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Development of a chiral assay for a novel, nonfluorinated quinolone, PGE-9509924, in dog plasma using high performance liquid chromatography with electrospray tandem mass spectrometry or fluorescence detection

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Abstract

PGE-9509924 is a nonfluorinated quinolone and is active against a variety of susceptible and drug resistant bacteria in vitro and in animal infection models. A method for determining both enantiomers of PGE-9509924 in dog plasma has been developed. The enantiomers are derivatized with a chiral derivatizing agent, (−)-1-(9-fluorenyl)ethyl chloroformate (FLEC) and the resulting diastereomers are separated by reverse phase chromatography. Plasma samples are prepared via solid phase extraction (SPE) in a 96-well format prior to being derivatized. Samples are then analyzed by electrospray-LC/MS/MS with multiple reaction monitoring or by HPLC with fluorescence detection. Results of a side-by-side validation of the method with LC/MS/MS and HPLC/Fl detection are presented. Over the range selected for validation (0.025–10 μg/ml), both methods give similar results with identical limits of quantitation. Due to the selectivity of LC/MS/MS and the use of a stable-isotopically labeled internal standard, significantly shorter chromatographic runtimes are achieved with LC/MS/MS, making it the method of choice for sample analysis.

Keywords: Chiral separation; FLEC; Derivatization; Nonfluorinated quinolone; Reverse phase chromatography; Electrospray HPLC/MS/MS; Fluorescence detection

1. Introduction

PGE-9509924, (**I**) 7-[3*S*-aminopiperidinyl]-1-cyclopropyl-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid, is a novel, nonfluorinated quinolone (see Fig. 1). In vitro, it exhibits good activity against gram-positive, gram-negative, and atypical bacteria [1–6]. It maintains its potency against a number of drug resistant bacteria [1,2,5]. PGE-9509924 contains one asymmetric carbon. The *S*-enantiomer is more potent as an antimicrobial agent. It is synthesized via stereoselective

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synthesis as a single enantiomer. Drug regulatory agencies require that the pharmacokinetics of enantiomeric drugs be evaluated with assays capable of quantitating the individual enantiomers.

A number of methods are available for separating and quantitating enantiomers. Enantiomers can be separated on chiral stationary phases (CSP). Methods for directly separating enantiomers of various quinolones have been reported using a bovine serum albumin CSP [7,8], an ovamucoid protein CSP [9,10], a crown ether CSP [11–14], and ligand-exchange chromatography [15]. While baseline resolution was observed with these systems, the efficiency of these separations was generally poor and in most cases the mobile phase is not compatible with MS detection. A number of liquid chromatographic CSPs were evaluated and none of them were able to efficiently separate the enantiomers of PGE-9509924.

Another technique for determining enantiomers is to react them with a chiral reagent and then separate the resulting diastereomers by achiral chromatography. A review on the use of enantiomeric derivatization for biomedical chromatography has been published and it lists a number of chiral reagents, which have been successfully used for this purpose [16]. Chiral derivatization procedures have been reported for a small number of quinolones. Lomefloxacin was derivatized with (*S*)-(+)-(1-naphthyl)ethyl isocyanate followed by ethyl chloroformate [17]. However, only partial resolution ($Rs = 1.13$) of the resulting diastereomers was obtained. Ofloxacin was coupled with L-leucinamide via diphenylphosphinyl chloride activation in a two step reaction [7]. A two step process was also used to couple temafloxacin with *S*-(-)-*N*-1-(2-naphthyl sulfonyl)-2-pyrrolidine carbonylchloride [10]. While the procedures used with ofloxacin and temafloxacin were successful in baseline resolving the respective diastereomers, the derivatization procedures were multi-step procedures. A fast, one-step derivatization procedure would be simpler to use. One of the chiral reagents listed in the previously mentioned review article is (−)-1-(9-fluorenyl)ethyl chloroformate (FLEC). The preparation and use of FLEC as a chiral derivatizing agent was first described by Einarsson et al. [18]. It reacts rapidly with amines in a one step reaction, forming highly fluorescent diastereomers. Both enantiomers are

