Short Communication

Preparative separation and analysis of the enantiomers of [^{14}C]Zileuton, a 5-lipoxygenase inhibitor

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ABSTRACT

Preparative resolution and quantitative methodology for the optical purity determination of [^{14}C]Zileuton, N-[1-^{14}C]1-(benzo-[*b*]thien-2-yl)ethyl-N-hydroxyurea, [^{14}C]Abbott-64077, a 5-lipoxygenase inhibitor with potential clinical applications in the treatment of inflammatory diseases, is described. The method involves the use of a chiral stationary phase composed of amylose tris(3,5-dimethylphenylcarbamate) chemically bonded to 3-aminopropyl silica gel (ChiralPak AD) and a mobile phase consisting of *n*-hexane–ethanol (90:10). Optical and radiochemical purities of >99% were achieved for both.

INTRODUCTION

Zileuton (Abbott-64077, Fig. 1) is a 5-lipoxygenase inhibitor with potential clinical applications in the treatment of inflammatory diseases [1,2]. In order to carry out absorption, distribution, metabolism and elimination (ADME) studies with emphasis on the fate of each enantiomer, several milligrams of radiolabeled enantiomers of Zileuton of high optical purities were required. To achieve this goal, we sought a chromatographic method that would separate these enantiomers which can be scaled up to preparative scale without the need for derivatiza-

tion. Zileuton was first separated on a Pirkle phenylglycine covalent column [3]. Later, using a second generation α_1-acid-glycoprotein (α_1-AGP) column, the analytical resolution was greatly improved [4]. These and other chiral stationary phases (CSP's) (for an excellent discussion of the CSPs mentioned here, see ref. 5) were investigated and the results of this investigation and the successful resolution of the labeled enantiomers are reported herein.
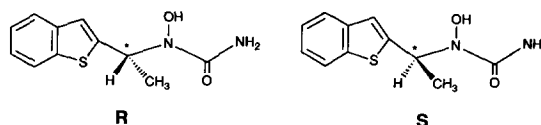

Fig. 1. [^{14}C]Zileuton: (left) [^{14}C]A-68967 (1,*R*), (right) [^{14}C]A-68968 (2,*S*). Asterisk denotes position of carbon-14 label.

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EXPERIMENTAL

Chemicals

n-Hexane (C_6H_{14}), methanol (CH_3OH), chloroform ($CHCl_3$), isopropanol (2-PrOH), ethanol (C_2H_5OH), tert.-butanol (t-BuOH), acetonitrile (CH_3CN), glacial acetic acid (HOAc), copper(II) acetate monohydrate [$Cu(OAc)_2$], triethylamine [$(C_2H_5)_3N$], ammonium acetate (NH_4OAc), potassium phosphate monobasic (KH_2PO_4), acetohydroxamic acid and perchloric acid were purchased from commercial vendors and used without purification. All solvents except the C_2H_5OH were HPLC grade and the chemicals were ACS reagent grade. The water used was from a Millipore Milli-Q water purification system. Scintillation cocktail (Flo-Scint III) was obtained from Radiomatic Instrument and Chemical Co., (Meriden, CT, USA). [^{14}C]Zileuton was synthesized in our laboratory. Zileuton, A-68967 and A-68968 "cold" reference materials were synthesized at Abbott Laboratories and used as received.

Analytical high-performance liquid chromatography

The chromatography mobile phase was delivered by a Perkin-Elmer Model 410 quaternary pump. Samples were injected using a Rheodyne Model 7125 syringe-loading sample injector with a 200-μl loop. Peaks were detected with an Applied Biosystems Model 785A UV programmable detector set at 235 nm connected in series with a Flo-One Beta Model 500 radioactivity detector (Radiomatic). Chromatograms were obtained by a Gateway 2000 computer (Gateway, N. Sioux, SD, USA) and a Panasonic Model KX-P1124i printer. A FIAtron CH-30 column heater was used for the high-temperature experiments. Analysis of the optical purity was performed using a ChiralPak AD (250 × 4.6 mm I.D.) column (Chiral Technologies, Drawer I

Fig. 2. Chiral stationary phase: cellulose tris(3,5-dimethylphenylcarbamate) bonded to silica gel (ChiralPak AD).

