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Separation of diastereoisomers of DuP 105, a novel oxazolidinone antibacterial agent, by supercritical fluid chromatography on a Chiralcel OD column

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Abstract

Oxazolidinones are a novel class of synthetic, orally-active antibacterial agents. DuP 105, which is representative of this class of compounds, is synthesized as a racemic mixture of two diastereoisomers. To ensure lot-to-lot consistency, separation and quantitation of these diastereoisomers is essential. Separation by reverse phase LC has not been achieved. This paper presents a supercritical fluid chromatography method using a Chiralcel OD column to resolve these diastereoisomers. A retention mechanism is briefly elaborated, based on the effects of temperature, pressure, percent modifier and type of modifier on resolution.

Keywords: Chiral analysis; Supercritical fluid chromatography; HPLC; Oxazolindinones; DuP 105

1. Introduction

Oxazolidinones are a new class of synthetic, orally-active antibacterial agents. DuP 105 (Fig. 1), which is representative of this class, contains two chiral centers [1–4]. The chiral center at carbon-5 is controlled through stereoselective synthesis while the second center is not, resulting in a racemic mixture of two diastereoisomers: *R,S*- and *S,S*- [1–4]. Only the *R,S*-isomer is pharmacologically active [1–4]. To ensure lot-to-lot consistency, an analytical method is needed to resolve and quantitate these diastereoisomers. Even though these diastereoisomers have different physical properties, their separation by reverse-phase LC has not been achieved (M. Alasandro, J. Brown and P. Hovsepian, unpublished results). Earlier attempts to use GC (M. Alasandro, unpublished results) or chiral LC on Bakerbond CrownPak CR, Bakerbond DNBPG, Chiralcel OD and Chiral AGP columns (R. Williams, unpublished results) were not successful. The poor resolution of the *R,S*- and *S,S*- isomers of DuP 105 may be attributed to the large distance between the two chiral centers (Fig. 1). The objec-

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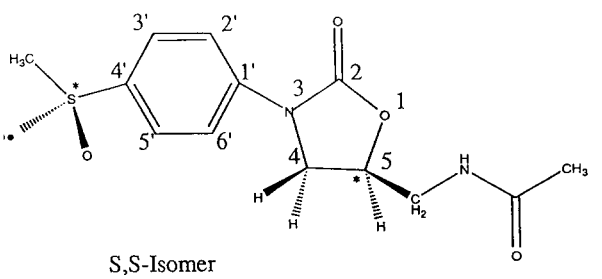
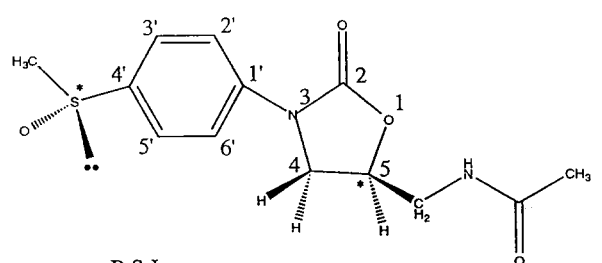
tive of this paper was to investigate chiral supercritical fluid chromatography (SFC) as a method for the direct resolution of the diastereoisomers of DuP 105 on a Chiralcel OD column. A comparison of chiral SFC with chiral LC for the separation of these diastereoisomers is also presented.

The Chiralcel OD stationary phase (Fig. 2) is finding increased use in the separation of pharmaceutical enantiomers [5–12]. Although this phase is coated onto the silica support, it still proves to be durable under LC and SFC conditions after multiple injections [6]. This chiral stationary phase (CSP) is also useful for a broader range of chiral compounds than other CSPs [7–12]. The higher diffusivity in SFC allows for faster and more efficient chiral separations than achieved by LC for some compounds. Also, SFC provides better selectivity for some compounds than is achieved by chiral LC [13]. With commercially available SFC systems and durable chiral stationary phases, SFC is becoming a practical tool for pharmaceutical separations of racemic mixtures.