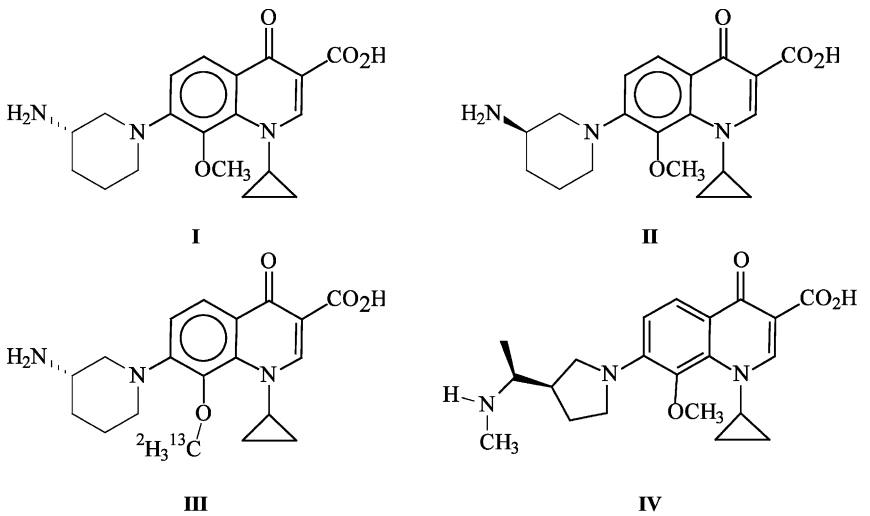

Fig. 1. Structures of (I) PGE-9509924 (*S*-enantiomer), (II) *R*-enantiomer of PGE-9509924, (III) $^{2}H_{3}^{13}C$-PGE-9509924, and (IV) PGE-510629.

available commercially in enantiomerically pure form. Procedures using FLEC have been described for amino acids [19,20], atenolol [21], and reboxetine [22]. A method based on the use of this reagent was developed for PGE-9509924 and its *R*-enantiomer. Both HPLC with fluorescence detection and electrospray-LC/MS/MS were evaluated and the results of a side-by-side validation are reported.

2. Experimental

2.1. Chemicals and reagents

PGE-9509924 (I), $^{2}H_{3}^{13}C$-PGE-9509924 (II), and PGE-510629 were synthesized in house [23]. Methanol (HPLC grade), acetonitrile (HPLC grade), phosphoric acid, boric acid and 50% sodium hydroxide were from J.T. Baker (Phillipsburg, NJ, USA). Glycine was from Gibco BRL (Rockville, MD, USA). Formic acid and (−)-FLEC were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Dog plasma was obtained from PelFreez Biologicals (Rogers, AR, USA). Purified water was obtained from a Milli-Q UV Plus system (Millipore Corp., Bedford, MA, USA).

2.2. Instrumentation

The Quadra 96 Multipipettor, Model 320 with the vacuum module (Tomtec Inc., Hamden, CT, USA) was used for transferring samples and performing the SPE extraction. Samples were taken to dryness in the SpeedVac, Model DSC250 with VaporNet (Savant Instruments Inc., New York, USA). The mass spectrometer was a PE-Biosystems API 3000 triple-stage quadrapole mass spectrometer (Concord, Ont., Canada). The HPLC system on the mass spectrometer consisted of a Gilson Gradient System (Model 305 pumps, Model 805 Manometric Module, Module 811C Analytical Mixer) (Gilson Inc., WI, USA), a Waters Solvent Degasser (Waters Corporation, Milford, MA, USA), and a LEAP HTS PAL autosampler (Leap Technologies, Carrbora, NC, USA). A divert valve (ThermoQuest Corp., San Jose, CA, USA) was installed between the column outlet and the mass spectrometer. The HPLC system used with the fluorescence detector consisted of a Waters Model 717 autosampler, Waters Model 616 gradient pump, a Waters Solvent Degasser, a Waters Model 474 fluorescence detector, Waters SAT/IN interface box, and the Waters Millennium Data System (Waters Corporation).

2.3. Chromatographic conditions

Waters Xterra MS, C18 columns (15 cm, 3.5 μm) were used. A 2.1 mm i.d. column was used with the LC/MS/MS and a 4.6 mm i.d. column was used with the HPLC/FL system. The mobile phase used with the LC/MS/MS system contained methanol:acetonitrile:water:formic acid, (40:40:20:0.1,v/v/v/v). A flow rate of 0.3 ml/min and an injection volume of 20 μl were used with the LC/MS/MS. Flow is diverted to waste for the first 3 min. of each run on the LC/MS/MS system. The mobile phase used with the fluorescence detection system contained methanol:acetonitrile: water:formic acid, (35:35:30:0.1, v/v/v/v). The flow rate was 1.3 ml/min and the injection volume was 20 μl. The gain on the Waters Model 474 Fluorescence Detector was set to 1000 and the filter was set to the digital filter mode with a time constant of 5 s. The excitation and emission wavelengths were 295 and 530 nm, respectively.

