



## An adaptable HPLC method for the analysis of frequently used antibiotics in ocular samples<sup>☆</sup>

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### ABSTRACT

Four different antibiotics, delivered individually to rabbit eyes via hydrophilic intraocular lenses soaked in the drug solution prior to implantation, were measured in aqueous and vitreous humor samples from the eyes. To meet this analytical need, we developed a sensitive, high performance liquid chromatographic (HPLC) method for measuring the concentrations of moxifloxacin, gatifloxacin, linezolid, and cefuroxime in the ocular tissue. Separations were carried out on a LichroSpher RP-18 column, maintained at room temperature. The fluoroquinolones were eluted with a mobile phase consisting of 20% acetonitrile, in 0.1% trifluoroacetic acid (pH 3.0) with 30 mM tetrabutylammonium chloride. Linezolid and cefuroxime were eluted with 25% acetonitrile in 25 mM Na acetate buffer, pH 5.0. All elutions were isocratic. With ultraviolet detection, the lower limit of quantitation (LLOQ) for these compounds approached 1 ng (on-column injection). By using fluorescence detection, the LLOQ for the fluoroquinolones improved to 200 pg. The overall accuracy of the method was  $\geq 90\%$ . With minor modifications, the method was optimized for each of the agents, and the resulting analytical sensitivity made the method suitable for clinical investigations of the ocular penetration of these drugs.

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### 1. Introduction

Cataract surgery is the most commonly performed surgery in the United States, numbering over 1.5 million annually. Endophthalmitis remains a rare complication of cataract extraction [1], but is potentially vision-threatening. The intracameral injection of intraoperative antibiotics during cataract surgery has recently been advocated as a means of providing prophylaxis against endophthalmitis, however, the achieved concentrations and duration of their effect is poorly defined [2]. A novel approach for intraocular antibiotic delivery utilizes a hydrophilic polymer intraocular lens

(IOL), soaked with antibiotics prior to implantation in the eye, to release the drug over hours or possibly days following the surgery [3].

In order to evaluate the ability of hydrophilic IOLs to deliver antibiotics to the eye, analytical methods for the precise measurement in ocular tissues of the antibiotics of interest were developed. Moxifloxacin, gatifloxacin, linezolid, and cefuroxime were the antibiotics selected based on their frequent ophthalmic use, as well as their antimicrobial activities against microorganisms commonly causing endophthalmitis, specifically gram-positive cocci. Moxifloxacin and gatifloxacin are bactericidal fluoroquinolones, which inhibit DNA gyrase and topoisomerase IV, and provide wide-spectrum coverage of gram-positive as well as gram-negative pathogens, including *Pseudomonas* [4,5]. Linezolid inhibits protein synthesis by binding to the bacterial 50S ribosomal subunit. It is bactericidal against most streptococci species and bacterostatic against staphylococci and enterococci [6,7]. Cefuroxime is a second generation cephalosporin that inhibits bacterial cell wall synthesis, with moderate coverage of gram-positive and gram-negative bacteria [8].

Methods for analysis of these antibiotics have been published, many employing reverse phase chromatography, e.g. for line-

Abbreviations: IOL, intraocular lens; MIC, minimum inhibitory concentration.

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zolid (with quantitation limits generally above 0.1  $\mu\text{g/ml}$ ) [7,9–14]; for the fluoroquinolones moxifloxacin [15–22] and gatifloxacin [23–27] (some of the methods with quantitation limits approaching 0.01–0.02  $\mu\text{g/ml}$ , but others much less sensitive); and for the  $\beta$ -lactam, cefuroxime (with one exception [28], all having quantitation limits of 0.1  $\mu\text{g/ml}$  or higher) [29–31]. Other analytical methods have also been utilized for these antibiotics, including liquid chromatography coupled to mass spectrometry (LC–MS) [32–36], capillary electrophoresis (CE) [37–41], capillary electrochromatography (CEC) [42,43], and spectrophotometry [44–48]. In addition to the insufficient analytical sensitivity of many of these methods for clinical use, few of the published methods are specifically designed for analysis of ocular samples. After reviewing these methods, a core analytical HPLC method was derived that could be adapted to the analysis of each of the four antibiotics with minor modification, accomplishing the dual aims of utilizing isocratic elution for simplicity and reproducibility, and maximizing sensitivity for all analytes. The present report describes the development of this adaptable and easily reproducible HPLC method using UV detection for the measurement of the four antibiotics in aqueous and vitreous humor ocular samples. For laboratories equipped with fluorescence detection, a modification of the method is described for analysis of the fluoroquinolones that provides additional detection sensitivity.