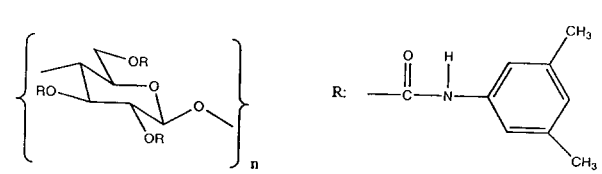


S,S-Isomer



R,S-Isomer

Fig. 1. Structures of DuP 105 diastereoisomers.



Chiralcel OD

Fig. 2. Structure of Chiralcel OD stationary phase.

Table 1
HPLC conditions (Chiralcel OD column, 25 cm × 0.46 cm i.d.)

Injection volume	5 μl
Column temperature	35°C
Detection	UV at 254 nm
Flow rate	1.0 ml min^{-1}
Mobile phase	Varied from 10/90 to 50/50% hexane/isopropanol

Table 2
SFC Conditions (Chiralcel OD column, 25 cm × 0.46 cm i.d.)

Injection volume	5 μl
Column temperature	Varied from 25 to 55°C
Detection	UV at 210 nm
Flow rate	1.0 ml min^{-1}
Pressure	Varied from 100 to 350 bar
Mobile phase	Varied from 95/5 to 75/25% carbon dioxide/methanol

2. Experimental

2.1. Apparatus

SFC was done on a Hewlett-Packard G1205A instrument (Little Falls, DE) equipped with a supercritical fluid pump, a modifier pump, and a fixed 5 μl volume external loop, a column oven, and a variable-wavelength UV detector.

Chiral LC studies were done on a Hewlett-Packard 1050 instrument (Little Falls, DE) equipped with a quaternary pump, a column oven, a variable injection volume auto sampler, and a variable-wavelength UV detector.

Both systems were equipped with MS-DOS-based Hewlett-Packard Chem Stations and interfaced to a Fisons Multichrom software program on a VAX 6000 series computer for generation of chromatograms and data reduction.

Table 3
Effect of temperature on retention time, selectivity, resolution and number of theoretical plates for the DuP 105 diastereoisomers

Pressure (Bar)	Temp. (°C)	Methanol (%)	Retention time of *S,S*-isomer (min)	Selectivity	Resolution	Theoretical plates
300	25	10	22.8	1.60	2.71	5290
300	35	10	23.5	1.12	2.32	6458
300	45	10	24.7	1.09	1.91	7108
300	55	10	26.7	1.07	1.38	7250

Table 4
Effect of pressure on retention time, selectivity, resolution and number of theoretical plates for the DuP 105 diastereoisomers

Pressure (Bar)	Temp. (°C)	Methanol (%)	Retention time of *S,S*-isomer (min)	Selectivity	Resolution	Theoretical plates
100	35	10	57.2	1.11	2.34	7790
150	35	10	42.2	1.12	2.35	7166
200	35	10	32.8	1.12	2.39	7066
250	35	10	27.2	1.12	2.36	6481
300	35	10	23.3	1.12	2.36	6447
350	35	10	20.8	1.13	2.35	6096

Table 5
Effect of the percentage of modifier on retention time, selectivity, resolution and number of theoretical plates for the DuP 105 diastereoisomers

Pressure (Bar)	Temp. (°C)	Methanol (%)	Retention time of *S,S*-isomer (min)	Selectivity	Resolution	Theoretical plates
300	35	5	116.2	1.13	2.64	7484
300	35	10	23.4	1.12	2.32	6155
300	35	15	10.4	1.11	1.93	5587
300	35	20	6.3	1.10	1.69	5078
300	35	25	4.6	1.09	1.01	4910

2.2. Samples

The drug substance was synthesized at DuPont Merck according to published procedures [1–4].

2.3. Chromatography

Liquid mobile phases and SFC modifiers were prepared from HPLC-grade isopropyl alcohol (IPA), hexane and methanol (all from EM Science, Gibbstown, NJ). SFC grade CO_2 was purchased from Scott Specialty Gases (Plumsteadville, PA). All mobile phase components were used without further purification. A Daicel Chemical Industries Chiracel OD 25 cm × 0.46 mm i.d. column, particle size 5 μm, was purchased from J.T. Baker (Phillipsburg, NJ). Details of the HPLC and SFC methods used are given in Tables 1 and 2. For the LC studies, the concentration of IPA was varied from 10% to 50%. For the SFC studies, pressure was varied