2.4. ESI–MS/MS conditions

Solutions of the analyte and internal standard were infused to determine the optimized MS/MS conditions for each compound. The TurboIonspray source temperature was set to 450 °C. The Ionspray voltage was 4020 V, the orifice was set to 74 V, the ring was set to 180 V, and the ST3 setting was −80 V. The nebulizer, curtain, and collision gases were set to 8 on a scale of 10, to 6 on a scale of 15, and to 5 on a scale of 12, respectively. The MS/MS detection scheme utilized collisionally activated dissociation (CAD) with selective reaction monitoring (SRM) of m/z 594–282 and m/z 598–286 for the analyte and IS, respectively, at optimized collision energy of −30 V (R02-Q0). The dwell time was 100 ms for each

ion reaction. The 282 and 286 m/z ions are the most abundant daughter ions, other than loss of water ions, which were not considered specific enough for use. Their formation involves a multi-step fragmentation and rearrangement process and the exact structure of these ions is unknown. No interference for this SRM scheme was detected in matrix blanks.

2.5. Preparation of standard solutions

Standards were prepared via serial dilution of a stock solution on a daily basis. Standards were prepared at the following concentrations: 0.005, 0.010, 0.025, 0.04, 0.1, 0.4, 1.0, 4.0, 7.5, 8.5, 10.0 μg/ml. The standards, including the stock solution were prepared in 20% methanol/80% 50 mM phosphoric acid adjusted to pH 3.1 with 50% sodium hydroxide solution.

PGE-510629 and $^{2}H_{3}^{13}C$-PGE-9509924 were used as the internal standards for the fluorescence and MS/MS detection methods, respectively (see Fig. 1). The concentration of the PGE-510629 internal standard solution was 0.5 μg/ml. The concentration of the $^{2}H_{3}^{13}C$-PGE-9509924 solution was 1.0 μg/ml. Both were prepared in 20% methanol/80% phosphate, pH 3.1.

2.6. Semi-automated SPE procedure

SPE was preformed on a Waters Oasis HLB 96-well extraction plate containing 10 mg of packing material (Waters Corporation). All solutions except the plasma were transferred using a TomTec Quadra 96 multipipettor. Individual plasma aliquots were transferred with a manual pipettor. The plate was conditioned with two aliquots of 400 μl of methanol and two aliquots of 400 μl of water. Plasma samples (50 μl), buffer or spiking solution (50 μl), and internal standard (50 μl) were loaded onto the plate. It was then washed with 400 μl of water and twice with 400 μl of 30% methanol/70% water. The samples were eluted with two 300 μl aliquots of 1% formic acid in methanol and then taken to dryness in a vacuum centrifuge. Vacuum was used to draw solution through the extraction plate. Additional details on the development of the SPE procedure will be published separately.

2.7. Derivatization procedure

A 0.36 mM solution of (−)-FLEC was prepared from the concentrated solution (18 mM in acetone) available commercially, by adding 20 μl of concentrated (−)-FLEC to 980 μl of acetonitrile. Aliquots of buffer solution (100 μl of 0.1 M borate, pH 8.2) and derivatizing reagent solution (200 μl of 0.36 mM (−)-FLEC) were added to each sample. The samples were vortexed and allowed to stand at room temperature for 30 min. A 100 μl volume of a glycine solution (100 μg/ml in water) was then added. The samples were vortexed and diluted 1:1 with a 50/50 mixture of acetonitrile and water. The diluted samples were then injected into the appropriate HPLC system. The Quadra 96 was used for these reagent additions and sample transfers steps. With the FLEC solution and the 50/50, acetonitrile/water solution, an aliquot of air (50 μl) is taken up into the pipette tips after picking up the solution to eliminate dripping.

2.8. Validation

Standard curves were prepared and assayed on three separate days. Quality control samples were prepared separately for PGE-9509924 and for the *R*-enantiomer of PG-9509924. Spiking levels were selected to mimic concentrations expected in real samples. QC samples for PGE-9509924 were prepared at high (5 or 7.5 μg/ml), medium (1.0 μg/ml), and low (0.1 μg/ml) concentrations. QC samples for the *R*-enantiomer were prepared at medium (0.4 μg/ml) and low (0.08 μg/ml) concentrations. Mixed QC samples were also prepared which contained a high (5 or 7.5 μg/ml) or medium (1.0 μg/ml) concentration of the *S*-enantiomer and a low (0.08 μg/ml) level of the *R*-enantiomer. On three separate days, three to four replicates of each QC sample were prepared and analyzed.

