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Short communication

# Chiral separation of MDL 73,005EF enantiomers using an $\alpha_1$-acid glycoprotein column

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**Abstract**

The enantiomeric separation of MDL 73,005EF (racemic mixture of two enantiomers) has been accomplished using a Chiral AGP ($\alpha_1$-acid glycoprotein) column. The enantiomers are baseline resolved with a runtime of less than 10 min. This separation is used to quantitate the enantiomers present in bulk drug samples, tabletted formulations, and drug used in pharmacological and toxicological studies. Variables found to have an effect on the enantiomeric separation were studied and include: mobile phase ionic strength, type and concentration of organic modifier added to the mobile phase, mobile phase pH, column temperature and the amount of analyte injected. The enantiomeric separation was optimized on the Chiral AGP column based on the effects that each variable had on the separation. Calibration curves for a standard were linear over a range of 0.24 to 61.2 $\mu$g/g with a correlation coefficient of better than 0.999. A detection limit of 0.012 $\mu$g/g and a quantitation limit of 0.24 $\mu$g/g were also found.

## 1. Introduction

The separation of enantiomers has become an important area in the pharmaceutical industry. The reasons for the separation and quantitation of enantiomers in a racemic mixture are being addressed by the regulatory agencies of the USA, Japan and the European Economic Community [1–5]. Areas where a chiral separation must be used for a racemic mixture are: bulk drug stability, drug product stability, pharmacological, toxicological and pharmacokinetic studies.

MDL 73,005EF, 8-azaspiro[4,5]decane-7,9-dione-8-(2- {[(2,3-dihydro-1,4-benzodioxin-2-yl)-methyl]amino}ethyl) monomethanesulfonate (Fig. 1), is a 5-$HT_{1A}$ (5-hydroxytryptamine) partial agonist based on the pharmacological activities in animal models and is being evaluated for the treatment of anxiety in man [6–8]. Compounds which act as partial 5-$HT_{1A}$ agonists represent a new class of anxiolytics devoid of the sedative and muscle relaxant effects that are observed with benzodiazepines [9]. This compound was developed based on the rational design of 5-$HT_{1A}$ receptor ligands using computer-assisted molecular modeling.

The ability to separate and quantitate the enantiomers present in a racemic mixture is important in pharmaceutical products, especially in the areas of both bulk drug and formulation stability and purity [1]. The chiral separation of

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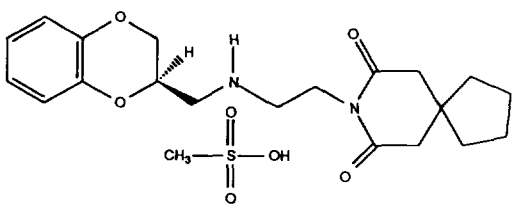
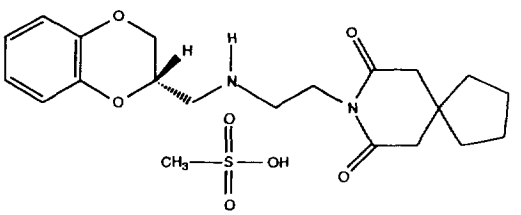


MDL 74,217EF



MDL 73,450EF

Fig. 1. Structures of MDL 73,005EF enantiomers.

the enantiomers present in MDL 73,005EF has been accomplished. The separation employs a commercially available protein column (Chiral AGP) that is composed of an $\alpha_1$-acid glycoprotein which is covalently bound to silica gel. A description of this column and its stability under various mobile phase conditions has been described elsewhere [10–14].

This paper will discuss the influence of various mobile phase parameters on the enantiomeric separation of MDL 73,005EF. An optimized separation for the enantiomers was developed based on the data that was collected. Calibration data, detection and quantitation limits are also presented.