Exton, PA, USA). The following columns were also investigated for enantiomeric resolution (Table I): β-Cyclodextrin (250 × 4.6 mm I.D.) (Advanced Separation Technologies, Whippany, NJ, USA), Pirkle's phenylglycine covalent (250 × 4.6 mm I.D.) (Regis, Morton Grove, IL, USA), Nucleosil Chiral-1 (250 × 4.6 mm I.D.) (Machery–Nagel, Germany), α_1-AGP (100 × 4.0 mm I.D.) (Chiral-AGP ChromTech AB, Norsborg, Sweden) and Merck cellulose triacetate (250 × 10 mm I.D.) (Merck, Darmstadt, Germany).

Radiochemical purity determination was performed using a Whatman Partisil 5 ODS 3 column (250 × 4.6 mm I.D.) (Whatman, Clifton, NJ, USA).

The mobile phase used for the optical purity determination consisted of n-hexane–ethanhol (90:10). The flow-rate was set at 1.0 ml/min. The mobile phase used for the radiochemical purity determination consisted of 70% NH_4OAc (0.1 M) containing 0.02% acetohydroxamic acid and 30% CH_3CN with pH adjusted to 2.0 with perchloric acid. The flow-rate was set at 1.0 ml/min.

Preparative high-performance liquid chromatography

Separations were carried out using a Waters Delta Prep 3000, a Perkin-Elmer auto sampler Model ISS 200 and a Waters Model 484 variable-wavelength absorbance detector operated at 235 nm. The chromatograms were obtained using a Waters Model 745B integrator. The chiral semi-preparative HPLC column used was a ChiralPak AD (250 × 20 mm I.D.) (Chiral Technologies).The mobile phase consisted of n-hexane–ethanol (90:10). The flow-rate was set at 20.0 ml/min.

For sample preparation, a portion of [^{14}C]Zileuton (2.25 mCi, 0.119 mmol, 28.1 mg) was dissolved in a mixture of C_2H_5OH and C_6H_{14} and injected onto the column at about 7 mg per injection.

Isolation of pure enantiomers

Fractions containing purified 1 were pooled and the solvent was removed in vacuo. The residue (0.82 mCi, 75% yield) was dissolved in C_2H_5OH. Fractions containing 2 were pooled, concentrated, dissolved in mobile phase and further repurified from radiochemical impurities using a YMC-pack 5-μm silica (250 × 22 mm I.D.) (YMC, Morris Plains,
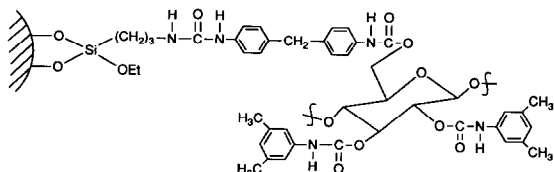
TABLE I

