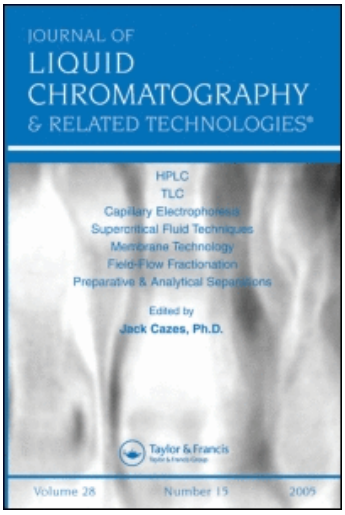

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Determination of Minor Impurities of (R)-1-Acetoxymethyl-3-(7-[(1-N,N-Dimethylcarbamoyl)-6-(4-Fluorophenyl)-Indol-3-Oyl]-1H,3H-Pyrrolo[1,2-c] Thiazol-3-yl)Pyridinium Chloride by High Performance Liquid Chromatography

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DETERMINATION OF MINOR IMPURITIES OF (R)-1-ACETOXYMETHYL-3-(7-[(1-N,N-DIMETHYLCARBAMOYL)-6-(4-FLUOROPHENYL)-INDOL-3-OYL]-1H,3H-PYRROLO[1,2-c] THIAZOL-3-YL)PYRIDINIUM CHLORIDE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Minor impurities of (R)-1-acetoxymethyl-3-(7-[(1-N,N-dimethylcarbamoyl)-6-(4-fluorophenyl)-indol-3-oyl]-1H,3H-pyrrolo[1,2-c]thiazol-3-yl)pyridinium chloride (I), a pro-drug, were determined using high-performance liquid chromatography. Manufacturing impurities, degradation products, and active drug, (R)-7-[(1-N,N-dimethylcarbamoyl)-6-(4-fluorophenyl)-indol-3-oyl]-1H,3H-pyrrolo[1,2-c]thiazole (II) were separated using a reversed-phase system with gradient elution. Detector response was linear for II to approximately 470 μg/mL which represents 47% of the drug concentration. The procedure provides relative standard deviations of 3.0% to 13.2% in typical bulk drug lots. A variety of reversed-phased columns were evaluated for the assay method with the optimum resolution achieved using a 5-μm Zorbax Rx C-8 packing.

INTRODUCTION

The synthesis of (R)-1-acetoxymethyl-3-(7-[(1-N,N-dimethylcarbamoyl)-6-(4-fluorophenyl)-indol-3-oyl]-1H,3H-pyrrolo[1,2-c]thiazol-3-yl)pyridinium chloride (I, See Figure 1), a platelet activating factor antagonist and pro-drug has been previously reported [1,2]. As reported recently [2], this class of compound has in vitro and in vivo activity in assay models for inflammation and septic shock. The synthesis of I is necessitated due to the lack of water solubility of (R)-7-[(1-N,N-dimethylcarbamoyl)-6-(4-fluorophenyl)-indol-3-oyl]-1H,3H-pyrrolo[1,2-c]thiazole (II), the active drug. This paper describes the use of high performance liquid chromatography (HPLC) for the quantitation of minor impurities in I bulk drug substance. Potential manufacturing impurities and degradation products are determinable by the procedure in addition to the quantity of active drug, II, in the bulk drug.

Although not a fluoroquinolone, the structure was viewed as being similar enough to other fluoroquinolones previously reported by this laboratory [3-5], which had been separated by gradient elution. Using these gradients as a starting point, and with the goal of separating the active drug from the pro-drug, the separation described was developed. The separation was further complicated by the vast structural differences between intermediates III and IV, with IV being a very lipophilic molecule and III being a very hydrophilic molecule. In this work a separation was pursued to adequately resolve the pro-drug and active drug and in the same chromatographic run quantitate minor impurities and degradates which can show marked differences in retention times using reversed-phase systems. The stability of I sample preparations was also an issue. Being a pro-drug, designed to hydrolyze rapidly to the active drug in the body, sample preparations had a limited usable lifetime.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Model SP-8800 ternary pump and chromjet data handling system (Spectra-Physics, Santa Clara, CA, USA). A