## 2. Experimental

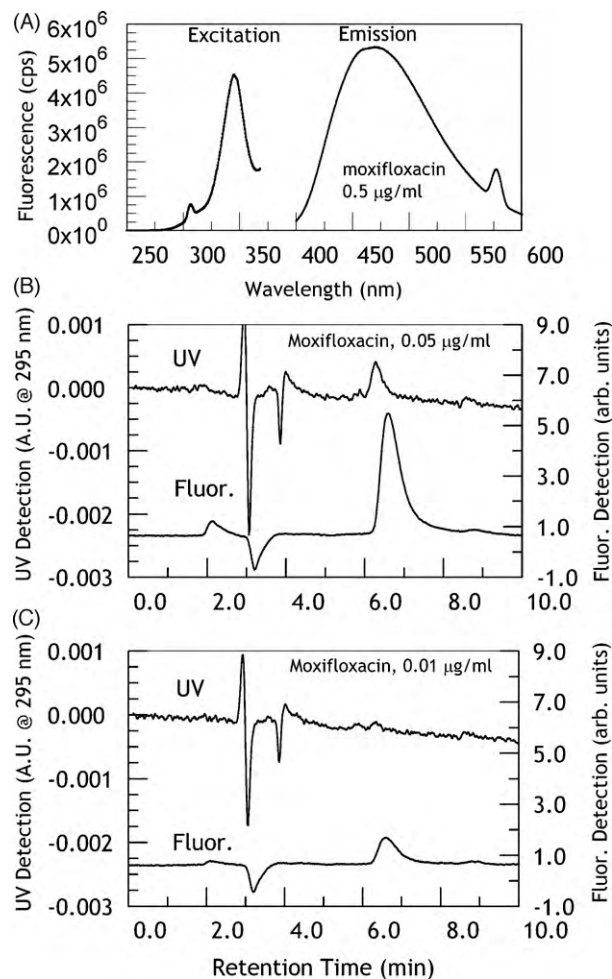
### 2.1. Chemicals

The water used for the mobile phases was double-distilled (Millipore Nanopure). Moxifloxacin was obtained as the formulation Vigamox (5 mg/ml) from Alcon Laboratories (Ft. Worth, TX) and gatifloxacin was obtained as the formulation Zymar (3 mg/ml) from Allergan Inc. (Irvine, CA). Linezolid was obtained as the injectable form of Zyvox (2 mg/ml) from Pfizer Inc. (New York, NY). Cefuroxime was supplied as a dry powder by Cura Pharmaceuticals (Eatontown, NJ), from which a quantity was weighed out and dissolved in sterile water to make a stock solution, which was stored frozen at  $-20^\circ\text{C}$  away from light. From the stock solutions of these drugs, fresh working dilutions were prepared before each analytical session to serve as standards. HPLC-grade acetonitrile (99.9% pure) was purchased from Sigma–Aldrich. Tetrabutylammonium chloride (TBA, >99% purity) was purchased from Sigma–Fluka, and HPLC/spectroscopy grade trifluoroacetic acid (TFA, 99.5% min) was obtained from Pierce. The fluoroquinolone antibiotics used as standards were ophthalmic formulations intended for topical application, and were used simply for convenience. With the exception of moxifloxacin, these ophthalmic preparations are not likely suitable for direct intracameral injection in humans due to the added preservatives.

### 2.2. Chromatographic conditions

Two analytical variants were employed, one for analyzing the fluoroquinolones, and the other for linezolid and cefuroxime. The mobile phase used for both moxifloxacin and gatifloxacin was 20% acetonitrile and 30 mM TBA, in 0.1% TFA, pH 3.0. The mobile phase used for both linezolid and cefuroxime was 25% acetonitrile in 25 mM sodium acetate buffer, pH 5.0. The mobile phases were vacuum-filtered before use.

