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Development of a chiral HPLC method to evaluate in vivo enantiomeric inversion of an unstable, polar radiosensitizer in plasma

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Abstract

A chiral HPLC method to quantify in vivo enantiomeric inversion of prodrug CI-1010 (**I*R***) or its drug **II*R*** (PD 146923), a radiosensitizer, upon X-irradiation of dosed rats was developed. These polar enantiomers were separated only by using normal-phase chiral HPLC. A Chiralpak AS column provided the best separation. Isolation of analytes from plasma employed solid-phase extraction (SPE), and required conditions that were compatible with normal-phase HPLC. Options for SPE were restricted by the chemically reactive nature of both prodrug and drug, which produced analyte losses as high as 100%. Acceptable recoveries using SPE required evaluation of conditions for analyte chemical stability. The validated method gave a lower-limit of quantitation (LLOQ) of 200 ng/ml for each enantiomer extracted from 0.15 ml of plasma. The LLOQ of the inverted enantiomer could be detected in the presence of 10 000 ng/ml of the dosed enantiomer. Precision (RSD) ranged from 14.2 to 4.4%, and from 24.2 to 5.1% for **II*S*** and **II*R***, respectively. Accuracy (RE) was $\pm$13.1 and $\pm$13.2%, respectively. Recoveries ranged from 44.3 to 71.4%, and from 40.7 to 67.9%, for **II*S*** and **II*R***, respectively.

Keywords: HPLC method; Plasma; Polar enantiomers; Chiral

1. Introduction

Chiral prodrug CI-1010 (**I*R***, Fig. 1. Note the format for stereochemical designation: **I** indicates compound **I**, in which chirality was not addressed, whereas **I*R*** or **I*S*** indicates specific enantiomers of **I**) produces chiral drug PD 146923 (**II*R***), an alkylating radiosensitizer for potential use to augment X-irradiation therapy for cancer [1]. Prodrug **I** is reactive in vivo, with a half-life of less than 2 min [1,2]. Drug **I** undergoes intramolecular

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cyclization to produce the aziridine drug **II**, or reacts with endogenous carbonate to produce oxazolidinone **III** (Fig. 2). Drug **II** also can be reactive; in addition to alkylating DNA, it produces metabolites **IV** and **V** in vivo [3]. It is reasonable to propose that the aziridine ring of prodrug **II** can be opened by attack of water to produce alcohol **IV** [4], which can fragment to form amine **V**.

I*R* and **II*R*** each contain one chiral center, of *R*-configuration. Some enantiomers have shown stereoselective adsorption, distribution, metabolism, excretion and/or toxicology [5]. It was important to evaluate the chiral stability in **I*R*** and **II*R*** in animals that had been dosed with **I*R*** and subsequently X-irradiated. Enantiomeric inversion of **I*R*** and/or **II*R*** to form **I*S*** or **II*S*** would indicate that pharmacokinetic parameters need to be determined not only for the *R*-enantiomers, but also for the *S*-enantiomers.

An achiral HPLC method [3] for quantitation of **II** and analogs used reverse-phase HPLC and an aqueous-based, mixed-mode solid-phase extraction (SPE) [6]. High recoveries in that achiral method were obtained, in part, because the aqueous SPE eluant was injected directly for reverse-phase HPLC. Options available for this sample preparation for this chiral separation were more restricted.

An SPE eluant that contained water or methanol could not be used as the injection solution for this chiral HPLC method [7]. For example, the combination of an aqueous acidic SPE elution followed by a drydown prior to reconstitution in mobile phase produced very low recoveries, presumably due to analyte degradation. Also, use of liquid–liquid extraction with direct injection of the organic layer was not practical for this chiral HPLC method due to very low recoveries resulting from unfavorable partition coefficients of the analytes.

The need to remove aqueous and/or acid components prior to chiral HPLC precluded the use of a protein precipitation. Water and/or acid could be removed at a reduced temperature by using vacuum assistance, but required several hours to attain complete dryness. This was too much time to allow processing the large number of samples expected. Alternatively, rapid solvent removal incorporating an elevated temperature, with or without vacuum, raised concerns about analyte instability.