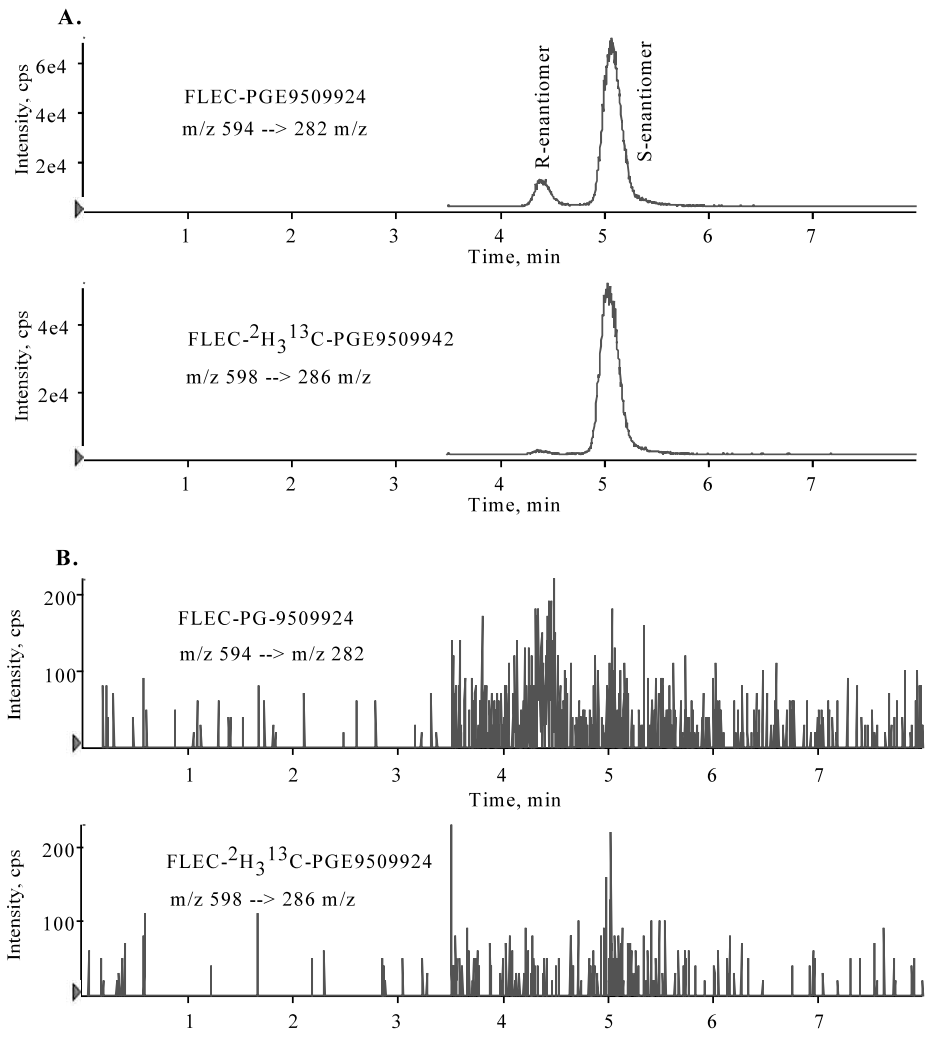

Fig. 2. (A) Chromatograms of a sample spiked with 0.05 μg/ml of the *R*-enantiomer and 0.5 μg/ml of the *S*-enantiomer on the electrospray HPLC/LM/MS system. The upper trace is the chromatogram for the two enantiomers (m/z 594 → m/z 282) and the lower trace is the chromatogram for the stable isotopically labeled internal standard (m/z 598 → m/z 286). (B) shows chromatograms for a plasma blank. Conditions are as listed in the Section 2.

3. Results and discussion

3.1. Selection of chromatographic conditions

The derivatized enantiomers of PGE-9509924 are diastereomers and therefore are structurally very similar. They can however, be separated on an achiral reverse phase system. The diastereomers needed to be baseline resolved, to ensure that the method would be able to quantitate low levels of each enantiomer in the presence of high levels of the other enantiomer. It was also desirable to complete the separation in a minimal runtime, hence a high efficiency separation was needed. Finally, the mobile phase needed to be compatible with MS detection. A number of columns and mobile phases were evaluated. Acidified water–organic mobile phases containing acetonitrile or methanol gave significantly different results. Mobile phases with acetonitrile gave