The HPLC system consisted of a Waters Empower workstation controlling a Waters 515 pump operated in isocratic mode at 0.5 ml/min and a Waters 717 autosampler. The chromatographic analyses were performed at room temperature with a LiChrospher RP-18, 5- $\mu\text{m}$  particle, 125 mm  $\times$  4 mm reversed-phase



**Fig. 1.** (A) Fluorescence excitation-emission characterization of moxifloxacin. The concentration of moxifloxacin was 0.5  $\mu\text{g/ml}$ , diluted in water from the stock solution. Data were obtained with a Jobin Yvon-Spex FL-3 spectrofluorimeter. (B and C) Comparison of UV and fluorescence detection of fluoroquinolones. There is an approximately fivefold increase in sensitivity for quantitation of moxifloxacin using fluorescence detection. Moxifloxacin is the peak with retention time at 6.2 min in the UV channel and 6.6 min in the fluorescence channel (see text for additional details). With UV detection, the LLOQ for moxifloxacin was 0.05  $\mu\text{g/ml}$  (B), while with fluorescence detection, the LLOQ was at least 0.01  $\mu\text{g/ml}$  (C). In (B and C), the left-hand ordinate shows the scale for the UV channel, and the right-hand ordinate shows the scale for the fluorescence channel.

column (Phenomenex, Torrance, CA), fitted with a guard column (a Waters Sentry Guard 3.9 mm  $\times$  20 mm,  $\mu$ Bondapak C18, 125  $\text{\AA}$ , 10  $\mu\text{m}$  cartridge, or a Phenomenex Security-Guard, 4 mm  $\times$  3 mm cartridge [part no. AJ0-4287] were used interchangeably). A Waters 996 photodiode array (PDA) was used for all of the analytes. For UV detection of the drugs, the following wavelengths were used: the two fluoroquinolones were detected at 295 nm, linezolid was detected at 255 nm, and cefuroxime was detected at 274 nm. To improve the sensitivity for the fluoroquinolones, a Waters 474 scanning fluorescence detector was connected in tandem with the PDA detector, with the excitation wavelength at 345 nm and emission wavelength at 470 nm (see Fig. 1).

### 2.3. Preparation of standards and calibration curves

Aqueous humor samples required no pre-processing other than dilution. Therefore, determination of all antibiotics in the aqueous humor samples was based on the external standard method, i.e. by comparison of analyte peak heights to those of dilutions of the authentic antibiotics. External standards were calibrated

over the range of 0.01–10.0 µg/ml for all of the antibiotics, except for gatifloxacin, due to saturation of the fluorescence detector at concentrations above 2.5 µg/ml. Because vitreous humor samples required pre-processing by solid phase extraction, they were quantitated by inclusion of an internal standard. Aliquots of vitreous samples were spiked with an internal standard, i.e. moxifloxacin and gatifloxacin served as the internal standard for the other, and cefuroxime and linezolid served as each other's internal standard. In each ocular tissue sample, only one of the antibiotics was present as the analyte, i.e. each of the analytes was measured independently, and thus it was appropriate to use one of the other antibiotics as the internal standard. Internal standards were calibrated by determining, after solid phase extraction of the sample, the ratio of the analyte peak height to the internal standard peak height, over an analyte concentration range of 0.01–10.0 µg/ml (2.5 µg/ml for gatifloxacin). When used as internal standards, moxifloxacin and gatifloxacin were spiked at a concentration of 0.5 µg/ml, and cefuroxime and linezolid were used at a concentration of 1.0 µg/ml.

#### 2.4. Aqueous humor and vitreous humor specimens

All animal procedures were performed at the University of Illinois at Chicago (UIC), using 2–3 kg New Zealand White rabbits, following an animal use protocol approved by the UIC Institutional Animal Care and Use Committee, and were in conformance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Each rabbit was sedated with ketamine, and both eyes were dilated with phenylephrine 2.5%, cyclopentolate 1%, and tropicamide 1%. Standard techniques for clear corneal incision cataract extraction were used. The nucleus excision was performed by phacoemulsification and aspiration. The lens of each eye was then replaced with an IOL made of a hydrophilic polymer (STAAR Colamer) soaked in an antibiotic (moxifloxacin, gatifloxacin, linezolid, or cefuroxime) for 1 h. Each eye of a rabbit received an IOL soaked in a different antibiotic [3]. Samples of aqueous humor were taken at 30 min, 2 h, 4 h, 6 h and 24 h. After 24 h, the animals were sacrificed, and a sample of the vitreous humor was obtained. Samples were frozen at –75 °C, and shipped to the University of Texas Health Science Center at San Antonio, where the chromatographic analysis was carried out. Samples were maintained at –75 °C until analysis. A detailed report of the results of the animal experiments will be published elsewhere (Nijm et al., in preparation).