This study describes the development and validation of a method to detect 5%, or more, in vivo enantiomeric inversion of prodrug **I*R*** or drug **II*R*** in rat plasma after dosing and X-irradiation. Mass spectrometric detection was not available. Method development was guided by a preliminary examination of representative samples, in which enantiomeric inversion of **I*R*** or **II*R*** was not evident. This suggested a focus on lower levels of inversion, rather than complete racemization.

2. Experimental

2.1. Chemicals and reagents

CI1010 (**I*R***), **I*S***, PD 146923 (**II*R***), **II*S***, internal standard and other analogs, were from Parke-Davis Pharmaceutical Research (Ann Arbor, MI). Hexane, methanol and acetonitrile (HPLC grades) were from Mallinckrodt (Paris, KY). Diethylamine (98%) and sodium phosphate (dibasic, 99.95%) were from Aldrich (Milwaukee, WI).

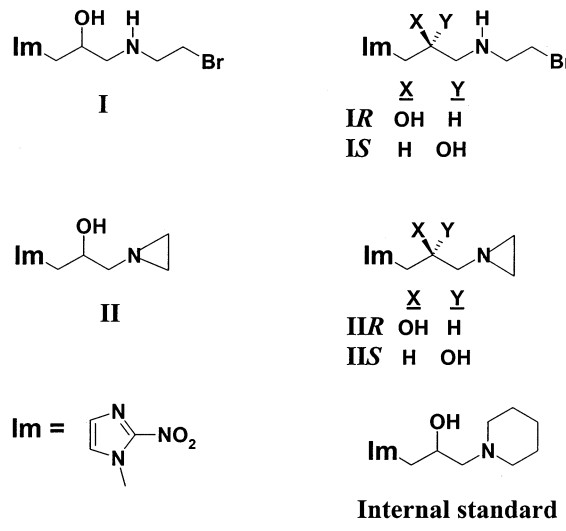

Fig. 1. Structures of analytes and internal standard. See text for further clarification regarding differences between chiral and achiral indications.

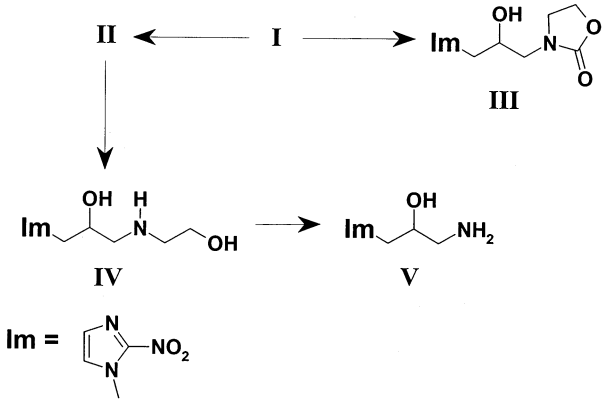

Fig. 2. Scheme indicating metabolism identified for prodrug **I**.

Phosphoric acid (85%), isopropanol, sodium acetate and toluene (all reagent grades) were from EM Science (Cherry Hills, NJ). Glacial acetic acid (HPLC grade) was from J.T. Baker (Phillipsburg, NJ). All reagents were used as received. Water was obtained using a Milli-Q System (Waters, Milford, MA). Rat plasma was prepared in-house by separating heparinized whole-blood collected from male Wistar rats (Charles River, Portage, MI).

2.2. Analyte isolation from plasma

Samples and controls were stored at −70°C. Within 1 h of assay, they were thawed for 2 min at 37°C, vortexed for several seconds, centrifuged ($2400 \times g$) at 4°C for 15 min, and kept on ice until extraction. Calibration standards were prepared using comparable conditions. The analytes were isolated from plasma by using solid-phase extraction (SepPak Plus C18, Waters, Milford, MA); all reagents were chilled to 0–4°C [7]. Solid-phase extraction cartridges were conditioned with 3 ml each of methanol, acetonitrile, water and 50 mM sodium phosphate (pH 6). Cartridges were loaded with a mixture of: (1) rat plasma (0.15 ml) from samples, calibration standards or quality controls; (2) 0.3 ml of the internal standard (2 (g/ml); and (3) 0.5 ml of potassium phosphate (50 mM, pH 6). After loading, sequential isopropanol and hexane treatments were used to wash and remove water from the cartridges. Methanol/2% acetic acid (0.5 ml) followed by 0.5 ml of methanol was used for elution. The eluant was evaporated at 20°C using compressed nitrogen gas and reconstituted in 0.3 ml mobile phase [7].

