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Application of quality by design approach in HPLC-UV va of ciprofloxacin in porcine ocular tissue and simulated ocu for quantification in an ex vivo ocular kinetic study

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hic method (HPLC-UV) for quantifying
ıre was developed and validated in
ccessfully used to determine the con-
AQbD) was used to develop extraction
ı was efficiently extracted through a
, with a 94.32 ± 0.61% recovery rate.
ıobile phase acetonitrile and disodium
ated method was discovered to be se-
veloped in this study, the drug's ocular
tration of eye drops and ointment. This
ı ocularcokinetics research.

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ABSTRACT

We present a novel high-performance liquid chromatograp ciprofloxacin after *ex vivo* administration. The proced accordance with ICH and FDA guidelines, and it was su centration of ciprofloxacin. Analytical quality by design techniques and optimize HPLC conditions. Ciprofloxacir single-step protein precipitation and extraction procedure All analytes were separated using isocratic elution and a n hydrogen phosphate over a 10-min run period. The valid lective, precise, and accurate. Finally, using the method de kinetic profiles were determined following *ex vivo* adminis validated HPLC-UV approach has potential applications ir

1. Rationale

acting vulnerable groups. Corneal
and account for 5% of preventable
uring of contact lenses in the United
g agricultural work [1]. Infectious
keratitis has reported that extended
uginosa [2]. Most of the cases of
ıinolone monotherapy or fortified

high patient acceptance with fewer

Corneal disorder remains the driving cause of global monocular blindness, particularly imp ulcers, commonly caused by bacterial keratitis, are the world's fifth leading cause of blindness a vision impairment in the world's least developed countries. Keratitis is also associated with the wea States, but in developing countries, it is more generally caused by eye damage suffered during keratitis can be caused by bacteria, viruses, or fungi. The Academy of Ophthalmology for bacterial k contact lens wearing is a common cause of bacterial keratitis induced by *Pseudomonas aeru* community-acquired infectious keratitis lead to empirical treatment with antibiotics. Fluoroqu aminoglycosides [2] are the most widely used therapies to treat this disorder.

Fluoroquinolones have broad antibacterial activity, deep ocular penetration, low tolerance, and

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side effects. Ciprofloxacin is a fluoroquinolone with a very good antibacterial profile. Since its acceptance, over 250 million patients are treated globally with a good safety profile, as reported in respected clinical publications [3]. Ciprofloxacin has been commonly used for bacterial keratitis in eye drops. Ophthalmic drug types have been one of the most important and widely known fields of pharmaceutical science and appear to be the best medication for bacterial keratitis [4].

In the evaluation of ocular dosage forms, *in vitro*, *in situ*, and *ex vivo* models have been developed. *In vitro* model is over-simplified by the complex visual anatomy to just one or a few cell layers; therefore, the visual barriers' function is most often not taken into account [5]. It takes time to build *in vitro* models using human/rabbit epithelial and endothelial cell lines to create a 3D culture, and epithelial resistance (a barrier permeability measure) is not comparable to human corneal tissue [6]. For *in vivo* animal research, the animal ethics committee requires intensive and detailed plans to ensure reports and a high degree of financial resources. Animal drug activity monitoring is referred to as "3Rs," which is abbreviated as "Reduction," "Replacement," and "Refinement". The use of *ex vivo* eye designs is an alternative to the concepts of 3R and can be specifically applicable in research on ocular drug delivery. This *ex vivo* model has similar permeability coefficients to the human eye as the porcine cornea [7], enhancing the model's predictivity, validity and, reproducibility between assays.

A pharmaceutical problem remains the ocular penetration of topically applied products. After topical application, the formation of structural barriers will prevent the absorption of drugs through the eye [5]. However, only 5-10% of the topically administered dose enters the inner ocular tissues [8]. To study the ocular pharmacokinetic profile of the drug, a suitable measurement method is required. Numerous researchers have defined *in vitro* and *in vivo* methods for determining the ciprofloxacin ocular pharmacokinetics. HPLC is an analytical tool for the analysis of ciprofloxacin. Samanidau et al. [9] reported a direct determination procedure of ciprofloxacin in pharmaceutical samples and blood serum. Drusano et al. [10] used a population method to analyze ciprofloxacin kinetics with multiple plasma samples and a single vitreous sample from rabbits using HPLC. Balguri et al. [11] have reported both *in vitro* and *in vivo* investigations of ocular disposition of ciprofloxacin from topically applied PEGylated nanostructured lipid carriers. However, there is no single method that can be used to analyze ciprofloxacin after *ex vivo* assay. Therefore, it is crucial to develop a new analytical method for quantification of ciprofloxacin for *ex vivo* studies.

In recent years, significant attention has been paid to developing new assay methods for drug analysis. The definition of quality by design (QbD) is gradually being applied to the development and validation of analytical techniques, which is referred to as analytical quality by design (AQbD). Since the USFDA implemented it, quality by design has been an essential pharmaceutical industry concept. Less experiment time is needed since design of experiment (DOE) methods are used to obtain possible parameter combinations and, it was recommended in the robustness test. In several studies, according to Rozet et al. [12], applications of AQbD to develop an HPLC analytical method were presented using the AQbD approach.

Ciprofloxacin is currently available as an eye drop and ointment for ocular problems. However, no bioanalytical method for detection/quantification of ciprofloxacin after *ex vivo* administration has been established. As a result, we have established a new HPLC-UV technique for measuring ciprofloxacin in porcine eye tissue matrices that's fast and accurate according to the US Food and Drug Administration (FDA) guidelines and the International Conference on Harmonization (ICH) in this report. Before analyzing ciprofloxacin with HPLC, we have developed and optimized a method with response surface methodology. For the first time, the validated method was then used to evaluate the drug's *ex vivo* ocular kinetic profiles after topical application to the eye.

2. Procedure

2.1. Chemicals and materials

Sigma–Aldrich Pte Ltd provided ciprofloxacin (purity: 99.9%), disodium hydrogen phosphate (analytical grade), and methanol (HPLC grade) (Singapore). Waters provided the HPLC column Xselect CSHTM C18 (Waters, 3.0 × 150 mm, 3.5 μm particle size) (Dublin, Ireland). Sigma–Aldrich Pte Ltd provided all of the other chemical reagents (Singapore). The remaining reagents were of analytical grade and purchased from regular commercial sources.

2.2. Preparation of stock, calibration standard and, quality control samples

Simulated tear fluid consisting of 6.7 g NaCl, 2.0 g $NaHCO_3$ and 0.08 g $CaCl_2{\cdot}2H_2O$ was initially prepared by dissolving components in 1 L deionized water (final pH of 7.4) [13]. To make ciprofloxacin standard solutions,100 mg of the ciprofloxacin was dissolved in methanol and diluted to 100 mL in a volumetric flask, achieving 1 mg/mL concentration. After that, the calibration and quality control (QC) solutions were prepared by diluting the standard working solutions with aquatic stock solutions in a series of steps. The calibration standards were prepared by dissolving aliquots of the stock solution in a mobile phase to achieve a final concentration range of 0.01–10 μg/mL. Porcine eye corneal tissue matrices were prepared by adding stimulated tear fluid to porcine eye corneal tissue with a ratio of 1:10. The samples were then homogenised using a homogenizer with a speed of 1000 rpm for 15 min, resulting in porcine eye corneal tissue matrices. The calibration requirements were prepared by mixing 100 μL of ciprofloxacin stock solutions into 900 mL of porcine eye corneal tissue matrices to achieve the concentration range of 0.01–10 μg/mL. All samples were processed at 25°C. Quality control solutions in porcine eye corneal were separately prepared in three different concentrations (0.03 g/mL as low, 3.5 g/mL as medium, and 7.5 g/mL as high), independent of the calibration standards.

