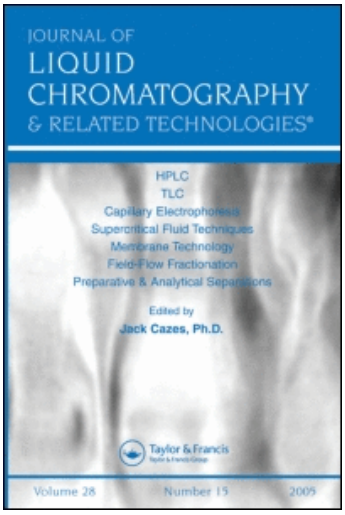


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# BUPROPION HYDROCHLORIDE: THE DEVELOPMENT OF A CHIRAL SEPARATION USING A CHIRAL AGP COLUMN

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# BUPROPION HYDROCHLORIDE: THE DEVELOPMENT OF A CHIRAL SEPARATION USING A CHIRAL AGP COLUMN

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## ABSTRACT

A chiral high-performance liquid chromatographic separation was developed for bupropion active ingredient. The mobile phase parameters that may influence the separation were studied and included: mobile phase pH, type and concentration of organic modifier, type and concentration of buffer, amount of analyte injected, and column temperature. The optimized chiral separation baseline resolved the two enantiomers in less than 10 minutes.

Method validation was also performed for the separation. The method was found to be linear over a range of 0.5 $\mu$g/g to 99.8 $\mu$g/g with a correlation coefficient greater than 0.999. The limit of detection was determined to be 0.25 $\mu$g/g while the limit of quantitation was found to be 0.5 $\mu$g/g.

Method precision was found to be 0.8% and 1.9% for the two enantiomers, while the system precision was found to be 0.4% and 0.5% for the two enantiomers.

## INTRODUCTION

Many new developments in the separation and analysis of chiral compounds have taken place over the past several years. One protein chiral stationary phase that has become popular is the $\alpha_1$-acid glycoprotein (Chiral AGP) column. The globular glycoprotein is composed of a single 181 amino acid residue polypeptide, two disulfide bonds, and five carbohydrate chains. The protein has an isoelectric point of 2.7 and a molecular weight of 40–44 kDa (1), with about 40 to 45% of the total mass attributed to the carbohydrate chains situated outside of the protein (2).

The predominant interactions between a drug and the AGP column appear to be hydrophobic (3–5). However, some investigators have proposed that electrostatic (6), van der Waal's (7), and hydrogen bonding (8) interactions may also participate in the retention process. Reports have shown that the structural properties of AGP are strongly dependent on the environmental pH (9), and that pH will influence the dissociation of the amino acid moieties. This, in turn, may influence any hydrogen bonding that may take place between the AGP, an analyte, and the organic solvent.

The AGP column has been used for the separation of basic, neutral, and acidic analytes, which suggests that multiple binding sites may be present (10–17). The enantioselectivity of this protein-based column may be due to the reversible conformational changes that are influenced by mobile phase pH and modifiers (13,14,18). Varying the mobile phase composition (pH, modifiers, and buffers) has provided separations that are influenced by apolar interactions (10,12,19,29), hydrogen bonding (21), ionic bonding, and ion pairing (11,12,20). Enantioselectivity may be the result of solute interactions with a hydrophobic core of the immobilized AGP (1,12,20). However, the exact nature of the enantioselective interactions between the analyte and the AGP is not completely understood.

In this study, a chiral separation was developed for bupropion. Bupropion, (*rac*)-2-*tert*-butylamino-3′-chloropropiophenone, is a second-generation, clinically efficacious antidepressant agent that was first marketed in the United States in 1989 (22,23). Bupropion is an aminoketone that contains a chiral center and is marketed as a racemic mixture. Many drugs that contain a chiral center are marketed as racemic mixtures even though the pharmacological activity resides with only one enantiomer (13,16,22).