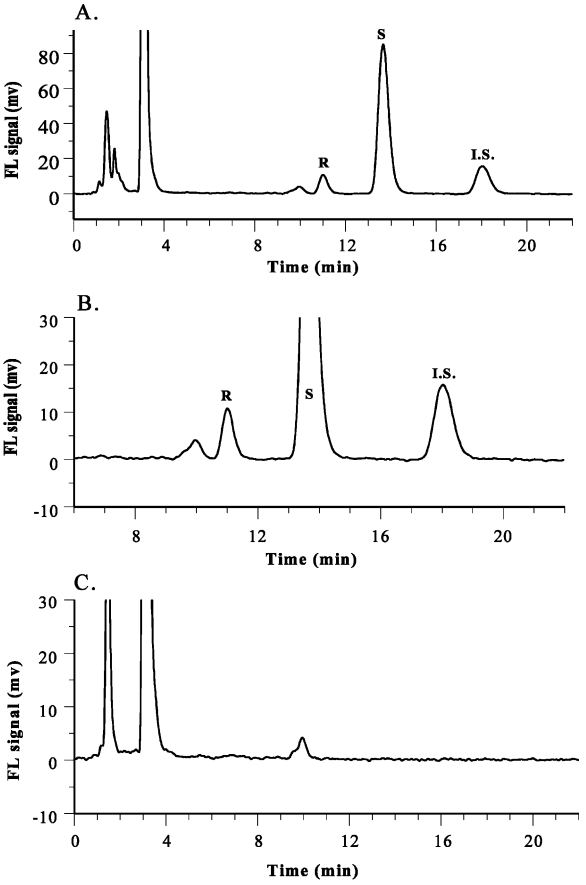

Fig. 3. (A) Full scale and (B) expanded scale chromatograms of a plasma sample spiked with 0.08 μg/ml of the *R*-enantiomer and 1.0 μg/ml of the *S*-enantiomer of PGE-9509924 on the HPLC system with fluorescence detection. (C) shows a chomatogram for a plasma blank. Conditions are as listed in the Section 2.

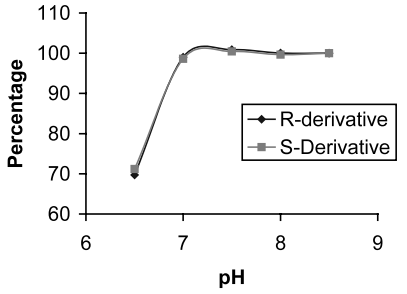

Fig. 4. Extent of derivatization as a function of pH. Conditions, 50 μl aqueous sample, 50 μl 0.1 M borate at pH 6.5, 7.0, 7.5, 8.0, and 8.5, 200 μl of 0.36 mM FLEC solution (in acetonitrile), room temperature, 30 min.

sharp, symmetric peaks but did not resolve the diastereomers in a reasonable runtime. With methanol in the mobile phase, the peaks were much better resolved, but the peaks were broad. Using a mixture of methanol and acetonitrile for the organic portion of the mobile phase results in a high efficiency separation with sharp, symmetric peaks and baseline resolution in a minimum runtime. For use with the more specific MS/MS detection, several brands of C8 and C18 columns were found to be suitable. A mobile phase containing 40% methanol, 40% acetonitrile, 20% water, and 0.1% formic acid was capable of baseline resolving the peaks of interest in 8 min. Fig. 2 shows sample chromatograms of a spiked plasma sample and a plasma blank on the LC/MS/MS system using the same brand of column selected for use with fluorescence detection (see below).

With fluorescence detection, several small peaks were observed in the blank. The separation of the peaks in the blank was dependent on the brand and type of column used. The Waters Xterra MS C18 column was best able to resolve all the peaks in the blank from the peaks of interest. Additionally, a mobile phase with 30% water was required to separate the peaks in the blank from the peaks of interest. Typical chromatograms for a spiked plasma sample and plasma blank with fluorescence detection are shown in Fig. 3. With the chemical internal standard used with fluorescence detection, total run time was 24 min.

3.2. Derivatization conditions

The reaction of FLEC with amines takes place rapidly at basic pH in mixtures of acetonitrile and water [18]. Derivatization reactions were run using 0.1 M borate buffer adjusted to pH 6.5, 7, 7.5, 8, and 8.5. Results of this study are shown in Fig. 4. At a pH of 7 or above, the reaction was rapid and quantitative. A buffer pH of 8.2 was selected, to ensure that any residual formic acid remaining from the SPE elution solvent would be completely neutralized.