2.3. Preparation of samples and analytes extraction

Preparation of sample and extraction of analytes was done using methanol extraction. Initially, methanol was added to porcine eye corneal tissue matrices spiked with g solutions. The mixture were then vortexted and sonicated for various period of times to optimize the extraction process. The samples were then centrifuged for 15 min. The obtained supernatant was put into a glass vial. The solvent was then evaporated in a fume hood for three hours to produce a dry residue. In the 100 µL mobile phase, the residue was then reconstituted and transferred to a 0.5 mL centrifuge tube. The ingredients were mixed by vortexing for 30 s before spinning for 15 min at a temperature of 20–25°C at 14,000 rpm. The obtained supernatant was collected, and 10 µL volume was injected into the HPLC for analysis. Fig. 1 illustrates the steps involved in the extraction of ciprofloxacin from corneal tissue. The Design Expert Software version 11 (State-Ease, Minneapolis, MN, USA) was used to optimize process parameters. The Composite Central Design (CCD) under response surface methodology was employed to optimize the sonication time, vortex time, and methanol volume. Additionally, the extraction performance and evaporation time were recorded as the responses (Table 1).

2.4. Instrumentation and optimization of HPLC–UV conditions

HPLC (Shimadzu Prominence, Shimadzu, Kyoto, Japan), Xselect CSH™ C18 column (Waters, 3.0 × 150 mm) particle size of 3.5 µm, fitted with a guard cartridge was used. Acetonitrile with disodium hydrogen phosphate 10 mM was used as the mobile phase.

The Central Composite Design (CCD) technique was used in conjunction with the Design Expert Software version 11 (State-Ease, Minneapolis, MN, USA) to optimize the chromatographic conditions, including mobile phase, flow rate, mobile phase pH, and acetonitrile concentration (Table 2). These parameters on retention time (RT), theoretical plates, and tailing factor were recorded as the response.

2.5. Analytical method validation

The established bioanalytical method was then validated in accordance with ICH and US FDA guidelines [14,15]. Parameters including selectivity, linearity, the lower limit of quantification (LLOQ), carry-over, dilution reliability, consistency, precision, extraction recovery, and stability have been validated.

2.6. Linearity, LOD and LLOQ

The linearity of the samples was checked by creating calibration curves with the standard working solution in the concentration range previously defined. The calibration curves were built on three different occasions at seven-level concentrations. The lower limit of quantification (LLOQ) and the limit of detection (LOD) was determined using the following equations after acquiring the standard deviation (SD) of the response and the slope of the calibration curve.

$$LOD = 3.3\sigma/S \quad (1)$$

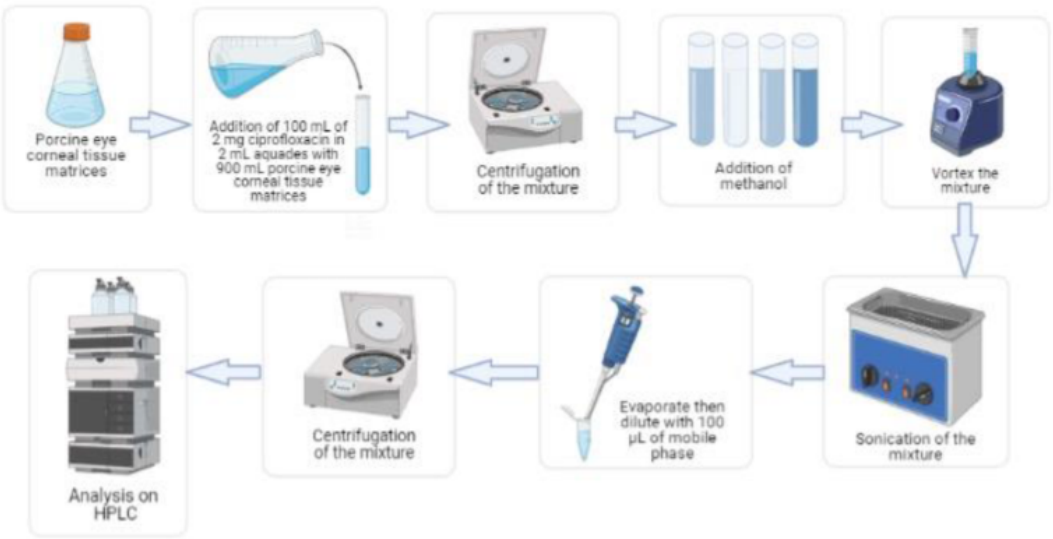

Fig. 1. Illustration of the steps involved in the extraction of analyte.

Table 1
Variables in CCD for optimization of analyte extraction.

Independent variables	Levels	
	-1	+1
Methanol volume (mL)	100	900
Vortex time (minute)	1	15
Sonication time (minute)	1	15

Table 2
Variables in CCD for optimization of HPLC method.

Independent variables	Levels	
	-1	+1
Acetonitrile concentration (%)	10	30
pH	2	4
Flow rate (mL/minute)	0.8	1

$$LLOQ = 10\sigma/S \quad (2)$$

Where σ = the SD of the data response and S = the slope of the calibration curve.

2.7. Precision and accuracy

In six replicates, at low, medium, and high concentrations, precision and accuracy were assessed for the LLOQ and QC samples. The accuracy of the solution was determined by measuring the relative errors (RE) and observing the relative standard deviation (RSD) of all solution responses. In this report, the intra-day and inter-day precision and accuracy were assessed. The RSD and RE for each sample replication should be less than 15% [14,15][15].

2.8. Carry-over and dilution integrity

The carry-over assessment was carried out by administering large concentrations of QC samples analyzed initially. A blank solution was injected afterward. The area of the blank solution was measured, and the area of the sample solution at LLOQ concentration could not be more than 20% of the area of the solution [14].

To evaluate the dilution integrity, a sample of analyte concentrations that are higher than the highest concentration of standard calibration solutions was analyzed. For this study, spiked samples of all analytes were prepared at a concentration of 250 g/mL for ciprofloxacin, followed by 5- and 10-times dilutions with porcine eye corneal matrices. The precision and accuracy were eventually measured.

2.9. Recovery of extraction

The recovery of extraction of all analytes was obtained from the sample matrices through a comparison of the measured value of all LLOQ analytes, low, medium, and high LLOQ QC samples with the same concentrations of samples prepared during the mobile process.

2.10. Stability

Ciprofloxacin stability test in porcine eye corneal fluid was performed under different treatment and storage conditions. For 48 h, the stability of all autosampler analyte solutions was evaluated. All analytes were tested for bench-top stability at room temperature for 24 h and long-term stability at -20°C for two weeks. Three freeze-thaw cycles were also used to test the stability at room temperature from -20°C storage. The initial responses of each solution were compared to the response to stability studies.