2.3. Chromatographic equipment

Chiral HPLC was performed on an HP 1090 L (Hewlett-Packard, Avondale, PA) fitted with a NewGuard Silica column (3.2×1.5 mm, 7 μm, Applied Biosystems, Foster City, CA) and two Chiralpak AS columns (250×4.6 mm, 10 μm, J.T. Baker, Phillipsburg, NJ) in tandem. The injection volume was 0.15 ml. A Lambda-Max spectrophotometer (Model 481, Waters, Milford, MA) was used for detection at 325 nm. The mobile phase (1 ml/min) was ethanol/hexane/diethylamine (11/89/0.2, v/v/v); an ethanol wash was used to flush the column prior to each injection [7]. Peak area ratios, relative to an internal standard, were used for quantitation. Conditions for the achiral chromatography using a Resolve CN column with an isocratic mobile phase of methanol/sodium perchlorate (10 mM) have been reported [3].

3. Results

3.1. Separation of enantiomers

A reverse-phase separation of enantiomers was preferred, due to the potential for its facile adaptation with an aqueous-based cleanup of plasma samples. However, solution standards of drug enantiomers **II*R*** and **II*S*** in isopropanol were not separated using a reverse-phase chiral stationary phase (cyclodextrin, ovomucoid or protein AGP columns). The use of chiral derivatizing reagents to produce diastereomers offered the potential for a reverse-phase separation using achiral columns, but was precluded by instability anticipated for the analytes during typical base-assisted derivatizations. Capillary electrophoresis using aqueous run buffers with chiral additives (vancomycin, rifamycin, or γ-, trimethyl-, or hydroxypropyl-cyclodextrin) also failed to separate **II*R*** and **II*S***.

Normal-phase chiral HPLC was evaluated next. Commercially available, polysaccharide-based columns were selected, based on literature searches indicating their success in separating compounds containing aromatic rings, or polar π-bonded groups such as carbonyl, nitro, sulfonyl, cyano or hydroxyl [8]. In addition, polysaccharide carbamate derivatives appeared to provide separation power for drugs containing highly substituted chiral centers, such as **I** and **II** [9]. This strategy was successful, as drug enantiomers were separated completely on a Chiralpak AS column (chiral stationary phase: tris (*S*)-methylbenzyl carbamate on amylose) using a mobile phase of hexane/ethanol with diethylamine. The elution of **II*S*** before **II*R*** was favorable for detecting trace **II*S***, and was used in formulation studies for the prodrug **I*R*** [10]. Separation was not as complete using alternative chiral columns, such as Chiralpak AD, or cellulose-based Chiralcel OD or OJ columns.

In contrast, although the best separation of prodrug enantiomers **I*R*** and **I*S*** was obtained with a Chiralpak AS column, the chromatography was less optimal. The peaks were quite broad, and overlapped, which may have been the result of analyte reactivity during chromatography. For comparison, the partially separated prodrug enantiomers eluted slightly before the completely separated drug enantiomers **II*R*** and **II*S*** on the Chiralpak AS column.

Because only the two drug enantiomers were separated completely, the strategy for evaluating in vivo enantiomeric inversion in samples from dosed rats involved quantitation of drug enantiomers **II*R*** and **II*S***; any prodrug had reacted in vivo or during sample preparation. The chiral stability of **I*S*** and **II*S*** in plasma in vitro was demonstrated. Therefore, the absence of **II*S*** in the plasma of animals dosed with **I*R*** was used to confirm chiral stability of both **I*R*** and **II*R***.

3.2. Analyte stability in buffered solution

Preliminary attempts to adapt a reverse-phase SPE sample cleanup to the chiral HPLC method resulted in analyte recoveries of less than 10%. Analyte degradation due to chemical reactivity of the sidechains was proposed as the cause of the low recoveries. Avoidance of conditions causing analyte degradation was essential for developing a method with acceptable recoveries.