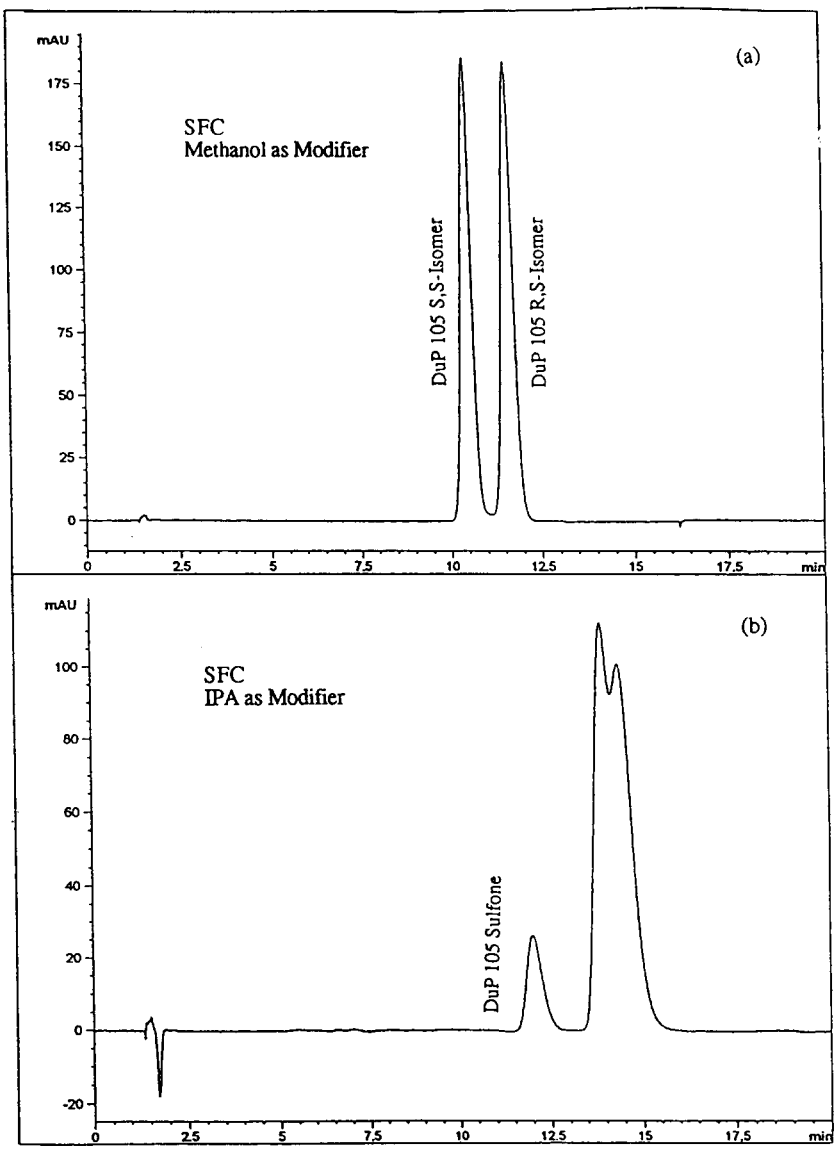


Fig. 3. SFC separation of DuP 105 diastereoisomers using either methanol or isopropanol as modifier. SFC conditions: (a) 300 bar, 35°C, 2 ml min^{-1}, 15% methanol; (b) 300 bar, 35°C, 2 ml min^{-1}, 25% isopropanol.

from 100 to 350 bar, temperature from 35 to 55°C, and percent methanol from 5% to 25%.

2.4. Performance monitoring

Resolution (R), selectivity (α), capacity factor (k), and the number of theoretical plates (N) were calculated using these respective equations: $R = 1.18(T_{R2} - T_{R1})/(P_{W1} + P_{W2})$; $\alpha = k_2/k_1$; $k = t_R - t_0/t_0$; $N = 5.54(t_R W_{1/2})^2$, where T_{R1} and T_{R2} are the retention times of the respective isomers, P_{W1} and P_{W2} are the peak widths at half-height, k_1 and k_2 are the capacity factors for the respective isomers, t_R and t_0 are the retention times of retained and non-retained compounds, and $W_{1/2}$ is peak width at half-height.