The effect of the concentration of the FLEC solution on the extent of derivatization is shown in Fig. 5. A concentration of 0.36 mM or higher is needed to achieve complete derivatization. Use of

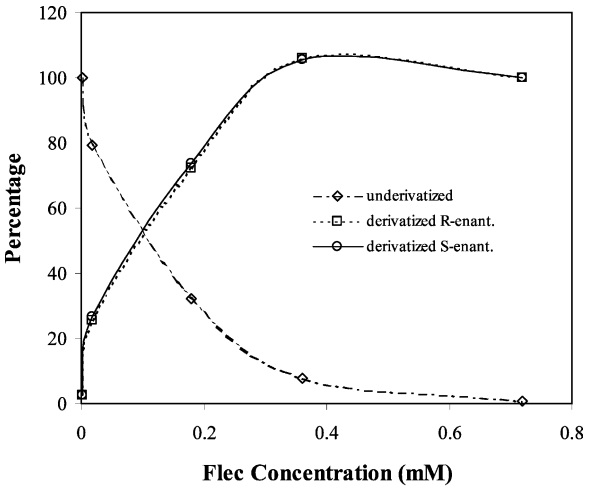

Fig. 5. Effect of the concentration of the FLEC solution on the extent of derivatization. Conditions, same as in Fig. 4 with pH 8.2 borate buffer.

a higher concentration results in larger peaks in the blank, which can adversely affect the performance of the assay. At room temperature, the reaction is quantitative after 30 min.

A solution of glycine (100 μl of 100 μg/ml glycine) is added after 30 min., to react with any excess (−)-FLEC. This stops the reaction of all the samples at a uniform time, which helps to insure good reproducibility. The HPLC/FL chromatograms are cleaner in the area where the peaks of interest elute when glycine is added. The FLEC derivative of glycine elutes early in the chromatogram and does not interfere with any of the peaks of interest. Adding glycine also protects the column from having reactive FLEC injected onto the column with every injection, which will increase the lifetime of the column.

3.3. Linearity

Spiked plasma standards for both enantiomers covering the range from 0.025 to 10.0 μg/ml were prepared and analyzed on 3 days. Using regression analysis, the concentration versus area ratio data (area of peak of interest over area of internal standard) was fit to a straight line, $y = mx + b$. For the HPLC/FL standard curves, a weighting factor of $1/x$ was used. The method is linear for PGE-9509924 over the entire range tested. For the *R*-enantiomer, the method is only linear to 7.5 μg/ml. At higher concentrations the peak was off scale. Table 1 shows the standard curve data for the three validation runs. The lower end of the curve, 0.025 μg/ml, represents the lower limit of quantitation. Replicate injections ($n = 5$) of the 0.025 μg/ml standards for the *R*-enantiomer and PGE-9509924 gave coefficients of variances (CV) of 4.0 and 7.1%, respectively. To evaluate the limit of detection of the method, spiked samples at concentrations of 0.005 and 0.01 μg/ml were prepared and analyzed. The 0.01 μg/ml standards

Table 1
Standard curve data

Run	HPLC/FL			LC/MS/MS		
	b	*m*	Correlation coefficient	*b*	*m*	Correlation coefficient
R-enantiomer						
1	0.0098	0.0724	0.99925	0.0125	0.0471	0.99749
2	0.0248	0.0861	0.99918	0.0104	0.0606	0.99535
3	0.0557	0.0659	0.99985	0.0693	0.0666	0.98173
S-enantiomer						
1	−0.0072	0.0659	0.99925	0.0174	0.0472	0.99287
2	0.0090	0.0789	0.99984	0.0082	0.0505	0.99702
3	0.0320	0.0748	0.99985	0.0077	0.0518	0.99875

A linear curve, $y = mx + b$, was used, where x = concentration (ng/50 μl of plasma) and y is the ratio of the area of the peak of interest to the area of the internal standard peak. Weighting factors of $1/x$ and $1/x^2$ were used for the HPLC/FL and LC/MS/MS curves, respectively.

Table 2
Results for samples spiked with PGE-9509924 or the *R*-enantiomer of PGE-9509924

		HPLC/FL				LC/MS/MS			
	Amount added (μg/ml)	*n*	Amount found (μg/ml)	Recovery (%)	CV (%)	*n*	Amount found (μg/ml)	Recovery (%)	CV (%)
PGE-9509924, high									
Run 1	7.55	4	7.54	100	2.0	4	6.87	91	4.7
Run 2	5.00	4	5.29	106	1.6	4	5.02	100	3.4
Run 3	5.00	4	5.35	107	2.7	4	5.15	103	3.7
PGE-9509924, medium									
Run 1	1.00	4	1.02	102	10.2	4	1.10	110	3.4
Run 2	1.00	4	1.07	107	2.0	4	1.07	107	3.8
Run 3	1.00	4	1.06	106	4.6	4	1.10	110	2.0
PGE-9509924, low									
Run 1	0.10	4	0.084	84	4.4	4	0.107	106	2.2
Run 2	0.10	4	0.0108	108	9.7	4	0.118	118	8.3
Run 3	0.10	4	0.094	94	10.5	3	0.113	113	3.7
R-enantiomer of PGE-9509924, medium									
Run 1	0.393	4	0.369	94	3.3	4	0.438	112	2.5
Run 2	0.397	4	0.436	110	6.6	4	0.454	114	3.7
Run 3	0.397	4	0.401	101	8.1	4	0.422	106	2.7
R-enantiomer of PGE-9509924, low									
Run 1	0.079	4	0.063	80	0.7	4	0.079	100	7.5
Run 2	0.080	4	0.089	112	8.0	4	0.090	113	3.8
Run 3	0.080	4	0.087	109	4.9	4	0.073	92	5.3

