was virtually complete for the sample heated at 60°C for 3 hours. Spectral analysis of the converted sample by proton nmr, C13 nmr, and mass spectrometry provided identification of this component as 7-chloro-6-fluoro-1-(4-fluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carbinol, a vinyl alcohol analog of CDFQ, resulting from loss of carbon monoxide (CO) from the carboxylate group. The chemical structures involved in the conversion of CDFQ to the vinyl alcohol are shown in Figure 6.

With the vinyl alcohol structure and mode of formation identified (ie. heating CDFQ in THF), the question remaining was why these two particular lots of CDFQ (A, B) were most susceptible to this reaction. Additional testing was performed on CDFQ lots A, B, and C in order to probe for differences (Table 2). The problem lots (A, B) had residues on ignition (0.1-0.2%), whereas lot C did not. Metals analyses by ICP and acetate determinations by gas chromatography revealed significantly higher potassium and acetate levels, respectively, in lots A and B (Table 2).

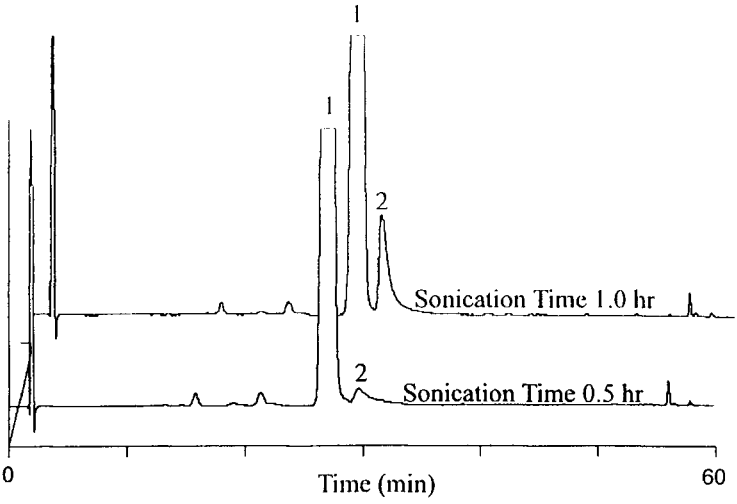


Figure 4. Chromatograms for CDFQ, lot A, sonicated for times indicated. Principal peaks: 1:CDFQ; 2:vinyl alcohol.

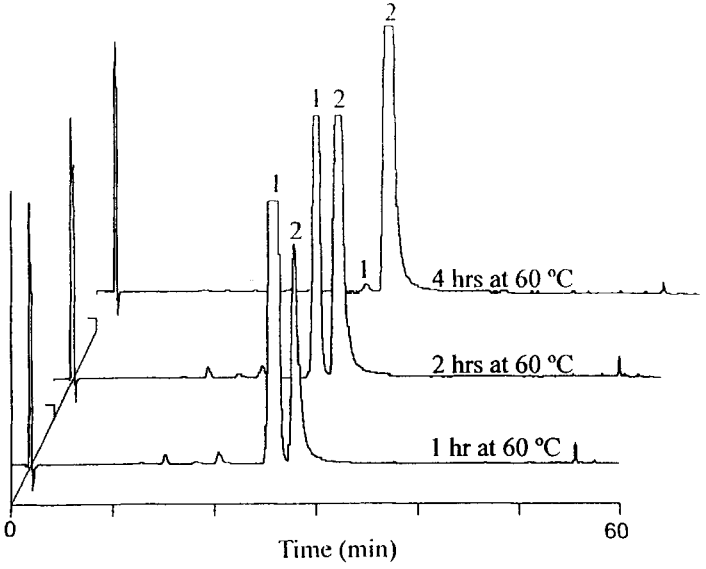
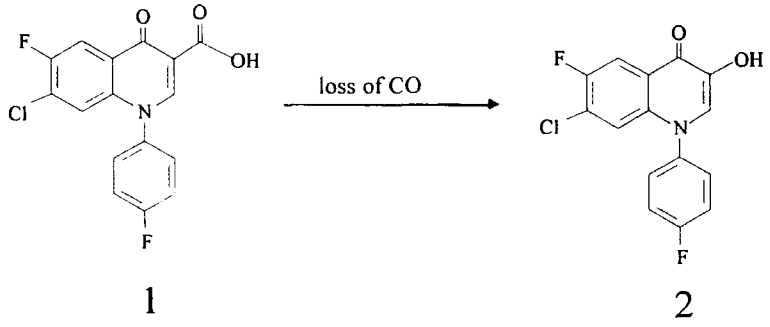


Figure 5. Chromatograms for CDFQ, lot A, heated at 60 °C for times indicated. Principal peaks: 1:CDFQ; 2:vinyl alcohol.