EFFECT OF CSP, MOBILE PHASE CONCENTRATION AND FLOW-RATE ON THE RESOLUTION AND ENANTIO-SELECTIVITY OF [^{14}C]Zileuton

CSP	Mobile phase	Flow-rate (ml/min)	$t_1{}^a$ (min)	$t_2{}^a$ (min)	$R_s{}^b$	α^c
ChiralPak AD (250 × 4.6 mm I.D.)	C_6H_{14}–C_2H_5OH (90:10)	1.0	11.2	19.3	7.7	2.01
	C_6H_{14}-2-PrOH (90:10)	1.0	9.8	15.7	5.0	1.92
Cyclobond 1 β-Cyclodextrin (250 × 4.6 mm I.D.)	$(C_2H_5)_3N$ (1%), pH 7 with HOAc–CH_3OH (60:40)	1.0	22.7	24.5	1.2	1.09
		1.5	14.7	15.8	0.9	1.08
	$(C_2H_5)_3N$ (1%), pH 4 with HOAc–CH_3OH (60:40)	1.0	15.3	16.1	1.1	1.08
		0.7	22.0	23.7	1.3	1.08
		0.5	30.6	33.0	1.5	1.08
	CH_3OH-H_2O (45:55)	1.0	29.1	31.3	1.9	1.17
	CH_3OH-H_2O (40:60)	1.0	25.9	28.8	1.2	1.12
Cellulose triacetate (250 × 10 mm I.D)	C_2H_5OH-2-PrOH (50:50)	0.5	49.5	65.5	1.1	1.42
Pirkle covalent phenylglycine (250 × 4.6 mm I.D.)	C_6H_{14}–C_2H_5OH–t-BuOH (90:1.5:8.5)	2.0	43.7	47.0	0.7	1.08
α_1-AGP (100 × 4.6 mm I.D.)	KH_2PO_4 (0.01 M), pH 3–2-PrOH (97:3)	1.0	6.0	18.6	7.9	3.63
Nucleosil Chiral-1 (250 × 4.0 mm I.D.)	$Cu(OAc)_2$ (1 mM)–CH_3CN (95:5)	1.0	20.1	22.8	1.22	1.14
	$Cu(OAc)_2$ (1 mM), pH 4.0–CH_3CN (95:5)	1.0	13.8	13.8	0	1.0
	$Cu(OAc)_2$ (1 mM)–CH_3CN (98:2)	1.2	23.4	26.6	0.94	1.15
	$Cu(OAc)_2$ (1 mM), pH 5.5–CH_3CN (95:5)	1.0	20.5	23.8	1.15	1.14

[a] t_1 and t_2 refer to the retention times of [^{14}C]A68967 (**1**) and [^{14}C]A68968 (**2**), respectively.
[b] R_s is the resolution factor.
[c] α is the enantioselectivity factor.

NJ, USA) semi-preparative column. The mobile phase consisted of C_6H_{14}-2-PrOH–$(C_2H_5)_3N$ (85:15:0.5). The flow-rate was set at 35.0 ml/min. Fractions containing **2** were pooled and evaporated to dryness in vacuo. The residue (0.62 mCi, 55% yield) was dissolved in C_2H_5OH.

RESULTS AND DISCUSSION

Table I lists several different approaches to the LC separation of the enantiomers of Zileuton. While all of these CSPs resolve the enantiomers to some extent, three of these, Nucleosil Chiral-1 [6], Pirkle's phenylglycine covalent and Merck cellulose triacetate column [7], suffer from poor resolution and long retention times. The drawback to the successful separation that was achieved using an (α_1-AGP) column is that α_1-AGP column has a very low loading capacity (0.1 μg) which makes preparative work using this stationary phase very impractical. The initial resolution achieved on the β-cyclodextrin column [8] was suitable for our needs but we discov-