Table 3
Result for samples spiked with both PGE-9509924 and its *R*-enantiomer

	R-enantiomer of PGE-9509924					PGE-9509924				
	Amount added (μg/ml)	*n*	Amount found (μg/ml)	Recovery (%)	CV (%)	Amount added (μg/ml)	*n*	Amount found (μg/ml)	Recovery (%)	CV (%)
HPLC/FL										
Low										
Run 1	0.079	4	0.088	111	7.1	7.55	4	7.70	102	4.4
Run 2	0.080	4	0.092	115	2.4	5.00	4	5.53	111	3.5
Run 3	0.080	4	0.082	104	5.8	5.00	4	5.39	108	3.8
Medium										
Run 1	0.079	4	0.069	88	3.2	1.00	4	0.97	97	0.9
Run 2	0.080	4	0.089	113	7.0	1.00	4	1.00	100	5.1
Run 3	0.080	4	0.085	107	5.5	1.00	4	1.01	101	3.9
LC/MS/MS										
Low										
Run 1	0.079	3	0.098	125	10.1	7.55	3	7.10	94	4.3
Run 2	0.080	4	0.095	120	13.5	5.00	4	4.80	98	4.6
Run 3	0.080	4	0.075	95	7.9	5.00	4	5.12	102	3.4
Medium										
Run 1	0.079	4	0.089	113	8.7	1.00	4	1.07	107	1.6
Run 2	0.080	4	0.091	115	6.4	1.00	4	1.01	101	4.4
Run 3	0.080	4	0.074	93	2.6	1.00	4	1.05	105	2.1

for both enantiomers were detected and had signal to noise ratios of 3 or greater in each of the three validation runs. This is considered the limit of detection of the method.

On the LC/MS/MS, both the *R*-enantiomer and the PGE-9509924 standards were linear from 0.025 to 10.0 μg/ml. A weighting factor of $1/x^2$ was used. The standard curve data for the three validation runs are listed in Table 1. Replicate injections ($n = 6$) of the 0.025 μg/ml standards gave CVs of 3.4 and 2.4%, for the *R*-enantiomer and PGE-0509924 standards, respectively. This is considered the limit of quantitation. The LC/MS/MS system was able to detect peaks from samples spiked with 0.005 μg/ml of either enantiomer with signal to noise ratios of 3 or greater. This is considered the limit of detection for MS/MS detection.

3.4. Precision

Replicate spiked QC samples ($n = 3$ or 4) were prepared and analyzed on three different days. Samples were spiked with the *R*-enantiomer at concentrations of 0.08 and 0.4 μg/ml or with PGE-9509924 at concentrations of 0.10, 1.0, and 5.0 or 7.5 μg/ml. Results of the analysis of these singly spiked samples are listed in Table 2. With HPLC/FL, all recoveries are within 80–112% and most are within 94–110%. The CVs are $\leq 10\%$. With LC/MS/MS, all recoveries are within 91–118% and the CVs are $\leq 8\%$.

Replicate samples ($n = 3$ or 4) that contained a relatively high concentration of PGE-9509924 and a low concentration of the *R*-enantiomer were also prepared and analyzed. The concentration of the R-enantiomer corresponds to 1.6 (1.3% in run 1) or 8% of the concentration of the *S*-enantiomer. Results for this study are shown in Table 3. For PGE-9509924, excellent recoveries and CVs are observed for both HPLC/FL and LC/MS/MS. For the *R*-enantiomer on HPLC/FL, recoveries range from 88 to 115% with CVs $\leq 7\%$. For the *R*-enantiomer on LC/MS/MS, recoveries range from 93 to 125% with CVs $\leq 13.5\%$. These results indicate that this method is able to detect and quantitate low levels of the *R*-enantiomer in the presence of high levels of the *S*-enantiomer.