2.11. Evaluation of the ocular kinetics after ex vivo administration of ciprofloxacin

Franz-type diffusion chambers with porcine cornea were used to perform experiments to determine ocular kinetics and drug permeation through the cornea. Immediately after slaughtering the animal, porcine eyes were collected and, corneas were carefully excised to prevent any damage. Fresh porcine eyeballs were collected for the purpose; the cornea was carefully removed with 2–4 mm of scleral tissue, washed with normal cold saline, and preserved till further use. By sandwiching the scleral layer between the rims of the two chambers, the freshly extracted and washed cornea was positioned between the donor and receptor compartments of the diffusion cell. Contrary to the endothelial surface, the epithelial surface faced upward towards the receptor compartment. The 10 ml capacity receptor compartment was filled with pH 7.4. simulated tear Fluid and kept on the magnetic stirrer slowly. At 37±2°C, the assembly temperature was maintained. In the donor compartment, ciprofloxacin dosage form was added [16]. At various interval

times, the ocular tissue was detached from the Franz cells and the excess of the formulation on the surface of the tissue was removed using deionized water. The tissue was handled as per method described in 2.3. Samples were stored at -20°C prior to analysis process. PK Solver's one compartment open model (China Pharmaceutical University, Nanjing, China) was used to determine the ocular kinetics profiles. The maximum drug concentration (Cmax), the maximum concentration-time (tmax), the drug concentration-time curve from 0 to 72 h (AUC), the mean half-life (t1/2), and the mean residence time (MRT) were all calculated.

2.12. Statistical analysis

The data ware presented as mean ± standard deviation (SD) Microsoft Excel® 2016 was used to measure mean, SD, %RSD and, % RE (Microsoft Corporation, Redmond, USA). PK Solver (Microsoft Excel add-in program) was used to determine non-compartmental pharmacokinetic parameters. Drug concentration curve vs. time profiles were developed. GraphPad Prism® version 8.3.0 (GraphPad Software Inc., San Diego, California) was used to statistically analyze results, with $p < 0.05$ as a significant difference.

3. Data, value and validation

3.1. Sample preparation and analytes extraction

Preparation of samples and extraction of analytes from biological matrices was carried out in this study using methanol protein precipitation, which has been previously shown to be simple to implement and use [17]. The sample preparation step was used to remove proteins and other compounds that can interact with the analyte of interest and cause column damage. The extraction performance and evaporation time were the parameters observed in this optimization process. Central composite experimental design was used to optimize the extraction method. The results showed that extraction performance and evaporation time in three parameters fitted to the quadratic model. The F-values for extraction efficiency and evaporation time were determined to be 10.64 and 1162.78, respectively.

Additionally, both *p*-values were <0.001, suggesting that extraction performance and evaporation time had a major effect on the optimization of this method. Fig. 2 depicts a representative three-dimensional graph demonstrating the impact of selected factors on extraction efficiency and evaporation time. As can be seen from these models, the performance of the extraction was directly proportional to the amount of the sonication time, vortex time, and methanol volume. The increase in the amount of sonication time, vortex time, and methanol volume in the extraction method generated higher extraction performance. As shown in Fig. 3, the evaporation time increased as the sonication time, vortex time, and methanol volume increased. The volume of methanol might cause it, as the more methanol was used for extraction, the longer it took for evaporation.

Finally, according to the software analysis, the methanol volume of 560 ml, vortex time of 15 min, and 3.2 min of sonication time

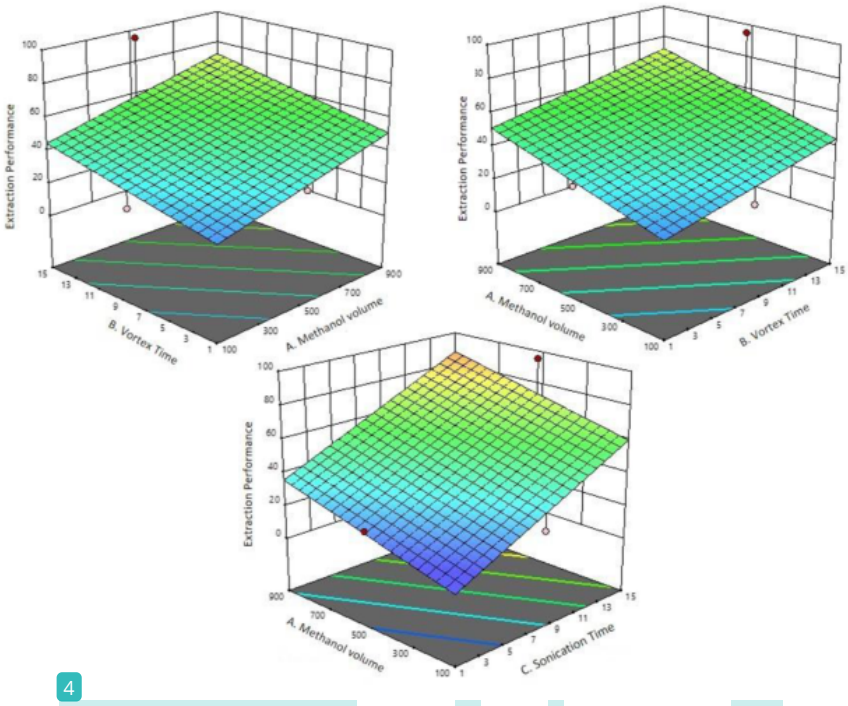

Fig. 2. Illustrations of representative response surface plots illustrating the impact of the selected factors on the extraction performance.

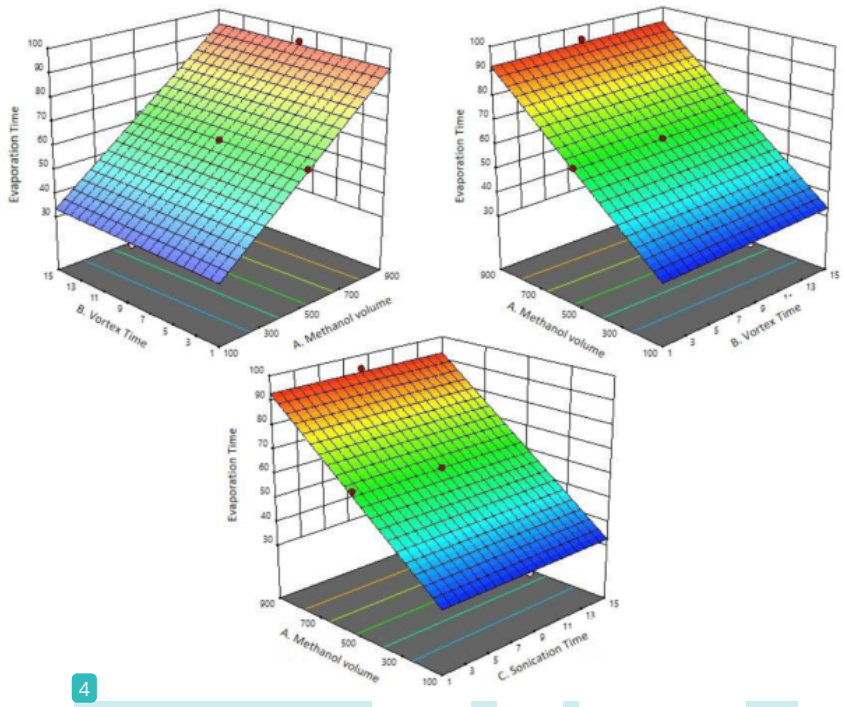

Fig. 3. Illustrations of representative response surface plots illustrating the impact of the selected factors on the evaporation time.

were recommended for the extraction method to obtain the optimum extraction performance evaporation time (Table 3).

3.2. Instrumentation and optimization of HPLC–UV conditions

The isocratic process RP-HPLC was used in this research. Ciprofloxacin was analyzed at 277 nm. Fig. 4 presents the HPLC chromatogram of blank porcine eye corneal tissue fluid and ciprofloxacin with porcine eye corneal tissue fluid with retention time 4.89 min. Due to its flexibility and suitability to analyze and separate ciprofloxacin, all analytes were separated on a reversed C18 column [18]. The C18 column demonstrated improved column efficiency and analyte elution with good resolution, tailing factor, and theoretical plate count.