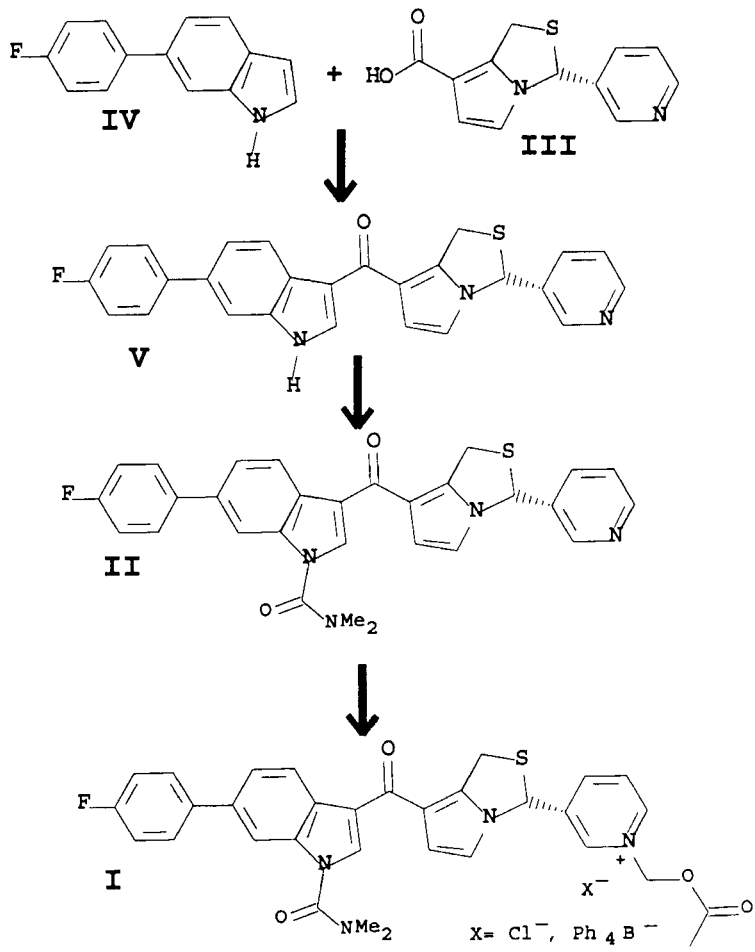

Figure 1. Synthetic Scheme for Preparation of I

Model 757 variable-wavelength UV detector (ABI Analytical Kratos Division, Ramsey, NJ, USA) and a Model SIL-10A autosampler with sample cooler at about 5°C (Shimadzu Scientific, Columbia, MD, USA) were used. Chromatographic separations described in this method were made using Zorbax Rx C-8 columns (5 μm, 80 Å) measuring 25 cm x 4.6 mm I.D. (Mac-Mod Analytical, Chadds Ford, PA). The following columns were also

evaluated for their suitability: Alltima C-18, 4.6 mm x 15 cm, 5 μm 100 Å and Nucelosil C-18, 5 μm, 4.6 mm x 15 cm (Alltech Associates, Deerfield, IL, USA); CSC-Nucelosil 5 μm, 4.6 mm x 15 cm, (Resolution Systems, Wilmette, IL, USA); Nucelosil C-18, 5 μm, 4.6 x 15 cm, (Column Resolution Inc., San Jose, CA, USA); Primesphere C-18HC, 4.6 mm x 25 cm, 5 μm (Phenomenex, Torrance, CA, USA); Spherisorb S10ODS C-18, 4.6 mm x 30 cm, 10 μm and Spherisorb S5ODS C-18, Hi-Chrom Rev, 5 μm, 4.6 mm x 25 cm (Regis, Morton Grove, IL, USA). Prior to use, the components of the eluent were filtered through 0.45 μm nylon membranes (Alltech).

Reagents

Acetonitrile (ACN) was Omni-Solv grade from EM Sciences (Cherry Hill, NJ, USA). The aqueous mobile phase was prepared by dissolving 2 mL of perchloric acid (Fisher Scientific) in 1 liter of water. Sodium pentane sulfonate was reagent grade from Aldrich Chemical Co. (Milwaukee, WI, USA). All bulk drug and related impurities were synthesized at Abbott Labs (North Chicago, IL, USA). Isolated compounds were characterized by ^{1}H, ^{13}C NMR and mass spectrometry. The diluent was prepared by mixing 1 liter of aqueous mobile phase and 1 liter of acetonitrile.