4. Conclusions

The method described in this report is able to quantitate the individual enantiomers of PGE-9509924 in dog plasma. Derivatizing PGE-9509924 and its *R*-enantiomer with the chiral reagent (−)-FLEC results in the formation of diastereomers, which are then separated by reversed phase HPLC. This single step reaction is quantitative and proceeds rapidly at room temperature. This is the first reported use of (−)-FLEC with a quinolone. The SPE clean-up procedure used on the plasma samples gives a very clean sample from which endogenous compounds that could react with the derivatization reagent have been removed. The use of the 96-well format and the 96-well pipettor to transfer samples speeds up the sample preparation procedure and increases the throughput of the assay. The enantiomer peaks are well enough resolved such that the detection limit for one enantiomer is not affected by high concentrations of the other enantiomer. Hence, this assay can detect low concentrations of the *R*-enantiomer in samples containing primarily the *S*-enantiomer and will be able to determine if any interconversion of the *S*-enantiomer to the *R*-enantiomer takes place in vivo. Results obtained with LC/MS/MS detection and fluorescence detection are essentially identical. Both methods are linear over similar ranges and exhibit good precision and accuracy. Due to the shorter runtime possible with LC/MS/MS detection, LC/MS/MS is the method of choice for those laboratories with access to this type of instrument. For those laboratories without access to this rather expensive type of instrument, fluorescence detection will give equivalent results, but a longer chromatographic runtime is required.

References

[1] S. Roychouldhury, C.E. Catrenich, E.J. McIntosh, H.D. McKeever, K.M. Makin, P.M. Koenigs, B. Ledoussal, Antimicrob. Agents Chemother. 45 (2001) 1115–1120.
[2] S. Roychouldhury, T.L. Twinem, K.M. Makin, E.J. McIntosh, B. Ledoussal, C.E. Catrenich, J. Antimicrob. Chemother. 48 (2001) 29–36.

[3] S.D. Brown, A.L. Barry, P.C. Fuchs, Abstract 549, 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 1999.
[4] S.D. Brown, P.C. Fuchs, A.L. Barry, Abstract 1510, 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 2000.
[5] D.F. Sahm, A. Staples, I. Critchley, C. Thornsberry, K. Murfitt, D. Mayfield, Abstract 1509, 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 2000.
[6] D. Felmingham, M.J. Robbins, C. Dencer, I. Mathias, H. Salman, G.L. Ridgway, Abstract 1511, 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 2000.
[7] K. Lehr, P. Damm, J. Chromatogr. 425 (1988) 153–161.
[8] L. Rabbaa, S. Dautrey, N. Colas-Linhart, C. Carbon, R. Farinotti, Antimicrob. Agents Chemother. 40 (9) (1996) 2126–2130.
[9] T. Nakagawa, M. Ishigai, Y. Nomiyama, M. Hayashi, T. Kawanishi, T. Okutomi, Y. Ishitani, A. Okazaki, Oyo Yakuri 49 (1995) 743–749.
[10] M. Matsuoka, K. Banno, T. Sato, J. Chromatogr. B 676 (1996) 117–124.
[11] M.H. Hyun, J.S. Hin, W. Lee, Bull. Korean Chem. Soc. 19 (1998) 819–821.
[12] M.H. Hyun, S.C. Han, J.S. Jin, W. Lee, Chromatographia 52 (2000) 473–476.
[13] W. Lee, C.Y. Hong, J. Chromatogr. A 879 (2000) 113–120.
[14] J. Brodfuehrer, S. Priebe, R. Guttendorf, J. Chromatogr. B 709 (1998) 265–272.
[15] S. Zeng, J. Zhong, L. Pan, Y. Li, J. Chromatogr. B 728 (1999) 151–155.
[16] S. Gorog, M. Gazdag, J. Chromatogr. B 659 (1994) 51–84.
[17] R.T. Foster, R.A. Carr, F.M. Pasutto, J.A. Longstreth, J. Pharm. Biomed. Anal. 13 (1995) 1243–1248.
[18] S. Einarsson, Josefsson, Moller, Sanchez, Anal. Chem. 59 (1987) 1191–1195.
[19] E. Okuma, H. Abe, J. Chromatogr. B 660 (1994) 243–250.
[20] N. Todoroki, K. Shibata, T. Yamada, Y. Kera, R. Yamada, J. Chromatogr. B 728 (1999) 41–47.
[21] M.T. Rosseel, A.M. Vermeulen, F.M. Belpaire, J. Chromatogr. 568 (1991) 239–245.
[22] E. Frigerio, E. Pianezzola, M.S. Benedetti, J. Chromatogr. A 660 (1994) 351–358.
[23] B. Ledoussal, J.K. Almstead, J.L. Gray, X.E. Hu, US Patent Number WO-9914214.