Parameters observed in this optimization process were theoretical plates, tailing factor, and retention time. The results indicated that the tailing factor, theoretical plates, and retention time were fitted to the quadratic model. The analysis's F-value was found to be 29.34, 6.09, and 40.48 for the tailing factor, theoretical plates, and retention time, respectively. Additionally, all *p*-values were less than 0.001, suggesting that the tailing factor, theoretical plates, and retention time are all important parameters for process optimization. Figs. 5, 6, and 7 display a representative 3D graph showing the effect of selected factors on the retention time, tailing factor, and theoretical plates. From these models, it can be seen that the tailing factor, theoretical plates and, retention time were directly proportional to the combination of acetonitrile concentration, pH and, flow rate.

Ciprofloxacin's pH–solubility profile indicates that its dissociation and isoelectric constants are as follows: pKa1 = 6.09, pKa2 = 8.62, and pI = 7.14. (isoelectric point, obtained by calculating the average of pKa1 and pKa2) [19]. This shows that ciprofloxacin comprises two ionizable functional groups. When ionizable analytes are involved, the pH of the mobile phase may have a major impact on the analyte's retention activity. Thus, the pH of the mobile phase is a critical parameter to be considered during method development. As a result, establishing a particular pH value is critical for achieving the desired separation. When the mobile phase pH is near or equal to the pKa of the compounds being analyzed, a prominent peak may cause a liquid chromatography problem. Additionally, a broad peak is often accompanied by a long tail, decreasing the process's accuracy [20]. The method was then optimized for the mobile

Table 3
Predicted and observed responses of the optimization of extraction method.

Factors			Responses	Predicted	Observed	Bias
Methanol volume (mL)	Vortex time (minute)	Sonication time (minute)				
560.000	15.000	3.200	Extraction efficiency (%)	93.756	94.32 ± 0.61 %	-0.602
			Evaporation Time (minute)	43.486	44.91 ± 2.18	-3.413

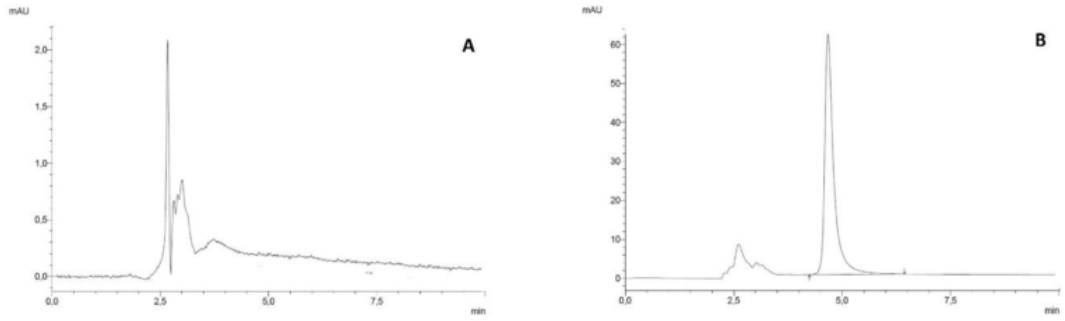

Fig. 4. Representative HPLC-UV chromatograms of blank porcine eye corneal tissue fluid (A) and ciprofloxacin with porcine eye corneal tissue fluid (B).

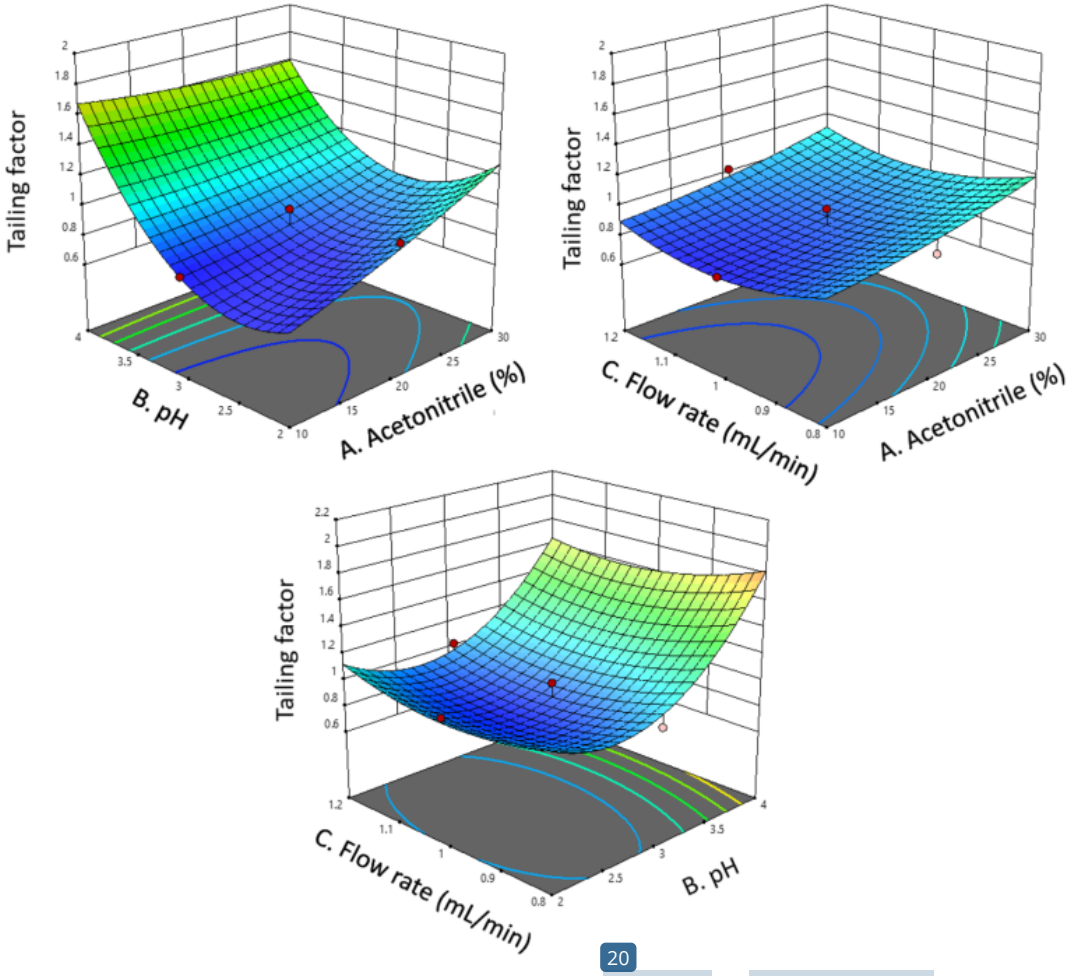

Fig. 5. Illustrations of representative response surface plots illustrating the impact of the selected parameters on tailing factor.

phase. The pH of the mobile phase should be at least one unit lower than the pKa of the compounds under investigation [21]. Disodium hydrogen phosphate at a concentration of 10 mM was used in this analysis. Additionally, appropriate pH selection improves retention, peak form, and sensitivity. Finally, according to the software analysis, the acetonitrile concentration of 19.75 %, pH of mobile phase of 2.25 and, flow rate of 1.2 ml/minute were recommended as optimized HPLC–UV conditions to obtain the optimum tailing factor, theoretical plates and, retention time (Table 4).