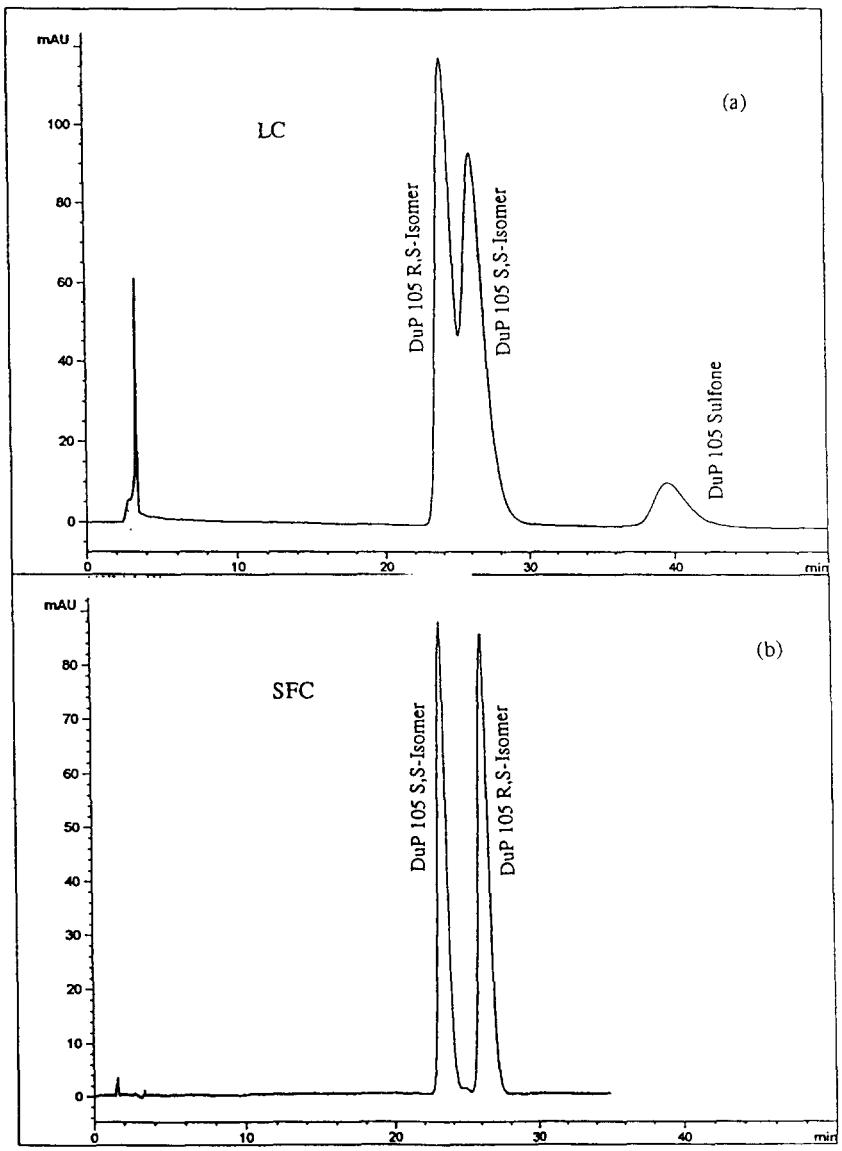


Fig. 4. Chiral SFC and HPLC separation of DuP 105 diastereoisomers. (a) HPLC conditions: 35°C, 1 ml min $^{-1}$, 70:30 hexane:isopropanol, Chiralcel OD 25 cm × 4.6 mm column; (b) SFC conditions: 300 bar, 35°C, 2 ml min $^{-1}$, 10% methanol, Chiralcel OD 25 cm × 4.6 mm column.

3. Results and discussion

3.1. SFC

To optimize the separation of the DuP 105 diastereoisomers and to understand the separation mechanism the effects of temperature, pressure, percent modifier and type of modifier on the SFC separation were studied.