Fundamentals of chemical reactivity suggested contrasting chemical stability in acid or base for these analytes **I** and **II**. Prodrug **I** is relatively stable in *acid* [1,10], whereas cyclization of **I** to **II** occurs readily in *base* [1,10,11]. In general, *acid* catalyzes a facile attack to open aziridine rings, but *basic* conditions require a strong nucleophile [4].

Chemical stability of **II** in buffer at pH 7, 6, 5 or 4 was evaluated. Drug loss in 0.1 M sodium phosphate at pH 7 or 6 demonstrated pseudo first-order kinetics, with half-lives of 26 or 17 h, respectively. In contrast, biphasic plots were obtained for drug loss in 0.1 M sodium acetate at pH 5 or 4. This suggested that drug was being lost through a more complex process, and raised concerns regarding the involvement of intermediate species; it was decided to minimize exposure to such conditions.

3.3. Analyte stability in organic solvents

Stability of stock solutions of **II*R*** or **II*S*** in isopropanol were evaluated. The responses were unchanged, and no chiral inversion was observed, after 1 week at −20°C. As a precaution, however, drug enantiomer solution standards in isopropanol were stored for less than 24 h at −20°C before use.

The chemical and enantiomeric stabilities in the injection solvent at room temperature were determined for **II*R*** (5 μg/ml) and the internal standard extracted from rat plasma. All responses assayed over a 29-h (the maximum time used for an HPLC run) were within 4% of the initial response. No enantiomeric inversion was observed.

3.4. Isolation of analytes from plasma

It would have been advantageous if the sample cleanup provided the analytes in a form that could be used directly for HPLC injection; i.e. compatible with the normal-phase, chiral HPLC. Liquid–liquid extraction was evaluated first, us-

ing solvents compatible with the mobile phase. Low recoveries (1%) indicated very unfavorable partition coefficients for these highly polar analytes. This approach was not pursued further.

Solid-phase extraction (SPE) had been used for the *achiral*, reverse-phase HPLC assay [3]. In that study, methanol/water/TFA (70/30/0.1) for SPE elution gave recoveries of 88% using phenyl SPE cartridges, and approximately 80% using C18, C8 or cyclohexyl cartridges. Cyano or C2 cartridges had produced recoveries of approximately 40%.

Conditions for producing the high SPE recoveries in the *achiral*, reverse-phase HPLC method could not be adapted directly to the *chiral* method. Use of the reverse-phase SPE eluant for injection using the normal-phase produced problems related to immiscibility of the two phases. Removing the methanol/water/TFA eluant via nitrogen gas drydown, followed by reconstitution in normal phase, gave less than 10% recovery. Acid-catalyzed analyte degradation during the prolonged time needed to remove water was proposed as the cause of the poor recoveries. Heating the samples would have expedited water removal, but was not pursued because of the corresponding increase in analyte degradation expected.

Nonaqueous conditions (alcohol/acid) were evaluated next for SPE elution in an effort to eliminate problems associated with water in the SPE eluant. Methanol/acid proved successful for analyte elution, but could not be used for HPLC injection due to severe peak broadening. An evaporation of the methanol/acid SPE eluant was facile enough to produce acceptable analyte recovery, but extensive method development was required. Although SPE removed all matrix peaks, it generated seven new nonmatrix interfering peaks [7]. All of these interfering peaks needed to be managed before acceptable lower limits of quantitation were obtained. These peaks were from analyte degradation products, residual water, SPE endcapping, and nonvolatile salts of bases originating from the SPE cartridges. Interfering peaks were managed by cartridge selection, thorough cartridge drying prior to elution, and chromatography [7].

3.5. Analyte stability in plasma

The *achiral* stabilities of prodrug **I** and drug **II** in rat plasma are known. Prodrug **I** was very unstable in plasma at 4°C; it was not detected at 2 h after spiking [3]. Drug **II** in plasma at 4°C was more stable, with at least 95% remaining after 7 h [3].