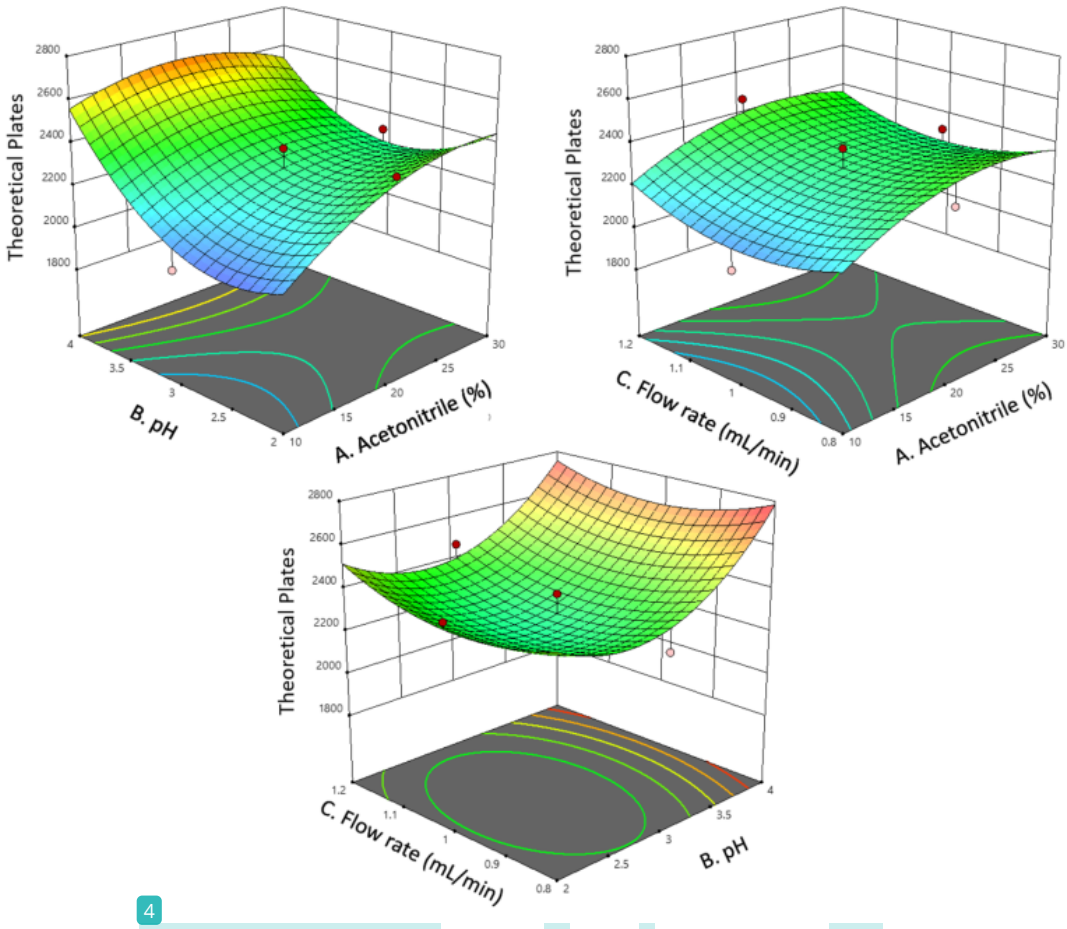

Fig. 6. Illustrations of representative response surface plots illustrating the impact of the selected factors on the theoretical plates and retention time.

4. Analytical method validation

4.1. Linearity, LOD and LLOQ

Table 5 gives the results of the method linearity, LOD and, LLOQ. We found that the method was linear (R = 0.999) when tested at concentrations starting from 0.01 to 10 μg/ml. The LOD and LLOQ also indicated an excellent sensitivity as we found that the method can detect ciprofloxacin at relatively low concentrations (Table 1). Several papers have also reported the HPLC method validation of ciprofloxacin for the determination of this substance in various types of matrices such as human plasma [22], prostate tissue [23] and, body fluids [24]. Considering these references, we found that the LOD's were between 0.01 and 0.04 μg/ml, while the LLOQ's were between 0.05 and 0.13 μg/ml. It supports our findings that this method's sensitivity was adequate to be applied in ciprofloxacin measurement in porcine eye tissue.

4.2. Accuracy and precision

To assess this method's precision and accuracy, intra- and inter-day measurements were performed. In evaluating inter-day accuracy and precision, ciprofloxacin was tested on three separate days. The intra-day evaluation was performed by conducting measurement on the same day using three replicates of ciprofloxacin samples. Our results suggested that the method is accurate to determine ciprofloxacin at a concentration between 0.02 to 7.5 μg/mL in intra-day and inter-day measurements (Table 6). The data was shown by the %RSD value that is not more than ± 15%. We also found that this method has good precision in intra- and inter-day evaluation using the same set of ciprofloxacin concentrations as above. We obtained that %RE fell between -10 and 13.33 %, which met the criteria for a precision measurement as they did not exceed ± 15% of the %RE limit (Table 6).

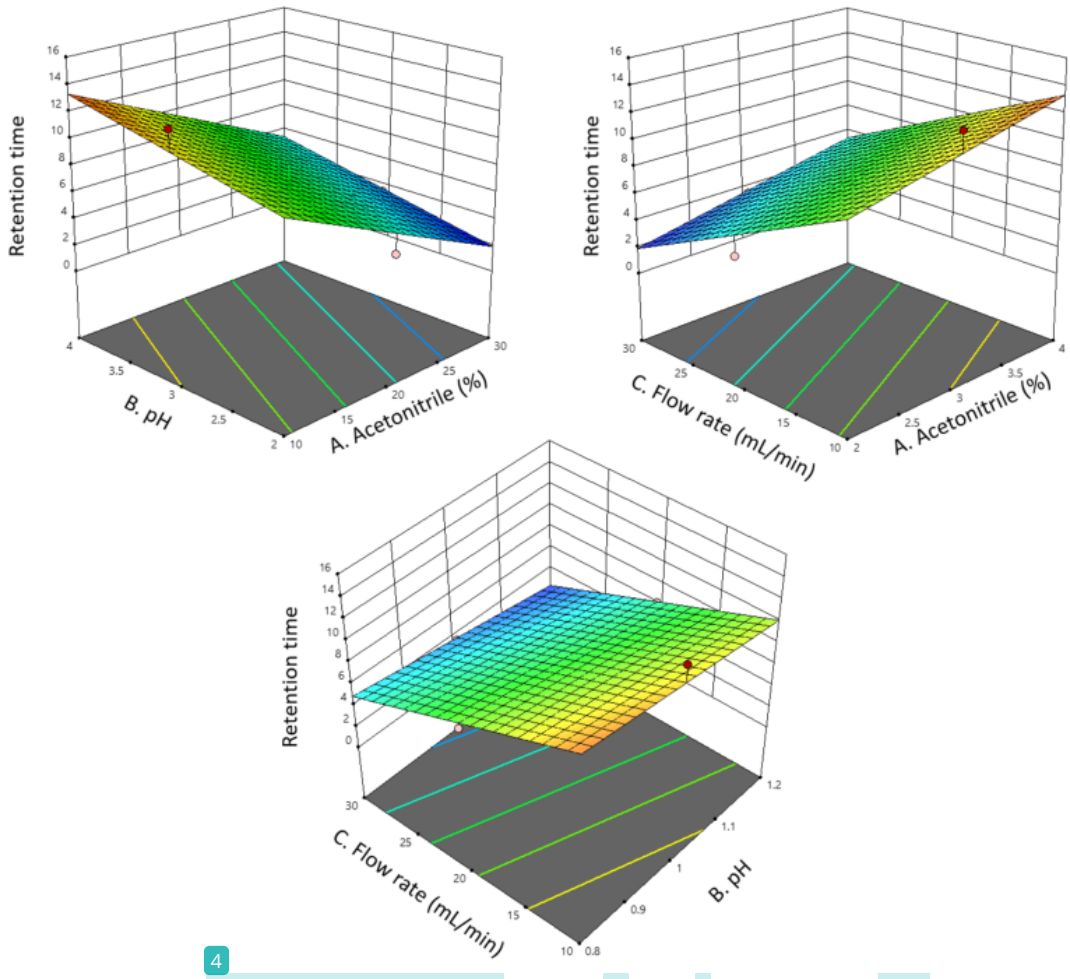

Fig. 7. Illustrations of representative response surface plots illustrating the impact of the selected factors on the retention time.