Very little has been published on the chiral separation of bupropion enantiomers (23). Therefore, a thorough study was done to develop and optimize a chiral separation for bupropion active ingredient on a chiral AGP column. The parameters that were found to influence the enantiomeric separation of bupropion on the AGP column were studied. The results that were found for each mobile phase parameter as well as the optimized enantiomeric separation are discussed.





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## EXPERIMENTAL

### Reagents and Instrumentation

Bupropion hydrochloride was purchased from Sigma (St. Louis, MO). Citric acid, sodium hydroxide, methanol, ammonium acetate, and acetonitrile were purchased from Fisher Scientific (Fairlawn, NJ, USA). Ethanol was purchased from Quantum Chemical Company (Newark, NJ, USA). HPLC-grade water was obtained by passing de-ionized water through a Barnstead Nanopure™ II water purification system (Dubuque, IA, USA). The instrumentation consisted of a Thermo Separations SCM1000 degasser, P4000 quaternary pump, AS3000 variable loop autosampler with built-in column oven, UV6000 photodiode array detector, and ChromQuest Data System (Thermo Separation Products, San Jose, CA, USA).

The Chromtech Chiral AGP column was purchased from Regis Chemical Company (Morton Grove, IL, USA).

### Procedures

Several standards were prepared at a concentration of 1 mg/g in Nanopure™-grade water. The working standards were prepared by diluting the 1 mg/g standards with Nanopure™-grade water. A sample size of about 100 $\mu$g/g (ppm) was typically used for all studies. A flowrate of 1.0 mL/min was used for all separations with (UV) detection at 230 nm, a column temperature of 35°C, and an injection volume of 50 $\mu$L, except where noted.

## RESULTS AND DISCUSSION

The chromatographic parameters that had a significant effect on the retention and resolution of bupropion (Fig. 1) enantiomers on the $\alpha_1$-acid glycoprotein (Chiral AGP) column were studied. The parameters studied include: the type and concentration of organic modifier, mobile phase pH, the type and concentration of buffer used, column temperature, and the amount of analyte injected into the chromatographic system. The effect that each parameter had on the separation is discussed.

### Effect of Organic Modifier

It has been documented that the organic modifier concentration will influence retention and resolution of enantiomers on the AGP column (9,12,16). Figure 2





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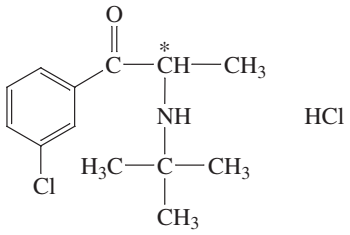


Bupropion, (*rac*)-2-*tert*-butylamino-3′-chloropropiophenone hydrochlorid

***Figure 1.*** Structure of bupropion.



shows the influence that the mobile phase concentration of methanol had on bupropion enantiomer retention. As the concentration of methanol in the mobile phase was increased, a corresponding decrease in retention was observed.

Table 1 shows the effect of the mobile phase methanol concentration on resolution and selectivity between the bupropion enantiomers. Resolution and selectivity between the enantiomers was found to decrease as the methanol concentration was increased. When the concentration of methanol was greater than 40%, little or no resolution was observed. These results were similar to what had been previously published (9,12,16).

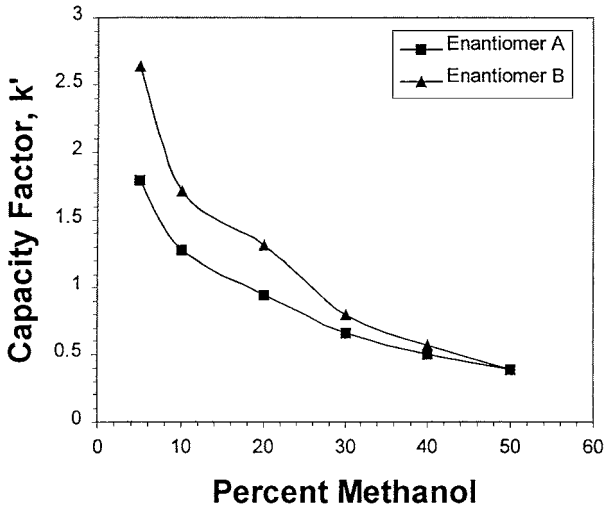


***Figure 2.*** The effect of methanol concentration on bupropion retention. (Mobile Phase: 20 mM citric acid, pH 5.5, methanol).