The *chiral* stability of each prodrug enantiomer in rat plasma was evaluated by spiking blank plasma at 0°C with 3000 ng/ml **I*R*** or **I*S*** and assaying after: condition A, 5 min, or condition B, storage at −78°C for 2 months followed by three freeze–thaw cycles. Neither **I*R*** nor **I*S*** were observed in extracts of the spiked plasma after conditions A or B. Instead, drug **II** was present in each extract as a single enantiomer, with the same stereochemistry as the spiking prodrug. Comparable results were obtained with 2000 ng/ml of **I*R*** at 4°C for 2 h. This demonstrated stability of the chiral centers of **I*R*** or **I*S*** in plasma, including freeze–thaw cycles.

The stability of drugs **II*R*** or **II*S*** in rat plasma on ice was determined in triplicate by spiking them at 400 and 2000 ng/ml, or 400 and 8000 ng/ml, respectively, followed by two freeze–thaw cycles. Mean concentrations ($\pm$S.D.) were $89.3 \pm 12\%$ and $97.3 \pm 8\%$, and $93.8 \pm 6\%$ and $90.4 \pm 24\%$, respectively, of initial mean values, indicating that **II*R*** and **II*S*** were stable under these conditions.

3.6. Chromatographic performance properties

The performance properties [12] were determined from a chromatogram of extracted rat plasma spiked with the internal standard and 2000 ng/ml of each drug enantiomer (Table 1, Fig. 3A). The relatively low number of theoretical plates is not uncommon for chromatography using a chiral stationary phase.

3.7. Calibration curves, linearity, precision and accuracy

The quantitation limit in a chiral assay that was developed to evaluate enantiomeric inversion when dosing with only one enantiomer may in-

Table 1
Chromatographic performance properties obtained from an extracted rat plasma calibration standard containing the internal standard and 2000 ng/ml each of **II*R*** and **II*S***

	Internal standard	**II*S***	**II*R***
Retention time (min)	16.3	25.3	32.3
Theoretical plates (N)[a]	5048	2560	2749
Capacity factor (k')[b]	1.7	3.2	4.4
Asymmetry factor (A)[c]	1.1	1.5	1.3
Resolution (R_s)[d]	6.2	4.5	4.5
Separation factor (α)[e]	1.9	1.4	

[a] Calculated as $N = 5.54\,[t_R/t_{w\frac{1}{2}}]^2$, where t_R is retention time, and $t_{w\frac{1}{2}}$ peak width at half height.

[b] $k' = (t_r/t_v) - 1$, where t_v is time required for unretained components to elute.

[c] $A = W_{0.05}/2f$, where $W_{0.05}$ is peak width at 5% peak height, and f is width between peak maximum and front edge of peak at 5% peak maximum.

[d] $R_s = 1.176\ (t_{r2} - t_{r1})/(t_{w\frac{1}{2},1} + t_{w\frac{1}{2},2})$, where t_{r1} and t_{r2} are retention times for peak of interest and nearest neighbor, and $t_{w\frac{1}{2},1}$ and $t_{w\frac{1}{2},2}$ are peak widths at half height for peak of interest and nearest neighbor.

[e] $\alpha = k'_2/k'_1$

volve more than just identifying the LLOQ for serial dilutions of a racemic mixture. Peak broadening occurs as analyte concentration increase, which produces a decrease in resolution for two analytes with similar capacity factors [12]. These conditions are not uncommon in the separation of enantiomers. When enantiomers might be present in unequal concentrations, the LLOQ of a chiral assay is described more completely as the minimum concentration of the trace enantiomer that can be detected in the presence of a maximum concentration of the major enantiomer [13].

The LLOQ, 200 ng/ml of **II*S***, could be quantitated in the presence of 10 000 ng/ml of **II*R***. This defined the usable range of this method. Performance was evaluated by preparing one quality control with concentrations close to these limits (400 ng/ml of **II*S*** and 8000 ng/ml of **II*R***, Fig. 3B). Also, plasma spikes containing either **II*R*** or **II*S***