The chromatographic resolution of the optical isomers of [^{14}C]Zileuton using the semi-preparative ChiralPak AD column is shown in Fig. 3. The first of the two enantiomeric peaks was **1** identified by
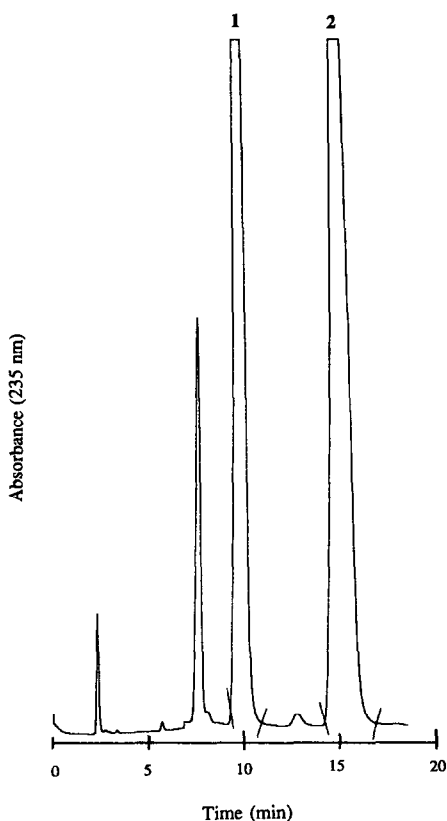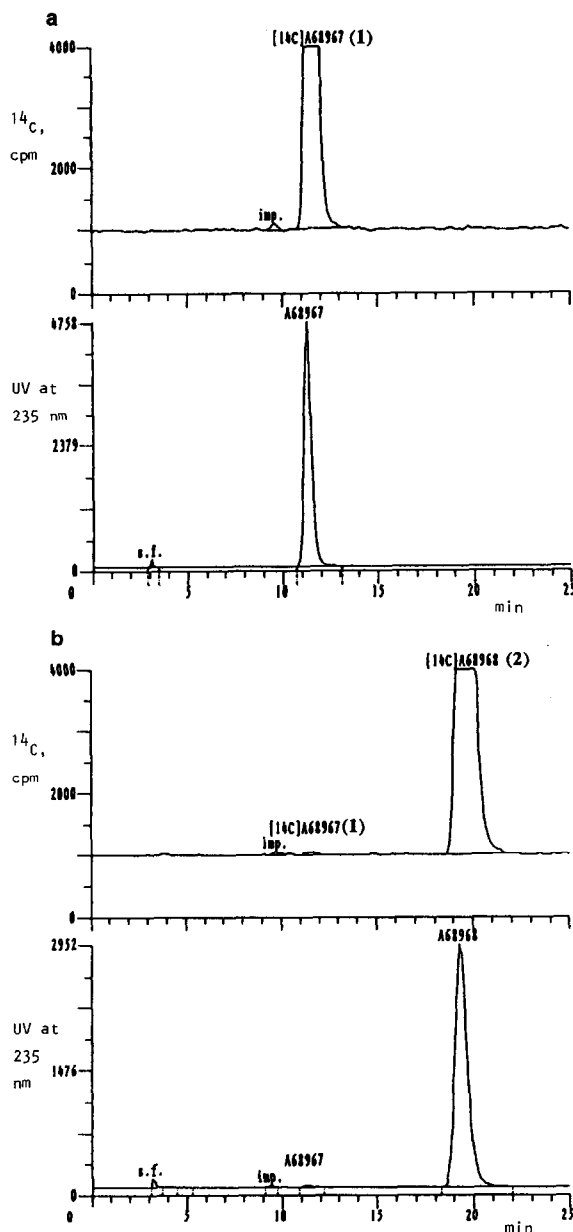

Fig. 3 Enantiomeric separation and resolution of [^{14}C]Zileuton on a Diacel ChiralPak AD, 250 × 20 mm I.D. column showing cutoff points for fraction collection during preparative runs. The mobile phase consisted of *n*-hexane–ethanol (90:10). Flow-rate was set at 20.0 ml/min.

ered over time that this CSP was inconsistent and unstable from one column to the other for the separation of Zileuton. Some batches achieved almost baseline separation while some batches did not. Also the retention time changed from one injection to another which led us to abandon the use of this column. Subsequently, we found that the ChiralPak AD gave good enantiomeric resolution with retention times under 25 min consistently from column to column. The CSP consists of tris(3,5-dimethylphenylcarbamate) cellulose chemically bonded to 3-aminopropyl silica gel via 4,4'-diphenylmethane diisocyanate (Fig. 2) [9,10]. Recently a preparative size column was purchased to resolve gram quantities of Zileuton and related compounds and it has displayed the same consistency and ruggedness shown in the smaller versions.

Fig. 4. Optical purity determination of (a) [^{14}C]A-68967 (**1**, >99%), (b) [^{14}C]A-68968 (**2**, >99%) on a ChiralPak AD, 250 × 4.6 mm I.D. column. The mobile phase consisted of *n*-hexane–ethanol (90:10). Flow-rate was set at 1.0 ml/min. s.f. = Solvent front; imp. = impurity.

comparison to authentic A-68967, followed by **2**, identified by comparison to authentic A-68968. The semi-preparative resolution of these enantiomers was uneventful owing to the excellent characteris-

tics of this CSP and the volatility of the mobile phase. A yield of 75% for **1** and 55% for **2** is good considering that **2** had to be purified further from some radiochemical impurities not separated in the chiral chromatography.