## 2. Experimental

### 2.1. Reagents and instrumentation

MDL 73,005EF, MDL 73,450EF and MDL 74,217EF (methanesulfonate salt) were obtained from Marion Merrell Dow Research Institute (Cincinnati, OH, USA). Acetonitrile, methanol, isopropanol, tetrahydrofuran and ethanol (HPLC grade) were obtained from Burdick and Jackson (Muskegon, MI, USA). Phosphoric acid, sodium dibasic phosphate, sodium hydroxide and triethylamine were obtained from Mallinckrodt (Paris, KY, USA). HPLC-grade water was obtained by passing deionized water through a Nanopure II water-purification system (Barnstead, Dubuque, IA, USA). The instrumentation consisted of a Spectra-Physics 8800 pump, Spectra-Physics 8875 autosampler, Spectra-Physics Spectra 100 UV detector (Fremont, CA, USA), Fiatron column heater (Oconomowoc, WI, USA). The Chrom Tech Chiral AGP column (100 × 4.0 mm, 5 μm) was purchased from ASTEC (Whippany, NJ, USA).

### 2.2. Procedures

Several formulated standards were prepared at a concentration of 1 mg/g of water. The working standards were prepared by diluting the 1 mg/g standards with water. A sample size of 2.5 μg/g was typically used for all of the studies. A flow-rate of 1.0 ml/min was used for all separations with UV detection at 210 nm. A column temperature of 30°C was used with an injection volume of 20 μl.

## 3. Results and discussion

The mobile phase parameters affecting the retention and resolution of the MDL 73,005EF enantiomers on the Chiral AGP column are: the type and concentration of organic modifier, ionic strength, mobile phase pH and column temperature. The amount of analyte injected into the chromatographic system also influenced enantiomer retention times. Each of these parameters were studied to evaluate their influence on the separation of the MDL 73,005EF enantiomers.

### 3.1. Organic modifier

The concentration of organic modifier in the mobile phase has a profound effect on the retention and resolution of enantiomers. Fig. 2 shows the influence of ethanol concentration on enantiomer retention. As the concentration of

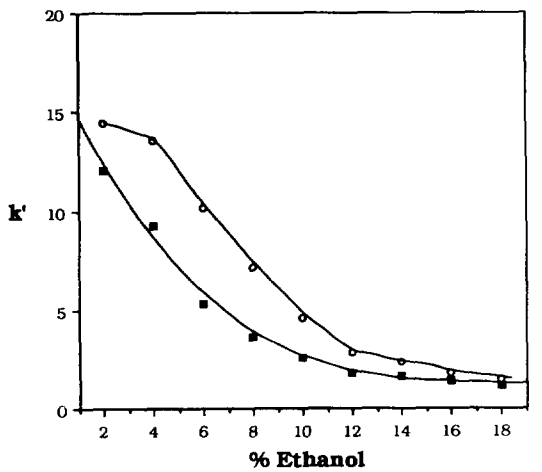



Fig. 2. Effect of ethanol concentration. Mobile phase: 50.0 m*M* $NaH_2PO_4$, pH 5.0, ethanol–water. ■ = MDL 74,217; ○ = MDL 73,450.

ethanol in the mobile phase was increased, analyte retention decreased. This is consistent with previous reports for other analytes on the Chiral AGP column [10].

Other organic modifiers were also studied and include: acetonitrile, isopropanol and methanol. The amount of organic modifier added to the mobile phase was adjusted so that the retention times of the enantiomers were similar. Table 1 compares the different organic modifiers for resolution, tailing factor and column efficiency. (The USP23 NF18 methods [15] were used for all calculations involving resolution, tailing factors and column efficiencies.) These data shows that methanol provides the best selectivity, resolution and peak shape for the two enantiomers.

### 3.2. Mobile phase ionic strength

The ionic strength of the mobile phase generally has an effect on enantiomer retention. Several different effects of ionic strength have been reported for the retention of enantiomers on a Chiral AGP column. Enantiomer retention may increase [10–12], decrease [11,12] or not change [13] with increasing ionic strength, depending on the nature of the analyte.

