

Separations of Fluoroquinolone Antibiotics by Reversed-Phase HPLC, Using Discovery Columns

Antibiotics from the fluoroquinolone subclass were studied and separated by reversed-phase HPLC on Discovery RP-AmideC16, Discovery C18, and Discovery C8 columns. These columns offer excellent resolution and selectivity.

Key Words:

fluoroquinolone • antibiotics • Discovery HPLC columns

More than 100 antibiotics are on the market today, and many more are in development. Bacterial resistance to antibiotics is a serious problem (1, 2). An antibiotic that takes a decade to bring to market can induce resistance within months of its introduction into clinical practice (3). The frequency of resistance in bacteria and the numbers of drugs to which bacteria are resistant are increasing. Therefore, it is critical to monitor the level of antibiotics given to humans and animals. HPLC is a powerful tool for isolation and quantitation of antibiotics because of its high specificity and sensitivity.

Fluoroquinolones are potent synthetic agents active against a variety of bacterial species (4,5). They have emerged as one of the most important classes of antibiotics of the past decades.

We analyzed a class of fluoroquinolones – ofloxacin, norfloxacin, ciprofloxacin, lomefloxacin – from powder form. To prepare these powder samples, we dissolved them in methanol, then diluted the components to appropriate concentrations with 25mM phosphate buffer (pH 3). The fluoroquinolones were separated by reversed phase HPLC, using isocratic elution (Figures A-C), on Discovery™ RP-AmideC16, Discovery C18, and Discovery C8 columns (each 15cm x 4.6mm ID, 5µm particles). The RP-AmideC16 column produced a baseline separation of ofloxacin and norfloxacin, which co-eluted on the Discovery C8 and C18 columns (Figures B & C).

Selectivity differences for ofloxacin and norfloxacin might be explained by the hydrogen bonding between the amide functionality of the RP-AmideC16 phase and the NH functionality of norfloxacin. Such differences show the advantage of using the amide column for a difficult separation.

Levofloxacin, sparfloxacin, grepafloxacin, and trovafloxacin were approved by the US Food and Drug Administration (FDA) during the 1990s (6-10). These fluoroquinolones are active against many pathogenic Gram-negative and Gram-positive bacteria and atypical pathogens. We studied a mixture of six fluoroquinolone (ranging from 200mg to 750mg) extracted from their respective tablets. First we crushed the tablets into powders and dissolved them in methanol; then we filtered the sample through 0.2µm PVDF filters to eliminate suspended particles. We then used

Figure A. Fluoroquinolone Antibiotics on a Discovery RP-AmideC16 Column

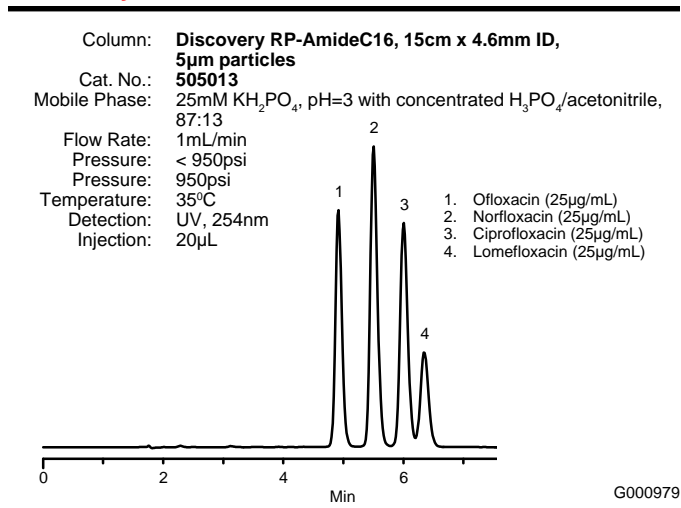
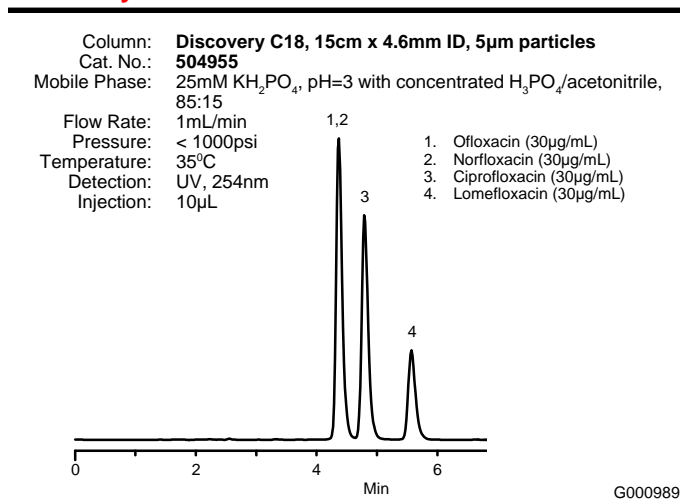