#### 2.5. Sample preparation

Aqueous humor samples were diluted up to 200-fold, depending on the drug and the sample time. Samples taken at earlier time points contained very high levels of drug and required more dilution. The gatifloxacin samples taken at early time points required 200-fold dilution; however, 5- to 20-fold dilutions were more typical for the other drugs. Because aqueous humor is a transparent material with very little protein, 20 µl aliquots of the diluted aqueous samples were injected directly into the analytical column. Vitreous samples were prepared by solid phase extraction (SPE) prior to analysis to remove proteins and other interfering compounds. A measured quantity (typically 50 or 100 µl) was removed from each sample, spiked with the internal standard, as described above, diluted with water to a final volume of 1 ml, and loaded on Waters Oasis HLB 1-ml SPE cartridges containing 30 mg of sorbent that had been preconditioned with 1 ml methanol followed by 1 ml distilled water for equilibration. The fluoroquinolones were washed with 1 ml 5% methanol, while the linezolid and cefuroxime samples were washed with 1 ml distilled water. For all compounds, the final elution was accomplished with 1 ml of 75% methanol–1%

NH<sub>4</sub>OH. Eluates were vacuum-dried, and resuspended in 100 µl of mobile phase prior to injection of a 20 µl aliquot into the column.

#### 2.6. Recovery, reproducibility, and accuracy

The recovery after SPE of the vitreous samples was determined by comparing peak heights of extracted and non-extracted test samples of the drugs. Vitreous sample recovery was >85% for linezolid and >90% for cefuroxime and the fluoroquinolones. Reproducibility was determined by comparing peak heights of repeated injections of calibration samples of the drugs, carried out over several days. Accuracy was determined by comparing the actual peak height of calibration samples of drugs against the expected response.

### 3. Results and discussion

#### 3.1. Optimization of detector parameters

In order to optimize the UV detection of the compounds, the optical absorption spectrum of each was measured in a spectrophotometer, and the wavelength of the largest absorption peak greater than 220 nm was determined. Using the photodiode array, linezolid was detected optimally at 255 nm, cefuroxime at 274 nm, and moxifloxacin and gatifloxacin were detected at 295 nm. For the fluoroquinolones, excitation–emission curves were obtained for each compound. The excitation–emission curve for a 0.5 µg/ml solution of moxifloxacin, diluted in water, is shown in Fig. 1A, indicating a peak excitation wavelength of 345 nm, and a peak emission wavelength of 470 nm. The excitation–emission scan of gatifloxacin showed similar wavelength maxima. Therefore, the fluorescence detector was set to these wavelengths to achieve the greatest sensitivity for these compounds. Fluorescence detection improved the measurement sensitivity for the fluoroquinolones by at least a factor of 5, improving the lower limit of quantitation (LLOQ) from 0.05 µg/ml to 0.01 µg/ml (compare Fig. 1B and C).

#### 3.2. Composition of mobile phases

The same column was used for all analyses, which simplified the method development. The mobile phases used in this study, which were adapted from two published methods for fluoroquinolones [21,49] and one reported for the analysis of cefuroxime [33], were similar for all four compounds, using 20–25% acetonitrile as the organic modifier, but with different buffers to adjust the pH for optimal separation of the specific compounds, i.e. pH 5.0 with an acetate buffer for cefuroxime and linezolid, and pH 3.0 with TFA for the fluoroquinolones. To improve peak shape for the fluoroquinolones, TBA was added as an ion-pair agent [21,49]. The incorporation of TBA limited the working lifetime of the mobile phase to about 3 weeks; therefore, the mobile phases were prepared in proximity to the anticipated time of analysis.