As the concentration of buffer was increased, retention of the stereoisomers also increased (Fig. 3). The selectivity between the two enantiomers also increased with higher buffer concentrations (Table 2).

### 3.3. Mobile phase pH

The pH of the mobile phase has been shown to have a strong influence on the retention and enantioselectivity of basic, acidic and non-protolytic compounds [14]. Schill et al. [16] reported that retention of enantiomers increased with increasing mobile phase pH, however, the pH range covered was only from 6.1 to 7.0. The effect of lower mobile phase pH values on analyte retention was not investigated. One study involving carboxylic acid enantiomers covered the pH range of 3 to 7.0 and showed that enantiomer retention increased from pH 3 to 4

Table 1
Comparison of different organic modifiers for the separation of MDL 73,450EF and MDL 74,217EF

| Organic modifier[a] | Resolution | Tailing factor MDL 74,217 | *N* | Tailing factor MDL 73,450 | *N* |
|---|---|---|---|---|---|
| A | 4.15 | 1.61 | 1747 | 1.89 | 1267 |
| B | 4.77 | 1.57 | 2029 | 1.73 | 1546 |
| C | 5.56 | 1.38 | 1844 | 1.40 | 1572 |
| D | 5.06 | 1.53 | 1729 | 1.16 | 1783 |

*N* = Efficiency.
[a] A = 5% isopropanol; B = 8.6% acetonitrile; C 27.5% methanol; D = 10% ethanol.

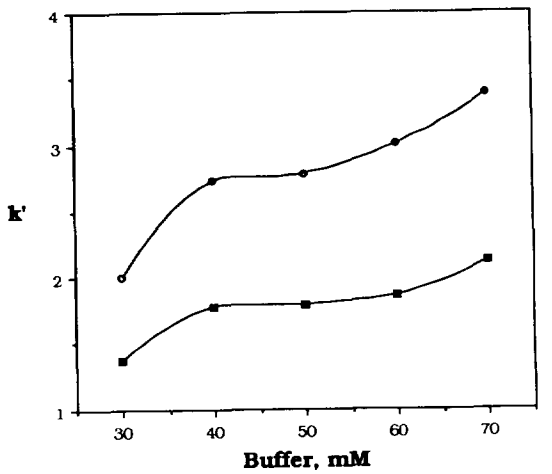



Fig. 3. Effect of ionic strength. Mobile phase: $NaH_2PO_4$, pH 5.0, ethanol–water (12:88). ■ = MDL 74,217; ○ = MDL 73,450.

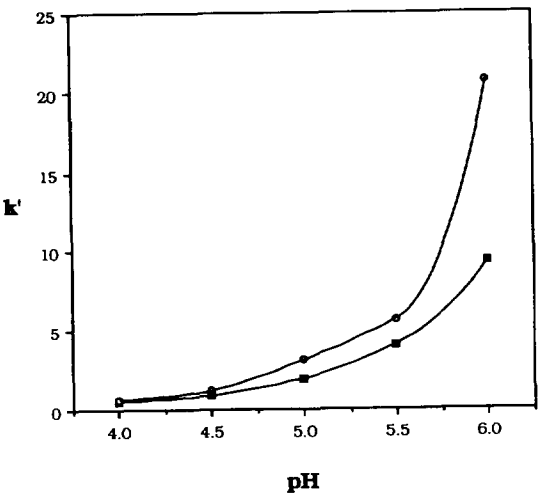



Fig. 4. Effect of mobile phase pH. Mobile phase: 50.0 m*M* $NaH_2PO_4$, ethanol–water (12:88, v/v). ■ = MDL 74,217; ○ = MDL 73,450.

and then decreased as the mobile phase pH was raised [10]. The change in retention was attributed to the hydrophobicity and ionization characteristics of the analytes and the stationary phase over the pH range studied.