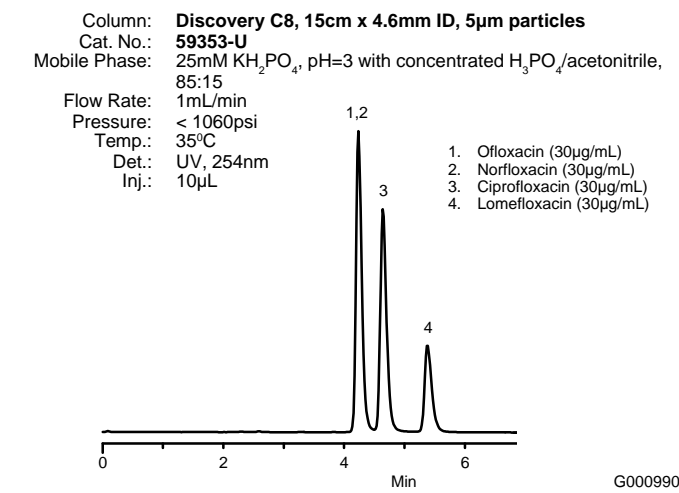
Figure B. Fluoroquinolone Antibiotics on a Discovery C18 Column



phosphate buffer to dilute the components to appropriate concentrations. We separated these fluoroquinolones using gradient elution with the same three Discovery columns (Figure D). Excellent resolution and peak shape are evident for some of the newly developed drugs in this class.

These studies show that, in the analysis of fluoroquinolone antibiotics, Discovery RP-AmideC16, Discovery C18, and Discovery C8 columns yield excellent resolution and selectivity.

Figure C. Fluoroquinolone Antibiotics on a Discovery C8 Column



Ordering Information:

Description	Cat. No.
Discovery HPLC Columns	
15cm x 4.6mm ID, 5µm particles	
Discovery RP-AmideC16	505013
Discovery C18	504955
Discovery C8	59353-U
Discovery Selectivity Packs¹	
5cm x 2.1mm ID Columns	55720-U21
15cm x 2.1mm ID Columns	55722-U21
5cm x 4.6mm ID Columns	55720-U
15cm x 4.6mm ID Columns	55722-U
25cm x 4.6mm ID Columns	55724-U

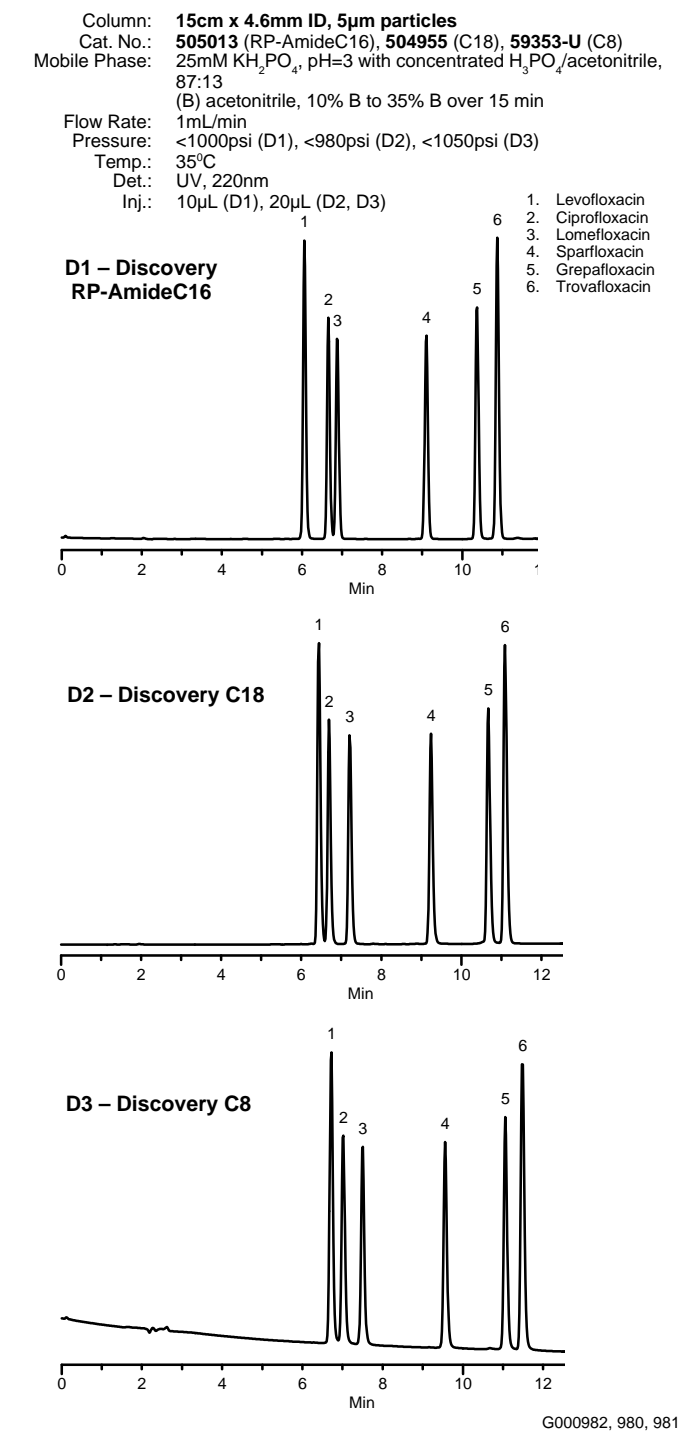
¹Four columns of equal dimensions, one of each Discovery phase (C18, RP-AmideC16, C8, Cyano).

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Figure D. Fluoroquinolone Antibiotics From Tablets



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