Increasing the temperature decreased resolution of the two diastereoisomers. Each 10°C increase in temperature caused a 15–28% reduction in resolution (Table 3). The higher temperatures decrease the selective interaction time between analytes and the chiral stationary phase, thereby decreasing resolution [7]. Increasing temperature increased efficiency, as indicated by the number of theoretical plates. In this case, the higher tempera-

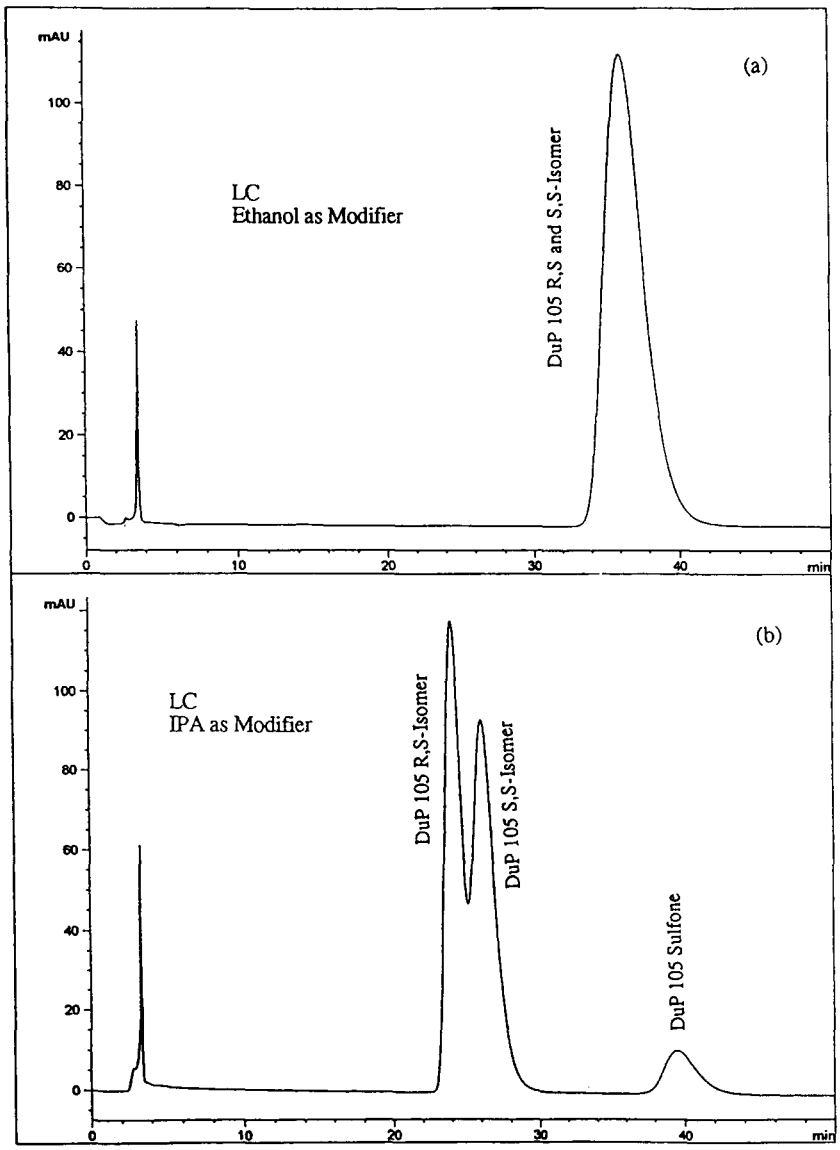


Fig. 5. Chiral HPLC separation of DuP 105 diastereoisomers: (a) 35°C, 1 ml min^{-1}, 80:20 hexane:ethanol, Chiralcel OD 25 cm × 4.6 mm column; (b) 70:30 hexane:isopropanol.

tures allow for a higher rate of mass transfer and therefore increase efficiency [7]. Since temperature has opposite effects on resolution and efficiency, a compromise was made to optimize these two parameters. In this study, the best resolution was achieved at a column temperature of 25°C. For routine use, however, a 35°C column temperature was chosen since cryogenic cooling was not necessary to accurately maintain this temperature.