#### 3.3. Calibration curves

Calibration samples were run for all four compounds, and linear regressions were made to each data set. In all cases, the correlation coefficients of the calibration curves were >0.999, indicating that the methods were highly linear over their respective working ranges. For the internal standards calibrations, the regressions of the ratio of the analyte peak height to internal standard peak height, against the concentration of the analyte, were also highly linear (correlation coefficients  $\geq 0.995$ ).

Because it was impractical to procure a sufficient amount of normal rabbit vitreous in which all standard dilutions could be prepared, a small quantity of vitreous was obtained and representative

**Table 1**  
Comparison of the chromatographic response of cefuroxime and linezolid dissolved in water and in mobile phase.

Analyte	Peak height, <sup>a</sup> water	Peak height, <sup>a</sup> m.p.	Percent difference <sup>b</sup>
Cefuroxime			
0.5 µg/ml	5.141 ± 331	5.547 ± 169	7.6
1.0 µg/ml	10.230 ± 381	11.178 ± 299	8.9
5.0 µg/ml	55.584 ± 3.315	58.451 ± 4.157	5.0
Linezolid			
0.5 µg/ml	2.805 ± 52	2.917 ± 60	3.9
1.0 µg/ml	5.739 ± 164	6.073 ± 279	5.6
5.0 µg/ml	28.505 ± 650	30.180 ± 1.350	5.7

<sup>a</sup> Peak height in detector units, mean ± range, *n* = 3.

<sup>b</sup> Calculated as  $[(PH_{aq} - PH_{mp}) / (PH_{aq} + PH_{mp}) / 2] \times 100$ .

**Table 2**  
Sensitivity and working range of method.

Analyte	LOQ (µg/ml)	Maximum concentration tested (µg/ml)	C.V. as internal standard ( <i>N</i> = 6) (%)
Cefuroxime	0.08	10	2.14
Linezolid	0.05	10	4.56
Moxifloxacin	0.01	10	4.90
Gatifloxacin	0.01	5	2.83

dilutions were made. Comparison of these vitreous standards (after SPE) to dilutions in water found negligible differences. Specifically, the retention times of cefuroxime and linezolid in vitreous were (mean ± range)  $3.9 \pm 0.02$  m and  $12.5 \pm 0.01$  m, respectively, while in water the corresponding retention times were  $3.9 \pm 0.17$  m and  $12.6 \pm 0.05$  m. A comparison of peak heights for these two analytes, dissolved in water or in mobile phase (to emulate the resuspension of the compound in mobile phase following SPE), indicated that the chromatographic response for these compounds was slightly lower in water than in mobile phase, however, the differences were consistently less than 10% (Table 1). In view of the limited supply of normal rabbit vitreous, these findings justified the use of water as a solvent for making calibration samples and for evaluating the method.

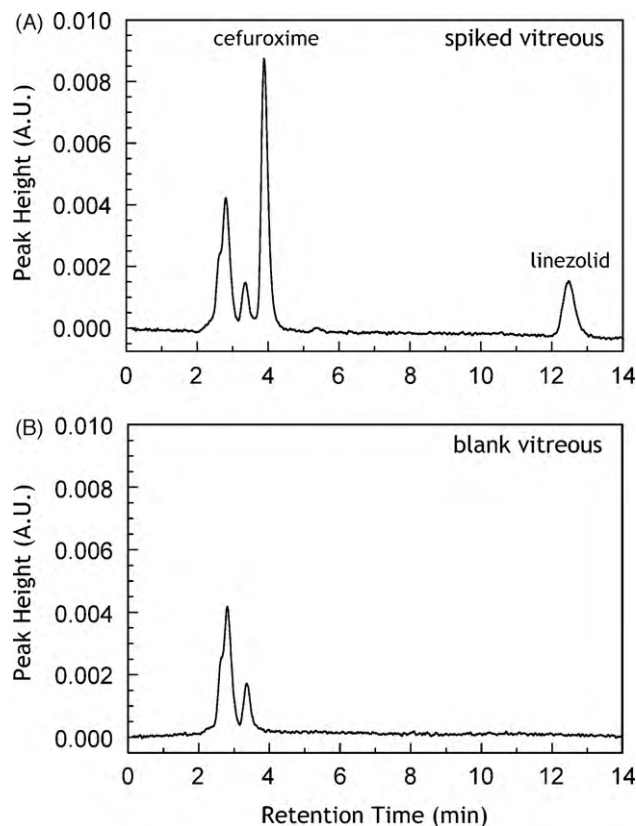
### 3.4. Performance of method

Each antibiotic was successfully detected in aqueous and vitreous humor samples from the rabbit eyes. Tables 2 and 3 summarize the performance of the methods. The LLOQ depended on the specific analyte, and varied between 0.01 and 0.08 µg/ml (Table 2). The performance of the method was assessed by comparing the