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***Table 1.*** The Effect of Methanol Concentration on Bupropion Enantiomer Resolution and Selectivity. (Mobile Phase: 20 mM Citric Acid, pH 5.5, Methanol)

| | Percent Methanol | | | | | |
|---|---|---|---|---|---|---|
| | 5.0 | 10.0 | 20.0 | 30.0 | 40.0 | 50.0 |
| Resolution | 1.40 | 1.40 | 1.31 | 0.74 | 0.5 | 0.0 |
| Selectivity | 1.49 | 1.47 | 1.38 | 1.21 | 1.14 | 0.0 |

The optimal concentration of methanol was found to be 10% (mobile phase: 20 mM citric acid, pH 5.5). However, two other organic modifiers, acetonitrile and ethanol, were studied to determine if either would baseline resolve the enantiomers. Acetonitrile did not adequately separate the two enantiomers, whereas a mobile phase that contained 2.5% ethanol separated the bupropion enantiomers; however, baseline resolution was not obtained. Figure 3 shows the separation obtained under the best mobile phase conditions that were studied using ethanol (2.5%) as the organic modifier (mobile phase: 20 mM citric acid, pH 5.5).

Figure 4 shows the best separation that was obtained when methanol was used, and was the only organic modifier studied that baseline resolved both enantiomers on the chiral AGP column (mobile phase: 20 mM citric acid, pH 5.5, 10% methanol). Lower concentrations of methanol showed improved resolution;

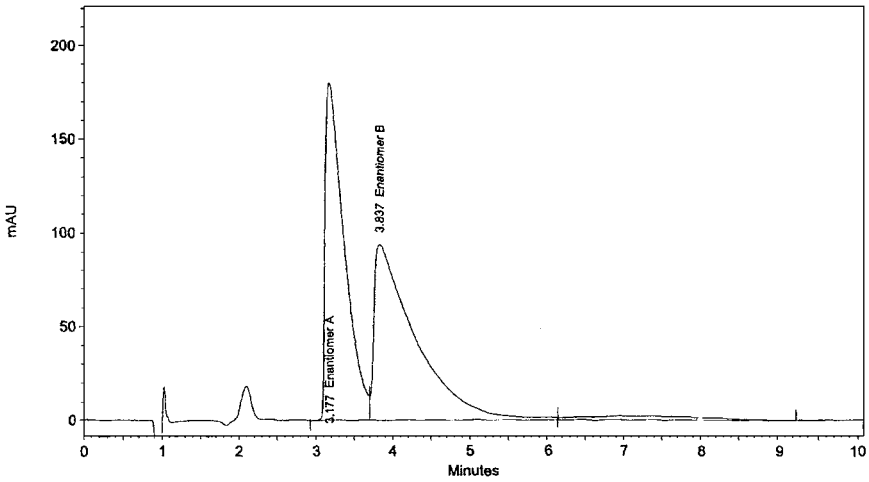


***Figure 3.*** The enantiomeric separation of bupropion using ethanol in the mobile phase. (Mobile Phase: 20 mM citric acid, pH 5.5, 2.5% ethanol).