Table 4
Predicted and observed responses for the optimization of the HPLC method.

Factors			Responses	Predicted	Observed	Bias
Acetonitrile (%)	pH	Flow Rate (mL/minute)				
19.75	2.25	1.2	Tailing factor	1	1	0
			Theoretical plates	2438.217	2483.32 ± 103.21	-1.850
			Retention time	5.082	5.19 ± 0.09	-2.125

Table 5
Properties of the calibration curve for quantification of ciprofloxacin with LOD and LLOQ values.

Slope	y-intercept	R	LOD (μg/mL)	LLOQ (μg/mL)
6685	7.25	0.999	0.015	0.02

4.3. *Carry-over and dilution integrity*

The carry-over effect was evaluated by injecting high concentrations of ciprofloxacin into the HPLC column and observing whether ciprofloxacin's signal appears in the subsequent measurement of a blank solution. There should be no traces of ciprofloxacin obtained in the detection of the blank. Our results suggested that there was no carry-over of ciprofloxacin in the sequential examination of blank solution. The HPLC profiles showed no carry-over. A blank solution exhibited no more than 20% of LLOQ in the corresponding

Table 6
Intra-day and inter-day precision and accuracy of ciprofloxacin in the porcine eye ($n = 6$).

Intra-day Precision and Accuracy				
Replication	Concentration added (μg/mL)	Concentration found (μg/mL) ± SD	Precision (%RSD)	Accuracy (%RE)
1	0.02	0.02±0.00	1.32	14
	0.03	0.03±0.00	7.14	-6.67
	3.5	3.71±0.32	8.63	6
	7.5	7.29±0.23	3.16	-2.8
2	0.02	0.02±0.00	10.88	-3.5
	0.03	0.03±0.00	6.25	1.33
	3.5	3.39±0.43	12.68	-3.14
	7.5	7.53±0.71	9.43	0.4
3	0.02	0.02±0.00	6.45	-7
	0.03	0.03±0.00	9.11	13.33
	3.5	3.82±0.33	8.34	9.14
	7.5	7.29±0.81	11.11	-2.8
Inter-day Precision and Accuracy				
Day	**Concentration added (μg/mL)**	**Concentration found (μg/mL) ± SD**	**Precision (%RSD)**	**Accuracy (%RE)**
1	0.02	0.02±0.00	2.11	-5
	0.03	0.03±0.00	9.68	3.33
	3.5	3.47±0.02	0.57	-0.86
	7.5	7.52±0.08	1.10	0.27
2	0.02	0.02±0.00	7.78	-10
	0.03	0.03±0.00	9.43	6
	3.5	3.54±0.03	0.90	1.14
	7.5	7.48±0.07	0.87	-0.27
3	0.02	0.02±0.00	9.54	10
	0.03	0.03±0.00	7.17	-2.33
	3.5	3.73±0.12	3.22	6.57
	7.5	7.86±0.43	5.48	4.8

retention time of ciprofloxacin when the blank solution was tested.

The dilution accuracy was examined by evaluating ciprofloxacin concentrations' integrity after the solution is made 5 and 10 times lowered than ciprofloxacin's concentrated solution. We discovered that the diluted ciprofloxacin solutions exhibited recoveries between 97.91 ± 3.21% and 101.19 ± 8.19% with the precision of 9.83%-11.23% when analyzed using our developed method. This result is considered as excellent dilution integrity as the standard for the satisfaction range for accuracy should be within 85 - 115 % and for precision is ± 15%.

4.4. Extraction recovery

Ciprofloxacin in this study was extracted from the porcine ophthalmic fluid and tissue. To evaluate the extraction recovery from the matrices, ciprofloxacin was added to the examined fluid at three different levels of concentrations: low (0.01 and 0.03 μg/mL), medium (1.5 μg/mL) and, high (3.75 μg/mL). Table 7 summarizes our results. The spiked sample recoveries were between 93.87 ± 8.43% and 94.10 ± 9.01%, corresponding for %RSD value range from 8.01 to 9.51%. By referring to the standard of extraction recoveries (±15% of RSD), it can be proposed that the data obtained in this study represents an acceptable value for a precise, reproducible as well as consistent method in determining ciprofloxacin extracted from the porcine eye fluid.

4.5. Stability studies

When added in porcine ocular fluid, ciprofloxacin was stable in all storage conditions (Table 8). Both low and high concentration samples exhibited recoveries that are above 95% with SD percentages not more than 15%, indicating acceptable values by ICH standards for stability validation [25].

Based on our literature search attempt, we found no report has published the stability of ciprofloxacin in porcine eye fluids. However, several studies have shown that ciprofloxacin in human plasma and rat plasma is stable after testing the same storage conditions as we did [26,27]. Although the stability of ciprofloxacin in porcine eye fluid should not be compared to its stability in plasma, there is an indication that ciprofloxacin is stable in many conditions. It was reported that ciprofloxacin showed no sign of

Table 7
Mean extraction recoveries of ciprofloxacin in porcine eye (n=6).

Concentration added (μg/mL)	% Extraction Recovery ± SD	% RSD
0.01	93.87±8.43	8.98
0.03	94.10±9.01	9.57
1.5	91.91±8.43	9.18
3.75	92.71±7.43	8.01

Table 8
Mean stability recoveries of ciprofloxacin at different storage conditions (n=3).

% Stability recoveries (mean ± SD) Concentration added (μg/mL)	Autosampler (48 h)	Bench-top (24 h)	Long-term (2 weeks)	Freeze-thaw (3 cycles)
0.01	98.19±7.41	96.03±8.01	95.03±9.32	99.31±9.09
0.03	99.11±3.98	97.49±7.98	100.92±8.44	100.75±7.59
1.5	101.81±9.15	102.94±7.19	97.23±7.02	101.43±9.34
3.75	100.75±8.32	101.11±8.93	99.39±8.69	95.43±8.84

degradation in the stability testing when spiked in prostate tissues, in bones, and in the presence of other compounds such as levofloxacin, moxifloxacin, rifampicin [28]. Interestingly, when challenged with 50% acidic degradation, ciprofloxacin can still be recovered at an acceptable amount [29].