**Table 3**  
Performance of method. Data are based on three replicates of calibration samples, from analyses performed on different days.

Analyte	Nominal conc. (µg/ml)	Measured conc. (µg/ml) Mean ± S.D.	Accuracy (%)	C.V. (%)
Cefuroxime	0.10	0.11 ± 0.00 <sup>a</sup>	108.48	3.22
	1.00	1.03 ± 0.10	102.51	9.27
	5.00	4.76 ± 0.60	95.19	12.53
Linezolid	0.05	0.05 ± 0.00 <sup>a</sup>	105.57	6.61
	1.00	0.96 ± 0.06	96.47	6.00
	5.00	4.88 ± 0.32	97.54	6.48
Gatifloxacin	0.05	0.05 ± 0.00 <sup>a</sup>	94.55	0.84
	1.00	0.98 ± 0.02	98.09	2.20
	2.50	2.52 ± 0.09	100.65	3.38
Moxifloxacin	0.05	0.05 ± 0.01	97.01	12.80
	1.00	0.97 ± 0.10	96.74	9.95
	5.00	4.87 ± 0.33	97.39	6.78

<sup>a</sup> Note: Round-off of decimal places resulted in standard deviation equal to zero.



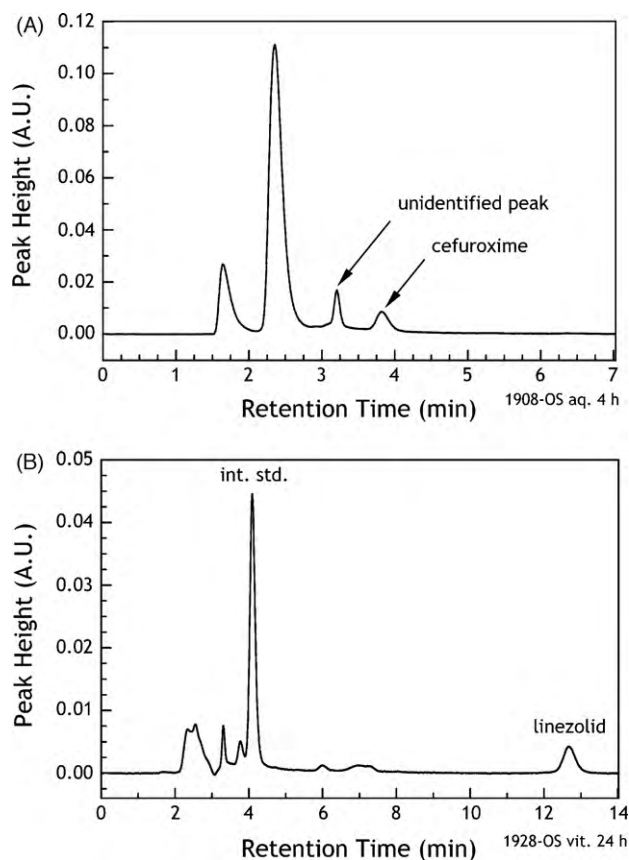
**Fig. 2.** Detection of cefuroxime and linezolid in normal rabbit vitreous humor. (A) Vitreous spiked with cefuroxime and linezolid, both at a concentration of 1 µg/ml. (B) Blank vitreous. For all of these analyses, 100 µl aliquots of rabbit vitreous were used and prepared by SPE, as described in the text, prior to injection into the analytical column.

results of three determinations of calibration samples, obtained in independent assays conducted on different days (Table 3). The reproducibility is expressed as the coefficient of variation (C.V. column in Table 3) of these measurements. Accuracy was estimated from the agreement of measured concentrations of the drugs with the nominal standard values, and is expressed as the average measured concentration as a percentage of the nominal standard concentration for three dilution levels (accuracy column in Table 3).