Chromatographic Conditions

A two step linear gradient was used, mixed with the ternary pump as shown in Table I. Other conditions were: flow-rate, 1.0 mL/min; pressure approximately 1800 psi; detector, 254 nm at 0.10 a.u.f.s., attenuation 128, and injection volume, 20 μL. All separations were performed at ambient temperature. The sample preparations were kept at about 5°C before injection.

Analytical Procedure

Bulk drug samples of I were prepared by dissolving approximately 100 mg of drug substance in 50 mL of diluent followed by dilution to 100.0 mL with diluent. A 1% standard of II was prepared by dissolving approximately

TABLE 1.

Linear Gradient for HPLC Eluent

Time (min)	Aqueous $HClO_4$ (%)	ACN (%)
0	90	10
5	90	10
15	60	40
65	05	95
66[a]	90	10
90[a]	90	10

[a] Used to re-equilibrate the column to the initial conditions.

100 mg in 20 mL of acetonitrile in a 100-mL volumetric flask, followed by dilution to volume with diluent. The above solution was serially diluted 5 mL to 50 mL then 5 mL to 50 mL with diluent. The amounts of impurities were estimated in the sample by comparing the corresponding peak areas in the samples and standard preparations. Impurity content was calculated on the anhydrous basis by correcting the sample concentration for the amount of water and residual solvents contained in the drug substance. The drug substance typically contains approximately 1% water, as measured by Karl Fisher titration and 1-5% isopropanol, as measured by gas chromatography.

Results and Discussion

Since compound I is a pyridium salt and II is the neutral base without the methylacetoxy portion, the chromatographic behavior of I is relatively independent of pH where as II is pH dependent as well as sensitive to the organic modifier of the eluent. The most symmetrical peak shapes for I and II were obtained in eluents containing aqueous solutions at pH values of approximately 2 to 4. The retention characteristics of the I, II, and related impurities did not vary significantly with the type of buffer. However, the order of elution of I and II could be reversed using trifluoroacetic acid (0.2%)

or sodium pentane sulfonate (0.2% w/v, pH=2.0 with H_2SO_4). The decision to use perchloric acid for the aqueous solution was based on the lack of interfering peaks in the blank solutions and lack of integration problems when II elutes before the drug I which were observed when II eluted after I. The separation of impurities and peak shapes for all three systems were not significantly different and the trifluoroacetic acid system and variations on it were in fact used for the LC-MS determination of minor impurities.

Single isocratic eluents failed to adequately resolve the drug and pro-drug and did not retain intermediate III at all or the retention of IV was unacceptably long (>60 min) and broad. For these reasons, a gradient elution system was developed. Single organic modifiers of tetrahydrofuran, methanol, and acetonitrile were used in this approach. Again, only acetonitrile modifier proved acceptable. Mixed organic systems also proved unacceptable. The gradient used provided the needed resolution between I and II and acceptable retention times for the other intermediates (Figures 2 and 3). If intermediate III could be eliminated, then the gradient could be varied in order to increase the resolution between I, II, and V. Because this was not the case for the synthetic scheme adopted, the system described proved to be the best.

A detection wavelength of 254 nm provides a very similar response for the impurities, the active drug (I), and pro-drug (II), providing an accurate estimation of unknowns quantitated versus II. The response of I versus II is approximately 0.8, which is the ratio of the molecular weights. This is not a surprising result considering the wavelength used and the functional groups in the back-bone of the drug. A response factor for V has not been determined at this time due the lack of an acceptably pure lot, but it would again not be expected to differ much from I and II. Chelating agents such as EDTA were evaluated because they have been used for other nitrogen containing drugs [3-5] due to the possible adsorption of the drug to metal surfaces or peak broadening in the presence of trace metals. The use of EDTA did not improve the separation and was not used further.