4.6. Evaluation of the ocular kinetics after ex vivo administration of ciprofloxacin

The developed method was then applied to determine *ex vivo* ocular kinetic profiles of two eye preparations: eye drop and ointment, both containing 0.3 mg ciprofloxacin. This is the first study investigating the ocular kinetic of ciprofloxacin following the administration of eye preparations. Fig. 8 depicts an overview of ciprofloxacin concentration changes in the tested aliquots after applying them to the *ex vivo* porcine corneal tissue from 0 to 6 h. Ciprofloxacin concentration rapidly reached its maximum concentration (Cmax) at around 0.5 h after applications in the tested eye drops. The application of eye ointment showed ciprofloxacin C_{max} at approximately 1 h. In the elimination stage, ciprofloxacin concentrations in eye drop decreased relatively faster ($T_{1/2} = 0.54 \pm 0.03$ h) than ciprofloxacin in the eye ointment ($T_{1/2} = 2.29 \pm 0.23$ h). Our further examination suggested that ciprofloxacin in the eye drop is almost eliminated from the corneal tissue before 6 h, while in the eye ointment, ciprofloxacin can still be traced (approximately ≥ 50 μg) at 6 h of application. The mean residence time (MRT) of ciprofloxacin in the corneal tissue also indicated that the eye ointment retained the drugs longer than the eye drop preparation. A side-by-side comparison of kinetic profiles for both preparations is given in Table 9. The distinctive pharmacokinetic profiles revealed in our finding further suggested that eye ointment can retain ciprofloxacin for a longer time in more concentration than eye drops.

The analytical method could be potentially useful in any *ex vivo* analysis of ciprofloxacin delivered to the ocular tissue from several drug delivery systems. Although the kinetic profiles have been successfully established using this method, it is crucial to consider that this method has not incorporated other physiological factors that may also affect the bioavailability of the ocular application of ciprofloxacin. As reported, factors such as tear drainage, tear dilution, conjunctival absorption, and melanin binding may have specific effects on the absorption, distribution, or elimination of drugs on ocular delivery [30]. Besides, since the actual applications of ciprofloxacin is to target the microbial-infected tissues, factors such as tissue inflammation, continuous lacrimation and, dilated blood vascular should also be taken into account when predicting the penetration of this drug [31]. Therefore, a more comprehensive experiment using *in vivo* model is required to understand how these physiological changes may contribute to the kinetic profiles of ciprofloxacin in ocular applications.

5. Conclusions

This research aimed to establish and validate an HPLC-UV method for concurrent ciprofloxacin analysis. The procedure was

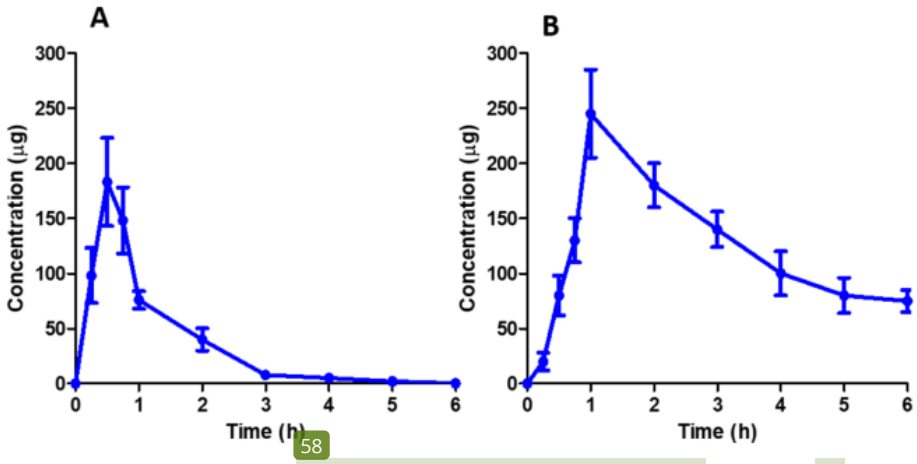

Fig. 8. The mean ex vivo corneal concentration and time profile of ciprofloxacin after administration of eye drop (A) and eye ointment (B) (means ± SD, $n = 6$ for each group).

Table 9
In vivo ocular kinetic parameters of ciprofloxacin after administration of eye drop and eye ointment (means ± SD, $n = 6$ for each group).

Parameters	Eye Drop	Eye Ointment
Dose (mg)	0.30	0.30
C_{max} (μg)	183.41 ± 23.21	245.01 ± 29.92
T_{max} (h)	0.50	1.00
AUC_{0-t} (μg.h)	191.80 ± 21.04	732.81 ± 56.32
AUC_{0-INF} (μg.h)	191.95 ± 20.29	927.00 ± 99.93
$T_{1/2}$ (h)	0.54 ± 0.03	2.29 ± 0.23
MRT (h)	1.02 ± 0.11	3.98 ± 0.27

successfully tested in accordance with ICH and FDA bioanalytical guidelines. The procedure was selective with apparent recovery, and all analytes found no matrix interference. This novel approach for measuring ciprofloxacin after *ex vivo* administration in porcine eye corneal tissue has many advantages, including simplicity, cost-effectiveness, high precision and accuracy, and high sensitivity and selectivity to study pharmacokinetics and biodistribution. Therefore, this bioanalytical approach provides a wide range of quantification of antibacterial drugs in potential pharmacokinetics, biodistribution research, and therapeutic drug monitoring applications. Eqs. 1-2

Specifications table

Subject area	*Chromatographic, Analytical Method Validation*
Compounds	*Ciprofloxacin*
Data category	*Chromatogram, ex vivo delivery, drug concentration, pharmacokinetics parameters*
Data acquisition format	*HPLC-UV Chromatogram*
Data type	*Analyzed*
Procedure	*We developed and validated the chromatographic method of CFZ in simulated tear fluid and porcine eye corneal tissue matrices for ex vivo kinetics studies. The method was successfully validated as per the ICH guidelines.*
Data accessibility	*Data is with this article*

CRediT authorship contribution statement

Nurul Muhlisah Maddeppungeng: Conceptualization, Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **Maria Mir:** Software, Validation, Visualization, Writing – review & editing. **Muhammad Raihan:** Methodology, Validation, Visualization, Writing – review & editing. **Elly Wahyudin:** Validation, Visualization. **Asma:** Methodology, Validation, Visualization. **Patricia Layadi:** Writing – review & editing. **Diany Elim:** . **Andi Dian Permana:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] L. Keay, F. Stapleton, O. Schein, Epidemiology of contact lens-related inflammation and microbial keratitis: a 20-year perspective, Eye Contact Lens 33 (2007) 346–353, https://doi.org/10.1097/ICL.0b013e318157c49d.
[2] M.D.P. Willcox, Management and treatment of contact lens-related Pseudomonas keratitis, Clin. Ophthalmol. 6 (2012) 919–924, https://doi.org/10.2147/OPTH.S25168.
[3] M. Vidyavathi, G. Srividya, A review on ciprofloxacin: dosage form perspective, Int. J. Appl. Pharm. 10 (2018) 6–10, https://doi.org/10.22159/ijap.2018v10i4.25315.