The LLOQ for linezolid was 0.05 µg/ml for both aqueous and vitreous samples. The LLOQ for cefuroxime was approximately 0.05 µg/ml for aqueous and 0.08 µg/ml for vitreous. For both of the fluoroquinolone drugs, the LLOQ with UV detection was about 0.05 µg/ml for both aqueous and vitreous samples, but improved to at least 0.01 µg/ml with fluorescence detection (Fig. 1B and C). All injections were made in a volume of 20 µl; therefore, these findings indicated an on-column sensitivity of about 1 ng for all four antibiotics using UV detection. With fluorescence detection, the sensitivity for the fluoroquinolones increased to 200 pg.

### 3.5. Analysis of ocular samples

A chromatogram of linezolid and cefuroxime, 1 µg/ml each, spiked into normal rabbit vitreous, is shown in Fig. 2A. For comparison, the analysis of an aliquot of blank vitreous is shown in Fig. 2B. There were no interfering peaks in the vitreous. Two examples of the analysis of samples from the rabbit IOL study are shown in Fig. 3. The analysis of cefuroxime in an aqueous humor sample taken 4 h following implantation of a cefuroxime-containing IOL implant is presented in Fig. 3A. As shown in this figure, in the cefuroxime-treated eyes only, an unidentified peak was found with a retention

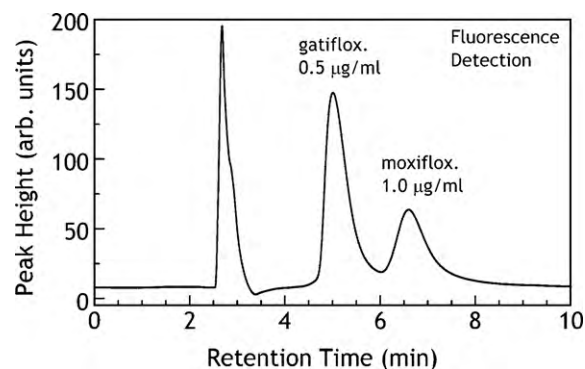


**Fig. 3.** Analysis of cefuroxime and linezolid in ocular tissues. (A) An aqueous humor sample, obtained from an eye 4 h following implantation with an IOL soaked in cefuroxime (no internal standard was used with the aqueous samples). The peak with RT of 3.2 min in this chromatogram was not identified, but was not related to cefuroxime. (B) A vitreous humor sample, from a different rabbit, obtained from an eye 24 h after implantation of an IOL soaked in linezolid. This sample was spiked with cefuroxime at 1  $\mu\text{g}/\text{ml}$ , as the internal standard.

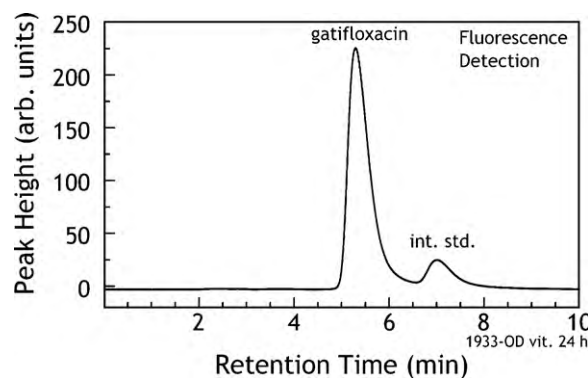
time of about 3.2 min. Additional LC–MS analysis indicated that this peak was not due to a cefuroxime metabolite or breakdown product. The chromatogram shown in Fig. 3B is from a vitreous humor sample taken at 24 h after implantation of a linezolid-containing IOL. This sample was spiked with cefuroxime as the internal standard.