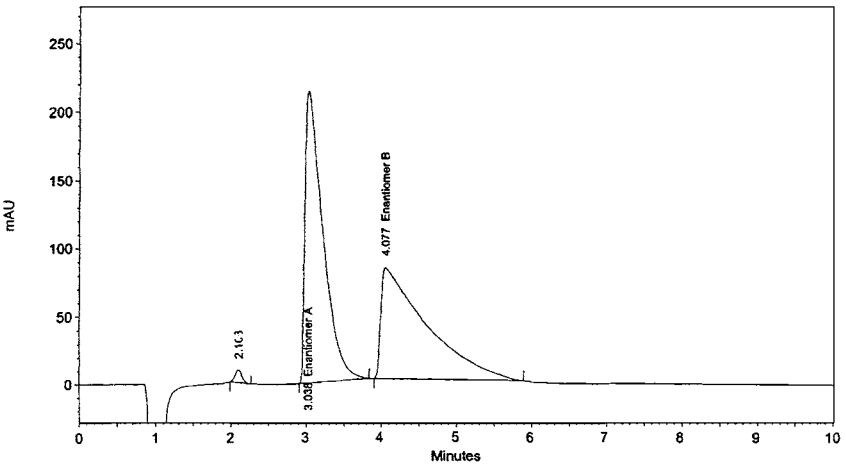


***Figure 4.*** The enantiomeric separation of bupropion using methanol in the mobile phase. (Mobile Phase: 20 mM citric acid, pH 5.5, 10% methanol).

however, excessive peak tailing and broadening were observed. Therefore, methanol was the organic modifier used for all additional studies.

## The Effect of Mobile Phase pH

Mobile phase pH plays a significant role in the retention and separation of enantiomers on a protein-based column. The isoelectric point of the $\alpha_1$-acid glycoprotein is 2.7 when measured in a phosphate buffer (13). Therefore, it should be expected that the stationary phase should have a net negative charge above this pH. Analytes that are acidic in nature have been shown to increase in retention as the mobile phase pH is decreased, whereas basic compounds decrease in retention (24,25). Retention of basic analytes at higher pH values is probably due to ionic bonding between the fully ionized analyte and the anionic groups in the binding sites of the protein (14). As the pH is decreased towards the isoelectric point of the protein, the protein becomes less anionic in nature and the result is lower retention of cationic drugs.

Figure 5 shows the effect that the mobile phase pH had on bupropion enantiomer retention. The results obtained were similar to that previously reported (14): retention increased as the mobile phase pH was raised from 4.5 to 7 (mobile phase: 20 mM citric acid, 10% methanol). It is interesting to note, that retention increased between pH 3.5 and 4.0 and then decreased at pH 4.5 before retention continued to increase with increasing mobile phase pH. This is likely due to ionic changes in the protein itself.

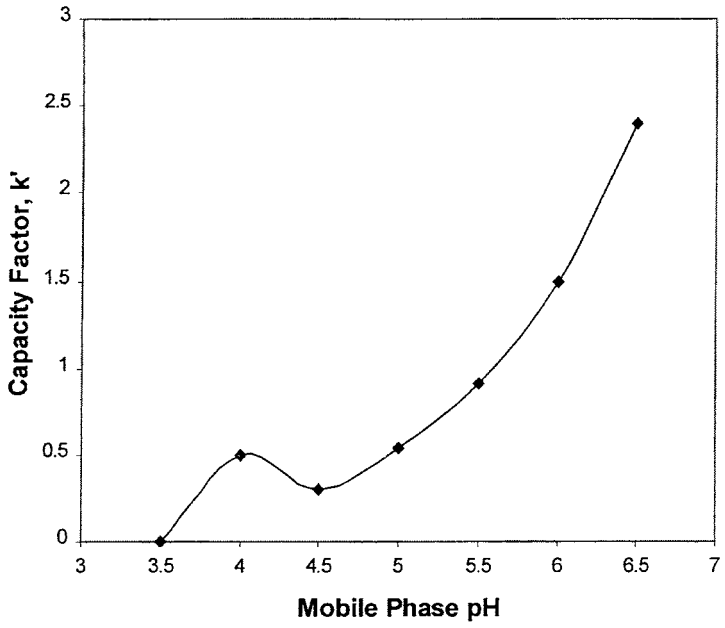


***Figure 5.*** The effect of mobile phase pH on bupropion retention. (Mobile Phase: 20 mM citric acid, pH, 10% methanol).