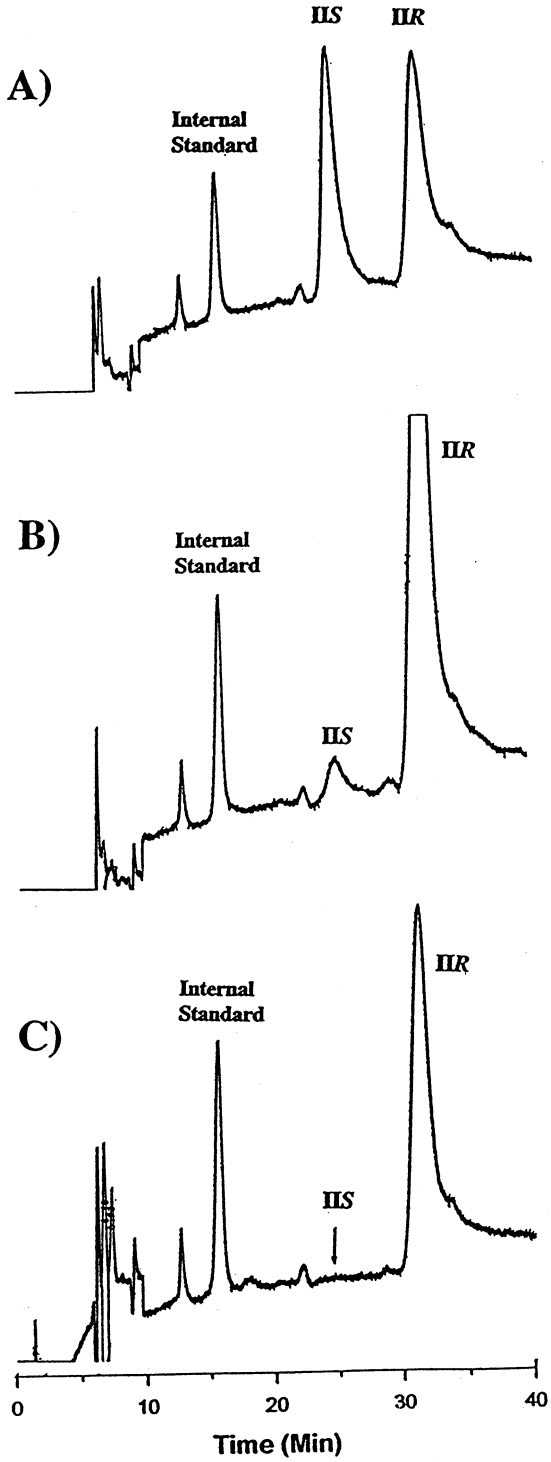

Fig. 3.

Fig. 3. Chromatograms obtained, using the same scaling factor, from extracted rat plasma. (A) A calibration standard that contained the internal standard and 2000 ng/ml each of **II*R*** and **II*S***. (B) A quality control that contained the internal standard and 400 ng/ml of **II*S*** and 8000 ng/ml of **II*S***. (C) A sample, spiked with internal standard, from a rat given X-irradiation 60 min after an intravenous injection of prodrug **I*R*** (40 mg/kg). Drug **II*R*** was present (2050 ng/ml); enantiomer **II*S*** was not observed.

at their highest concentration in the standard curves, were extracted to verify analyte purity and chiral stability during the assay.

The assay was validated with seven rat plasma standards run in triplicate in one batch run. Extracted plasma contained 200–3000 ng/ml of **II*S***, and 200–10 000 ng/ml of **II*R***. The range for **II*S*** was selected to emphasize the lower concentrations expected for this analyte. The best fit line was determined by least-squares linear regression of the calibration data. Standard curves, described by a first-order polynomial using a weighting factor of 1/concentration, were linear with correlation coefficients (R) of 0.994 and 0.987 for **II*R*** and **II*S***, respectively. Reproducibility was evaluated by the variation of individual back-calculated standards from the regression line. Relative standard deviations (RSD) for **II*S*** ranged from 14.2 to 4.4%, with relative errors (RE) of -13.1 to 13.0%. The RSD for **II*R*** ranged from 24.2 to 5.1%, with RE of -6.2 to 13.2%.

Repeatability (%RSD) of peak area determined ($n = 6$) for 500 ng/ml of drug enantiomers **II*R*** and **II*S*** was 3.3 and 3.0%, respectively; for 5000 ng/ml of drug enantiomers **II*R*** and **II*S*** ($n = 6$) it was 3.5 and 3.5%.