The fluoroquinolone agents were detected very efficiently in both aqueous and vitreous humor samples. In the standard dilutions, detected in the UV channel, gatifloxacin had a RT of approximately 4.8 min, and moxifloxacin had a RT of 6.2 min. A much stronger detector response was obtained with fluorescence detection. Because the fluorescence detector was situated downstream from the optical detector, the gatifloxacin and moxifloxacin peaks in this channel were delayed to 5.1 and 6.6 min, respectively (Fig. 4, also compare Fig. 1B and C). Possibly because of the additional fluid path, the peaks were slightly broadened in the fluorescence channel, compared to the PDA channel, resulting in some overlap; however, the peaks for each analyte were separated sufficiently for analysis. The analysis of a vitreous humor sample, obtained 24 h after implantation of an IOL soaked in gatifloxacin, is shown in Fig. 5.

As noted earlier, the detailed pharmacokinetic results obtained with this HPLC analytical methodology will be presented elsewhere. Briefly, the validated HPLC method was used to measure the time course of antibiotic appearance and clearance from aqueous humor over a 24 h period, following implantation of a drug-loaded IOL, as well as the penetration of the drug into the



**Fig. 4.** Gatifloxacin (0.5  $\mu\text{g}/\text{ml}$ ) and moxifloxacin (1.0  $\mu\text{g}/\text{ml}$ ) standards. The chromatographic conditions were as described in Section 2.2, using fluorescence detection of the fluoroquinolones with  $\lambda_{\text{Ex}} = 345 \text{ nm}$  and  $\lambda_{\text{Em}} = 470 \text{ nm}$ .



**Fig. 5.** Analysis of fluoroquinolones in vitreous humor. Fluorescence detection of gatifloxacin in vitreous humor obtained from a rabbit eye 24 h after implantation of an IOL soaked in this drug. The internal standard was moxifloxacin, 0.5  $\mu\text{g}/\text{ml}$ .

vitreous humor at 24 h. For each antibiotic studied, the mean drug concentration remained above the minimum inhibitory concentration (MIC) for most endophthalmitis-causing bacteria for at least 6 h. Of note, gatifloxacin concentrations in aqueous humor remained above relevant MICs for at least 24 h after IOL implantation.

### 3.6. Optimization of fluorescence detection

In order to achieve maximal detection sensitivity, the optimal parameters must be used. With respect to fluorescence detection of fluoroquinolones, there has been considerable variation in the wavelengths selected by analysts for the detector settings. For example, the excitation wavelength has frequently been set in the range of 280–295 nm [17,18,21,26,49,50], but longer excitation wavelengths, e.g. 325 nm in a CE instrument [49], and even 515 nm [22], have been reported. For measurement of the fluorescence emission, wavelengths have ranged from 400 nm [21] to 450–460 nm [17,49,50] as well as 500 nm and longer [18,22]. In view of this wide range of detection parameters, the fluorescence excitation and emission wavelengths used in this study, 345 and 470 nm, respectively, were selected from the direct measurements of the fluorescent properties of moxifloxacin and gatifloxacin. The resulting detection sensitivity was at least 0.01  $\mu\text{g}/\text{ml}$  (200 pg on-column) for both fluoroquinolones. Gatifloxacin appeared to have higher quantum efficiency, compared to moxifloxacin, and it is possible it may be detected at a lower concentration with further method optimization.

#### 4. Conclusion

This paper describes a straightforward HPLC method that was adapted to the assay of four antibiotics, cefuroxime, linezolid, gatifloxacin, and moxifloxacin, with only relatively minor adjustments to the composition of the mobile phase. The method is robust and exhibits greater than 90% accuracy for all four compounds. Although tandem UV and fluorescence detection was used in this study, laboratories with access to a UV detector only may still utilize this method, with approximately a fivefold loss of sensitivity for fluoroquinolone detection, as illustrated in Fig. 1. Nevertheless, both detection methods yield sufficient sensitivity, equal to or better than 0.05 µg/ml, to be adequate for most clinical pharmacokinetic studies of these agents, especially in ophthalmic research, in which very limited specimen volumes are typically available [51,52].

The pharmacokinetic results from our research show therapeutically effective drug concentrations were achieved in ocular tissue for all four antibiotics using IOL delivery. Gatifloxacin and moxifloxacin were especially well delivered by this technique (Nijm et al., in preparation). The novel approach of using antibiotic-soaked IOLs has great clinical potential, and may provide a safer and more economical method of preventing infectious complications of cataract surgery, now one of the most commonly performed surgeries worldwide.

#### Conflict of interest

The authors have no financial or proprietary interests in any of the products or methods described in this report.

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