Several C-8 and C-18 reversed-phase packings were evaluated for the determination of pro-drug impurities. For this evaluation, identical gradient profiles were used as described in the text. The results are summarized in

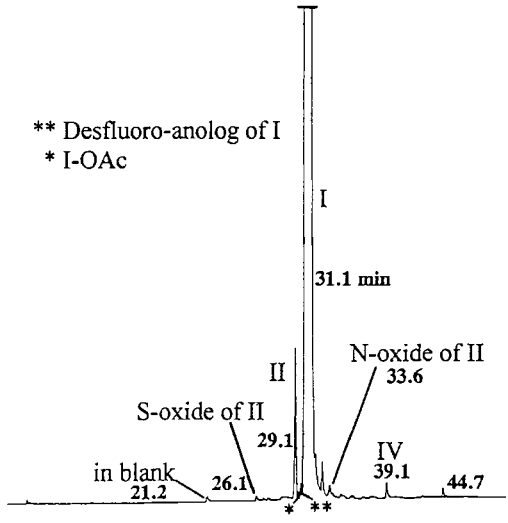

Figure 2. Typical Chromatogram of a Representative Lot of I

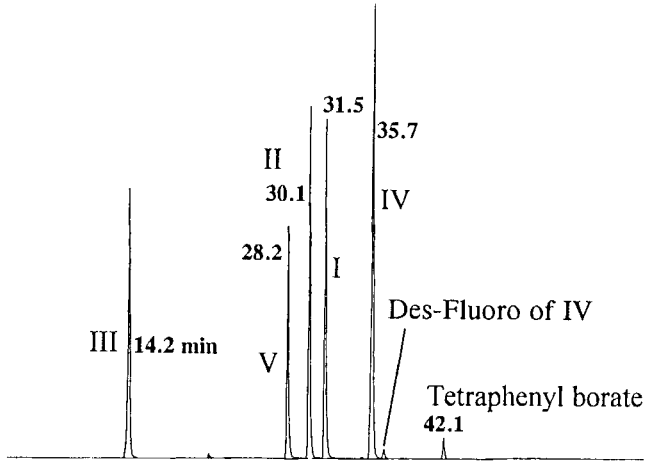

Figure 3. Synthetic Mixture of Intermediates

TABLE 2

Compariton of Column Packing on the Resolution of I and II and Tailing Factor of I

Packing	Resolution Factor[a]	Tailing Factor[a]
Nucelosil, Alltech 4.6 mm x 15 cm, 5 μm	2.0	1.4
Alltima C-18 4.6 mm x 15 cm, 5 μm	2.6	2.2
Nucelosil, CSC 4.6 mm x 15 cm, 5μm	4.0	1.0
Nucelosil, Column Resolution 4.6 mm x 15 cm, 5 μm	2.9	1.7
Primesphere C-18HC 4.6 mm x 25 cm, 5 μm	6.0	1.0
Spherisorb S5ODS 4.6 mm x 25 cm, 5 μm	7.3	2.5
Workhorse S10ODS 4.6 mm x 25 cm, 10 μm	7.3	1.5
μ-Bondapak C-18 3.9 mm x 30 cm, 5 μm	2.4	1.5
Zorbax Rx C-8[†] 4.6 mm x 250 cm, 5 μm	4.8	1.0

[a]U.S. Pharmacopia XXIII, p. 1777.
[†]System desribed in text.

Table II. The comparison of resolution factors calculated between I and II, which is a critical separation in our application, demonstrates that the separation is largely a function of the type of reversed-phase column used. Some of the other columns evaluated did produce greater resolution between I and II but sacrificed peak shape to obtain more resolution. The only other column that appeared acceptable was the Primesphere C-18 column, which had

a resolution of 6.0 (typical R=4-6). The choice of the Zorbax RxC-8 column over the Primesphere C-18 column was based on this laboratory's experience with the stability of the Zorbax column and its multiple uses in this laboratory. In our limited use the Primesphere performed equally as well and most likely could be substituted.

The system used in the procedure is also more useful than others evaluated in the resolving more impurities, the most important of which is the desfluoro-analog of the active drug. It's relative retention time was 0.99 and in the best scenario was a leading shoulder on the main drug peak. The peaks that can be seen in a typical lot of I are shown in Figure 2.

Detector response for II was linear from 0.5 μg/mL to 470 μg/mL and for I was linear from 1.1 μg/mL to 430 μg/mL (correlation coefficients >0.999). Linearity curves of concentration versus response essentially intersected the origin for both compounds, allowing the use of one-point calibration for quantitation of impurities. Assay precision was assessed by performing the procedure on one lot of I. Two analysts performed the determinations on different days. The assay precision (relative standard deviation values) ranged from 3.0% to 13.2% for impurities having mean values of 0.48% to 0.11%.

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