Fig. 4 shows the effect of pH on the retention of the enantiomers. As the pH was raised from 4.0 to 6.0, retention of the enantiomers increased. Resolution and selectivity also increased with higher mobile phase pH, however, resolution of the enantiomers and peak shape were optimal at a mobile phase pH of 5.0. The selectivities between the two enantiomers at different mobile phase pH values is shown in Table 3.

### 3.4. Column temperature

Column temperature has been reported to influence enantiomer retention, enantioselectivity and resolution [10,14]. When the column temperature was increased, enantioselectivity decreased while column efficiency increased. The

Table 2
Selectivity between the MDL 73,005EF enantiomers at different mobile phase buffer concentrations

| Buffer concentration (m*M*) | *k'* (capacity factor) | | *α* |
|---|---|---|---|
| | MDL 74,217 | MDL 73,450 | |
| 30 | 1.37 | 2.00 | 1.46 |
| 40 | 1.78 | 2.74 | 1.54 |
| 50 | 1.79 | 2.79 | 1.56 |
| 60 | 1.86 | 3.01 | 1.62 |
| 70 | 2.12 | 3.38 | 1.60 |

Table 3
Selectivity between the MDL 73,005EF enantiomers at different mobile phase pH values

| Mobile phase pH | *k'* | | *α* |
|---|---|---|---|
| | MDL 74,217 | MDL 73,450 | |
| 4.0 | 0.46 | 0.56 | 1.46 |
| 4.5 | 0.86 | 1.16 | 1.34 |
| 5.0 | 1.91 | 3.07 | 1.61 |
| 5.5 | 4.05 | 5.66 | 1.40 |
| 6.0 | 9.34 | 20.7 | 2.22 |

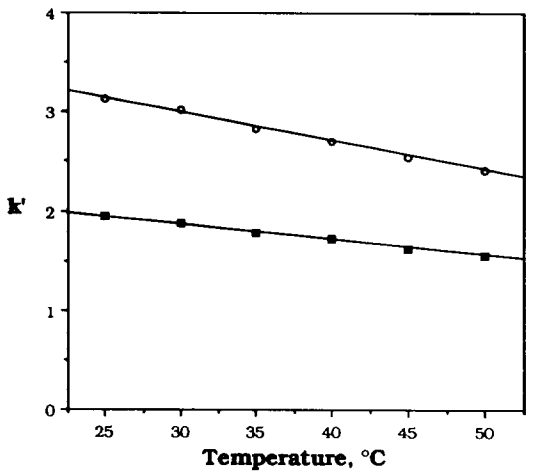



Fig. 5. Effect of column temperature. Mobile phase: 50.0 m*M* $NaH_2PO_4$, ethanol–water (12:88, v/v). ■ = MDL 74,217; ○ = MDL 73,450.

increase in efficiency has been attributed to faster transfer kinetics between the stationary phase and the enantiomers [10,14].

Similar results were found for the two 73,005EF enantiomers, where retention and selectivity decreased with increasing temperature and peak shape improved (Fig. 5). A column temperature of 30°C was chosen based on peak shape, retention, selectivity, resolution and maximum column life. The selectivity between the enantiomers was found to change only slightly as the temperature was decreased, with an $\alpha$ value of 1.62 at 25°C and 1.55 at 50°C.

### 3.5. Amount of analyte injected

The amount of sample injected onto the Chiral AGP stationary phase influenced both peak tailing and resolution (Table 4). Peak tailing and resolution were improved when smaller amounts of analyte were injected. Selectivity was found to decrease with increasing amounts of analytes that were injected ($\alpha$ value of 2.31 at 0.55 $\mu$g/g of analyte injected to 2.25 at 44.5 $\mu$g/g analyte injected). Therefore, the AGP column was sensitive to the amount of sample injected and this should be taken into account when determining how much sample may be chromatographed.