[4] A. Austin, T. Lietman, J. Rose-Nussbaumer, Update on the management of infectious keratitis, Ophthalmology 124 (2017) 1678–1689, https://doi.org/10.1016/j.ophtha.2017.05.012.
[5] G. Begum, T. Leigh, E. Courtie, R. Moakes, G. Butt, Z. Ahmed, S. Rauz, A. Logan, R.J. Blanch, Rapid assessment of ocular drug delivery in a novel ex vivo corneal model, Sci. Rep. (2020) 1–12, https://doi.org/10.1038/s41598-020-68254-1.
[6] C. Loch, S. Zakelj, A. Kristl, S. Nagel, R. Guthoff, W. Weitschies, A. Seidlitz, Determination of permeability coefficients of ophthalmic drugs through different layers of porcine, rabbit and bovine eyes, Eur. J. Pharm. Sci. 47 (2012) 131–138, https://doi.org/10.1016/j.ejps.2012.05.007.
[7] J. Barar, M. Asadi, S.A. Mortazavi-Tabatabaei, Y. Omidi, Ocular drug delivery; Impact of in vitro cell culture models, J. Ophthalmic Vis. Res. 4 (2014) 238–252, https://doi.org/10.1007/978-3-319-29346-2_21.
[8] A. Patel, Ocular drug delivery systems: an overview, World J. Pharmacol. 2 (2013) 47, https://doi.org/10.5497/wjp.v2.i2.47.
[9] V.F. Samanidou, C.E. Demetriou, I.N. Papadoyannis, Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC, Anal. Bioanal. Chem. 375 (2003) 623–629, https://doi.org/10.1007/s00216-003-1749-9.
[10] G.L. Drusano, W. Liu, R. Perkins, A. Madu, C. Madu, M. Mayers, M.H. Miller, Determination of robust ocular pharmacokinetic parameters in serum and vitreous humor of albino rabbits following systemic administration of ciprofloxacin from sparse data sets by using IT2S, a population pharmacokinetic modeling program, Antimicrob. Agents Chemother. 39 (1995) 1683–1687, https://doi.org/10.1128/AAC.39.8.1683.
[11] S.P. Balguri, G.R. Adelli, K.Y. Janga, P. Bhagav, S. Majumdar, Ocular disposition of ciprofloxacin from topical, PEGylated nanostructured lipid carriers: Effect of molecular weight and density of poly (ethylene) glycol, Int. J. Pharm. 529 (2017) 32–43, https://doi.org/10.1016/j.ijpharm.2017.06.042.
[12] E. Rozet, P. Lebrun, P. Hubert, B. Debrus, B. Boulanger, Design Spaces for analytical methods, TrAC - Trends Anal. Chem. 42 (2013) 157–167, https://doi.org/10.1016/j.trac.2012.09.007.
[13] K. Al Khateb, E.K. Ozhmukhametova, M.N. Mussin, S.K. Seilkhanov, T.K. Rakhypbekov, W.M. Lau, V.V. Khutoryanskiy, In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery, Int. J. Pharm. 502 (2016) 70–79, https://doi.org/10.1016/j.ijpharm.2016.02.027.
[14] Bioanalytical Method Validation Guidance for Industry, Food Drug Adm. (2018) 1–41.
[15] E.M. Agency, European medicines agency: an unacceptable choice, Prescrire Int. 20 (2011) 278.
[16] A.D. Permana, R. Nurul, P. Layadi, A. Himawan, N. Juniarti, Q. Kurnia, E. Utomo, S. Aulia, A. Arjuna, R.F. Donnelly, Thermosensitive and mucoadhesive in situ ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis, Int. J. Pharm. 602 (2021), 120623.
[17] D. Ramadon, A.J. Courtenay, A.D. Permana, I.A. Tekko, E. McAlister, M.T.C. McCrudden, H.O. McCarthy, R.F. Donnelly, A sensitive HPLC-UV method for quantifying vancomycin in biological matrices: Application to pharmacokinetic and biodistribution studies in rat plasma, skin and lymph nodes, J. Pharm. Biomed. Anal. 189 (2020), 113429.
[18] S. Imre, M.T. Dogaru, C.E. Vari, T. Muntean, L. Kelemen, Validation of an HPLC method for the determination of ciprofloxacin in human plasma, J. Pharm. Biomed. Anal. 33 (2003) 125–130, https://doi.org/10.1016/S0731-7085(03)00151-1.
[19] P.C. Sharma, A. Jain, S. Jain, R. Pahwa, M.S. Yar, Ciprofloxacin: Review on developments in synthetic, analytical, and medicinal aspects, J. Enzyme Inhib. Med. Chem. 25 (2010) 577–589, https://doi.org/10.3109/14756360903373350.
[20] A.D. Permana, I.A. Tekko, H.O. McCarthy, R.F. Donnelly, New HPLC–MS method for rapid and simultaneous quantification of doxycycline, diethylcarbamazine and albendazole metabolites in rat plasma and organs after concomitant oral administration, J. Pharm. Biomed. Anal. 170 (2019) 243–253.
[21] A.D. Permana, E. Wahyudin, M.N.AmirA. Ismail, M. Raihan, Q.K. Anjani, E. Utomo, P. Layadi, R.F. Donnelly, New and sensitive HPLC-UV method for concomitant quantification of a combination of antifilariasis drugs in rat plasma and organs after simultaneous oral administration, Anal. Methods. 13 (2021) 933–945.
[22] J. Vella, F. Busuttil, N.S. Bartolo, C. Sammut, V. Ferrito, A. Serracino-Inglott, L.M. Azzopardi, G. LaFerla, A simple HPLC-UV method for the determination of ciprofloxacin in human plasma, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 989 (2015) 80–85, https://doi.org/10.1016/j.jchromb.2015.01.006.
[23] O. Szerkus, J. Jacyna, A. Gibas, M. Sieczkowski, D. Siluk, M. Matuszewski, R. Kaliszan, M.J. Markuszewski, Robust HPLC–MS/MS method for levofloxacin and ciprofloxacin determination in human prostate tissue, J. Pharm. Biomed. Anal. 132 (2017) 173–183, https://doi.org/10.1016/j.jpba.2016.10.008.
[24] G.J. Krol, G.W. Beck, T. Benham, HPLC analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids, J. Pharm. Biomed. Anal. 14 (1995) 181–190, https://doi.org/10.1016/0731-7085(95)01611-2.
[25] ICH, Validation of analytical procedures: text and methodology Q2 (R1), ICH harmonised tripartite guideline, 2005.
[26] H. Choudhury, B. Gorain, A. Paul, P. Sarkar, S. Dan, P. Chakraborty, T.K. Pal, Development and validation of an LC-MS/MS-ESI method for comparative pharmacokinetic study of ciprofloxacin in healthy male subjects, Drug Res. (Stuttg). 67 (2017) 94–102, https://doi.org/10.1055/s-0042-116593.
[27] E.S. Zimmermann, B.G.S. Torres, T. Dalla Costa, Validation of a sensitive HPLC/fluorescence method for assessment of ciprofloxacin levels in plasma and prostate microdialysate samples from rats, Biomed. Chromatogr. 30 (2016) 330–336, https://doi.org/10.1002/bmc.3552.
[28] B. LLopis, C. Funck-Brentano, N. Tissot, A. Bleibtreu, S. Jaureguiberry, E. Fourniols, A. Aubry, N. Zahr, Development and validation of a UPLC-MS/MS method for simultaneous quantification of levofloxacin, ciprofloxacin, moxifloxacin and rifampicin in human plasma: application to the therapeutic drug monitoring in osteoarticular infections, J. Pharm. Biomed. Anal. (2020) 183, https://doi.org/10.1016/j.jpba.2020.113137.
[29] K.A.M. Attia, M.W.I. Nassar, M.B. El-Zeiny, A. Serag, Stability-indicating methods for the analysis of ciprofloxacin in the presence of its acid induced degradation product: a comparative study, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 159 (2016) 219–222, https://doi.org/10.1016/j.saa.2016.01.056.
[30] V. Agrahari, A. Mandal, V. Agrahari, H.M. Trinh, M. Joseph, A. Ray, H. Hadji, R. Mitra, D. Pal, A.K. Mitra, A comprehensive insight on ocular pharmacokinetics, Drug Deliv. Transl. Res. 6 (2016) 735–754, https://doi.org/10.1007/s13346-016-0339-2.
[31] T. Velpandian, Intraocular penetration of antimicrobial agents in ophthalmic infections and drug delivery strategies, Expert Opin. Drug Deliv. 6 (2009) 255–270, https://doi.org/10.1517/17425240902798119.

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