Increasing pressure had no significant effect on resolution (Table 4). Increasing the pressure did, however, significantly decrease retention times. Retention times decreased from 60 to 20 min by changing the pressure from 100 bar to 350 bar (Table 4). It is likely that the higher pressures increase the distribution of the solute in the mobile phase and therefore decrease retention time. Since resolution is not significantly

decreased with increasing pressure, higher pressures can be used to decrease retention times without sacrificing resolution.

Increasing the concentration of modifier decreased both resolution and retention time (Table 5). Resolution decreased linearly as the percent modifier increased from 10 to 20% methanol modifier but then decreased more rapidly on going from 20% to 25% methanol modifier (Table 5). This effect of modifier on resolution can be explained in terms of adsorption of the methanol into the stationary phase such that the high methanol concentration saturates the stationary phase and prevents solute interaction.

The type of mobile phase modifier used had a pronounced effect on resolution. Changing from methanol to IPA caused a significant decrease in resolution (Fig. 3). Considering the two proposed separation mechanisms for the Chiralcel OD stationary phase, i.e. steric fit discrimination within the chiral cavity and/or formation of transient diastereomeric complexes through hydrogen bonding interactions [9], it is likely that the branched chain alcohol IPA blocks access to the chiral cavity more than the smaller linear alcohol methanol and therefore decreases resolution [9].

Table 6
Day-to-day injection precision for the quantitation of the *S,S*-DuP 105 isomer

Injection	Day 1	Day 2	Day 3
	% *S,S*-isomer	% *S,S*-isomer	% *S,S*-isomer
1	46.65	46.70	46.78
2	46.52	46.58	46.68
3	46.65	46.50	46.56
4	46.59	46.62	46.40
5	46.83	46.70	46.35
Mean	46.65	46.62	46.55
%RSD	0.25	0.19	0.39
Grand mean	46.61 ± 0.1		

Table 7
Quantitation of the *S,S*-DuP 105 isomer in five lots of DuP 105 drug substance

Isomer	Lot				
	1	2	3	4	5
% *S,S*-	47.49	47.23	47.36	46.70	47.66
% *S,S*-	47.49	47.34	47.40	46.55	47.57
% *S,S*-	47.49	47.43	47.36	46.71	47.51
Mean =	47.49	47.33	47.37	46.65	47.58
%RSD =	0.00	0.21	0.05	0.20	0.17

3.2. LC

If the molecular size of the mobile phase constituents effects resolution, then a more pronounced effect should be seen in the LC mode, in which hexane is used instead of CO_2. Hexane is a larger and a more bulky molecule than CO_2 and should be more difficult to displace from the chiral cavity [9]. Our observations support this, as poorer resolution was obtained using LC (Fig. 4). Changing from a bulky modifier such as IPA to a smaller linear alcohol such as ethanol decreased resolution (Fig. 5), which is opposite to the effect seen in SFC. Also, the order of elution of the isomers in LC is reversed compared with that seen with SFC (Fig. 5). This result suggests a different retention mechanism for the enantiomers of DuP 105 in LC and SFC. Different separation mechanisms between SFC and LC have been noted by others for some compounds [9].

Changing from LC to SFC conditions necessitated that the Chiralcel OD column equilibrated at ambient temperature and pressure after flushing the column with the SFC mobile phase. Continuous flushing of the column with the SFC mobile phase did not improve separation. It was only after overnight equilibration under ambient conditions that resolution of the diastereoisomers was achieved. This equilibration time seemed necessary to fully solvate the stationary phase or possibly to allow the polymer to swell. Once this equilibration was achieved, the column proved efficient and reproducible from day to day.

3.3. Validation of SFC method

Day-to-day injection precision was excellent. The RSD between injections for SFC was sub-

stantially less than 1% (Table 6). Response was linear over the range tested from 0.04 to 1.00 mg ml^{-1} with a correlation coefficient of 0.999. Using SFC, five different lots of DuP 105 were assayed. The variation of the *S,S*-isomer content was less than 1% (Table 7), indicating good control of the synthetic process.

4. Conclusion

Based on the results presented in this paper and by others [6–13], SFC can offer better selectivities than LC for some compounds. Considering the high cost of these chiral columns, the capability of using them under either LC of SFC conditions is attractive, especially when the order of elution can be reversed based on the technique used.

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