It should be noted that several internal standard candidates, including the one selected, had partition properties similar to the analytes. However, none also possessed a chemical reactivity similar to the analytes. Use of such an internal standard might have been advantageous for improving linearity, precision and accuracy of the method.

3.8. Analyte recovery from SPE

Recoveries of **II*R*** and **II*S*** from rat plasma were determined in triplicate by comparing the responses of each calibration standard with the response of the solution standard used to prepare it. Mean recoveries (%RSD) for **II*R*** ranged from 40.7 ± 57.5 to 67.9 ± 9.6%, and those for **II*S*** ranged from 44.3 ± 11.3 to 71.4 ± 14.9%. Although these recoveries were less than ideal, they were sufficient for addressing the questions of interest.

3.9. Evaluation of in vivo enantiomeric inversion

Rats were dosed with either: (1) a single, 40-mg/kg dose of **I*R*** injected into the tail vein, or (2) 3-min daily infusions of 10, 40 or 80 mg/kg of **I*R*** into the tail vein for 5 days. Selected rats then also underwent X-irradiation of the hind limb. Samples were removed from all rats, and assayed using the validated chiral HPLC method. Concentrations observed for **II*R*** ranged from 200 to 7000 ng/ml for rats given a single dose of **I*R***, and from 580 to 8200 ng/ml for rats infused with **I*R*** over several days. Enantiomer **II*S*** was not observed in any of the chromatograms from plasma samples (Fig. 3C), indicating that in vivo enantiomeric inversion did not occur in dosed rats, with or without X-irradiation.

4. Conclusions

An HPLC UV detection method was developed to evaluate in vivo enantiomeric inversion of prodrug **I*R*** or its drug, **II*R*** from plasma after X-irradiation of dosed rats. The enantiomers could be separated only using normal-phase chiral HPLC. Method development was restricted by the chemical reactivity of the analytes. Evaluating the effect of pH on analyte stability provided insight into identifying optimal conditions for SPE. A nonaqueous elution and a rapid dry-down minimized analyte degradation. The method provided a detection limit of 200 ng/ml of the inverted enantiomer in the presence of 10 000 ng/ml of the dosed enantiomer. A study conducted in dosed, X-irradiated rats demonstrated the absence of in vivo enantiomeric inversion.

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References

[1] M. Binger, P. Workman, Cancer Chemother. Pharmacol. 29 (1991) 37.
[2] M.I. Walton, P. Workman, Cancer Chemother. Pharmacol. 22 (1988) 275–281.
[3] W.W. Bullen, D.T. Rossi, K.L. Hoffman, A. Suri, C.D. Lathia, J. Pharm. Biomed. Anal. 16 (1997) 47–55.
[4] H.H. House, Modern Synthetic Reactions, 2, Benjamin/Cummings, Menlo Park, 1972, p. 542 Ch. 9.
[5] FDA's Policy Statement for the Development of New Stereochemical Drugs, Chirality 4 (1992) 338–340.
[6] D.T. Rossi, S.K. Overmyer, R.C. Lewis, P.K. Narang, J. Chromatogr. 566 (1991) 257–265.
[7] J.R. Kagel, D.T. Rossi, C.D. Lathia, J. Chromatogr. A 376 (1997) 129–137.
[8] I.W. Wainer, Drug Stereochemistry, 2, Marcel Dekker, New York, 1993 Revised and Expanded, Ch. 6.
[9] A.M. Krstulovic, Chiral Separations by HPLC, Ellis Horwood/Wiley, New York, 1989 Ch. 13.
[10] A.S. Kearney, S.C. Mehta, G.W. Radebaugh, Int. J. Pharm. 102 (1994) 63–70.
[11] G.R. Newkome, W.W. Paudler, Contemporary Heterocyclic Chemistry: Synthesis, Reactions, and Applications, Wiley, New York, 1982, pp. 347–350 Ch. 25.
[12] L.R. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2, Wiley, New York, 1979 Chs. 2 and 5.
[13] J.R. Kagel, V.J. Stella, C.M. Riley, J. Pharm. Biomed. Anal. 11 (1993) 793–802.