Table 4
Effect of sample loading on peak tailing and resolution

| Analyte ($\mu$g/g) | Peak tailing[a] (column efficiency[b]) | | $\alpha$[c] | Resolution[d] |
|---|---|---|---|---|
| | MDL 74,217 | MDL 73,450 | | |
| 0.44 | 1.28 (1606) | 1.20 (1543) | 2.33 | 7.2 |
| 0.55 | 1.48 (1435) | 1.20 (1584) | 2.31 | 7.1 |
| 1.10 | 1.34 (1533) | 1.20 (1699) | 2.32 | 7.3 |
| 2.25 | 1.29 (1570) | 1.20 (1599) | 2.31 | 7.2 |
| 4.45 | 1.31 (1589) | 1.29 (1477) | 2.31 | 7.0 |
| 8.9 | 1.35 (1589) | 1.37 (1463) | 2.30 | 6.9 |
| 11.1 | 1.38 (1564) | 1.41 (1395) | 2.30 | 6.8 |
| 22.2 | 1.48 (1454) | 1.67 (1103) | 2.28 | 6.1 |
| 31.1 | 1.58 (1360) | 1.81 (964) | 2.27 | 5.7 |
| 44.5 | 1.73 (1222) | 2.10 (769) | 2.25 | 5.2 |

[a] Calculated using USP XXIII peak tailing method.
[b] Calculated using USP XXIII column effeciency method.
[c] Calculated using USP XXIII selectivity method.
[d] Calculated using USP XXIII resolution method.

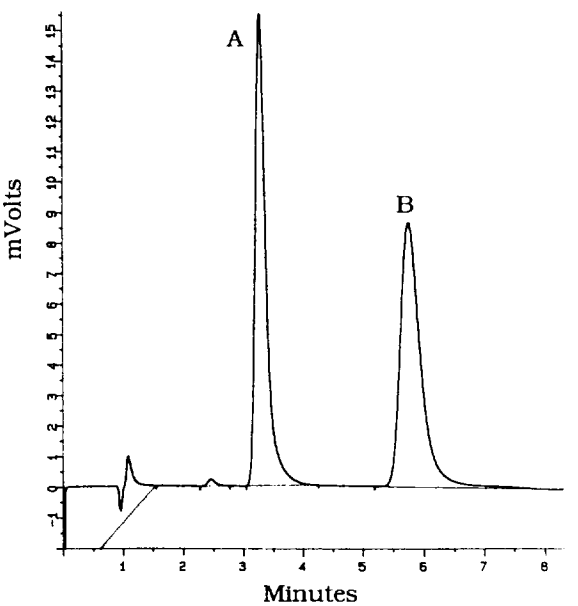



Fig. 6. Optimized separation of MDL 73,005EF enantiomers. Mobile phase: 50.0 m*M* $NaH_2PO_4$, pH 5.0, methanol–water (27.5:72.5, v/v); column temperature: 30°C. Peaks: A = MDL 74,217; B = MDL 73,450.

### 3.6. Separation

The optimized separation for the enantiomers of MDL 73,005EF is shown in Fig. 6. The mobile phase was composed of $NaH_2PO_4$ (50 m*M*, pH 5.0 adjusted with NaOH) and a solvent composition of methanol–water (27.5:72.5). A flow-rate of 1.0 ml/min and a column temperature of 30°C were used. The enantiomers were baseline resolved with a runtime of 10 min.

### 3.7. Calibration curves and sample loading

Calibration curves were established over the range 0.24 to 61.2 μg/g. Correlation coefficients of greater than 0.999 were found, with a detection limit of 0.012 μg/g (3:1 signal to noise) and a quantitation limit of 0.24 μg/g. A minimum of four injections of each standard was performed. The method precision was 1.0% while the system precision was 0.28%. This chromatographic system was rugged and reliable with minimal changes in retention or resolution occurring after several hundred injections.

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