1 2

Figure 6. Chemical structures for the conversion of CDFQ (1) to the corresponding vinyl alcohol (2).

Table 2

Potassium and Acetate Determinations on Blended CDFQ Lots

Lot ID	Potassium (%)	Acetate (%)
A	0.07	0.05
B	0.14	0.07
C	0.007	0.005

The extent to which a residual amount of potassium acetate in CDFQ promotes the formation of the vinyl alcohol was explored. For this experiment, CDFQ lot C, unspiked and spiked with 0.1%, 0.5%, and 1.0% (w/w) potassium acetate was sonicated for 60 minutes in THF. Unspiked CDFQ formed only 0.5% of the vinyl alcohol under these stringent conditions, whereas the preparations spiked with potassium acetate produced greatly increased amounts of this reaction product. A 50-fold increase in vinyl alcohol formation resulted from the addition of only 0.1% potassium acetate. These results (Table 3) clearly demonstrate that low levels of potassium acetate promote vinyl alcohol formation from CDFQ sonicated in THF.

The peroxide content of the THF used for CDFQ sample preparations was also suspected to impact vinyl alcohol formation. To examine the effect of peroxides, the peroxide concentration in four THF lots was determined using peroxide test strips.

Table 3

Dependence of Vinyl Alcohol Formation on % Potassium Acetate for CDFQ, Lot C

Sample Number	% KOAc	Vinyl Alcohol PA%
1	0.0	0.5
2	0.1	2.5
3	0.5	96
4	1.0	88

Table 4

Dependence of Vinyl Alcohol Formation on Peroxide Content in THF for CDFQ, Lot A

Sample Number	Peroxides ppm	Vinyl Alcohol PA%
1	25	32
2	50	39
3	70	95
4	125	95

CDFQ (lot A) was sonicated for 60 minutes in each of these four THF lots. Results summarized in Table 4 show that CDFQ solutions in THF containing higher peroxide concentrations produced significantly more of the vinyl alcohol.

It was desired to change the sample preparation procedure to prevent formation of the vinyl alcohol. This was accomplished by omitting THF from the sonication step: 60 mg CDFQ was dissolved by sonication in 100 mL acetonitrile then diluted to 200 mL with 1:3 THF/water. With this procedure, CDFQ dissolved readily and did not recrystallize upon cooling. HPLC analysis showed the vinyl alcohol was not present in either lot A (Figure 7) or lot B.

When CDFQ samples were prepared in this fashion, both of the problem lots (A, B) passed HPLC testing requirements for assay and impurities. To further ensure that vinyl alcohol formation would not be a problem for the

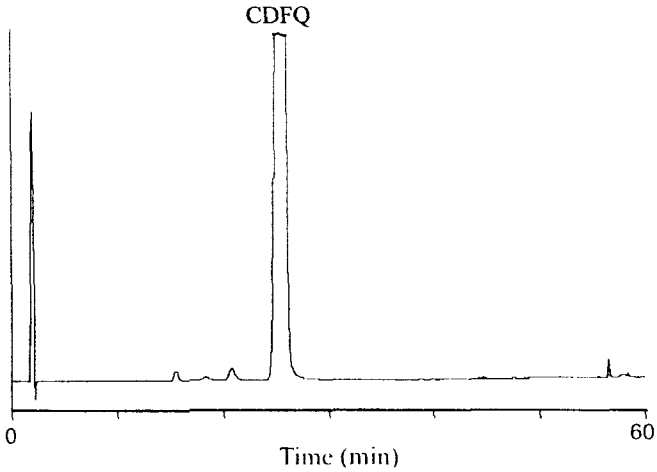


Figure 7. Chromatogram for CDFQ, lot A, prepared by modified procedure.

future HPLC analyses of CDFQ, a maximum limit of 50 ppm was set for total peroxides in the THF. We urge caution when THF is used for HPLC sample preparations, particularly when the solutions are heated or sonicated. Although no attempt was made here to generalize this reaction, in principle it could occur for quinolones other than CDFQ.

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