Optical purities of **1** and **2** were determined to be greater than 99% (Fig. 4a and b). The radiochemical purities of **1** and **2** were determined to be greater than 99% and the radioactive peaks corresponded to the UV peaks of A-68967 and A-68968, respectively, at 8.7 min (Fig. 5a and b). Assays of the effluents collected during the analyses of **1** and **2** showed that 101.6% and 98.8% of the radioactivity were recovered from the column, respectively.

In conclusion, the effectiveness of the cellulose tris(3,5-dimethylphenylcarbamate) CSP in the separation of the enantiomers of [^{14}C]Zileuton has been demonstrated. The preparative procedure described above allowed the resolution of mg quantities of the enantiomers of the labeled Zileuton of high optical purities.

Fig. 5. Radiochemical purity determination of (a) [^{14}C]A-68967 (**1**, >99%), (b) [^{14}C]A-68968 (**2**, >99%) on a Whatman Partisil ODS 3, 5 μm, 250 × 4.6 mm I.D. column. The mobile phase consisted of ammonium acetate (0.1 M) containing 0.02% acetohydroxamic acid–acetonitrile (70:30), pH adjusted to 2.0 with perchloric acid. The flow-rate was set at 1.0 ml/min.

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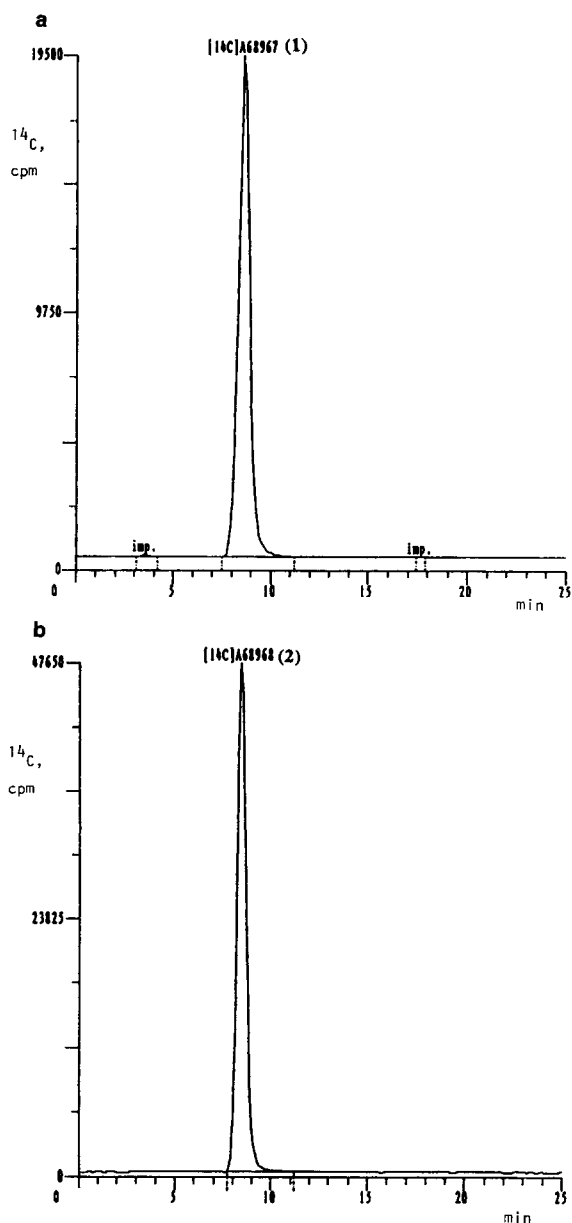10 Y. Okamoto, R. Aburatani, S. Miura and K. Hatada, *J. Liq. Chromatogr.*, 10 (1987) 1613.