Table 2 shows how the mobile phase pH affected resolution between the two enantiomers. At a mobile phase pH of 5.0, the enantiomers were partially separated but not baseline resolved. Resolution between the enantiomers reached a maxima at pH 5.5. As the mobile phase pH was raised above 5.5, resolution was found to decrease between the two enantiomers even though retention increased. This is due to significant peak tailing for the enantiomers as the pH was increased. Therefore, a mobile phase pH of 5.5 was chosen since this pH would provide good retention, selectivity, and resolution for the bupropion enantiomers.

***Table 2.*** The Effect of Mobile Phase pH on Bupropion Enantiomer Resolution. (Mobile Phase: 20 mM Citric Acid, pH, 10% Methanol)

| | Mobile Phase pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 |
| Resolution | 0.0 | 0.6 | 0.0 | 0.64 | 1.31 | 1.15 | 1.08 |

### The Effect of Citric Acid Concentration

Enantiomeric retention on a chiral column may be influenced by the concentration of buffer in the mobile phase, depending on the types of interaction that take place between an analyte and the stationary phase. It has been reported that retention and selectivity of enantiomers may be influenced by the concentration of buffers (21,26).

Figure 6 shows the effect of citric acid concentration on enantiomer retention. The citric acid concentration ranged from 1.0 to 100 mM with a mobile phase pH of 5.5 and 10% methanol. The enantiomers showed a significant decrease in retention from 1.0 to about 10 mM of citric acid, and then showed only a slight decrease in retention. This indicates that the major retention mechanism at this pH is ion exchange (14). Table 3 shows the resolution and selectivity data for this study. Resolution and selectivity did not change significantly over the citric acid concentration range studied.

### The Effect of Column Temperature

The separation of enantiomers on a protein-based column was shown to be influenced by column temperature (13,24). Typically, a slight decrease in resolution

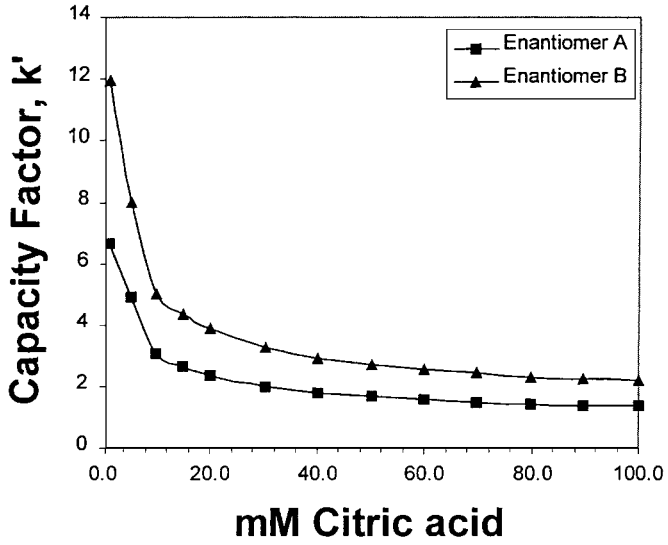


***Figure 6.*** The effect of citric acid concentration on bupropion retention. (Mobile Phase: citric acid, pH 5.5, 10% methanol).

***Table 3.*** The Effect of Citric Acid Concentration on the Resolution and Selectivity of Bupropion Enantiomers. (Mobile Phase: mM Citric Acid, pH 5.5, 10% Methanol)

| | Citric Acid (mM) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 5.0 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| Rs | 1.7 | 1.7 | 1.7 | 1.6 | 1.6 | 1.4 | 1.3 | 1.4 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 |
| $\alpha$ | 1.8 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |

Rs-Resolution.
$\alpha$-Selectivity.



occurs when the temperature is increased, with a 20 to 30% decrease in retention for each 10°C increase. However, these enantioselectivity changes are usually small (24).

The effect that column temperature had on enantiomer retention, resolution, and selectivity was studied over the range of 30 to 50°C when a mobile phase of 20 mM citric acid, pH 5.5, and 10% methanol were used. Figure 7 shows the results that were found. Enantiomeric retention was found to decrease with increasing temperature, whereas resolution and selectivity were found to increase (Table 4). This result was unexpected and is the opposite of what has been observed for other basic compounds (13,24). The improvement in resolution is the result

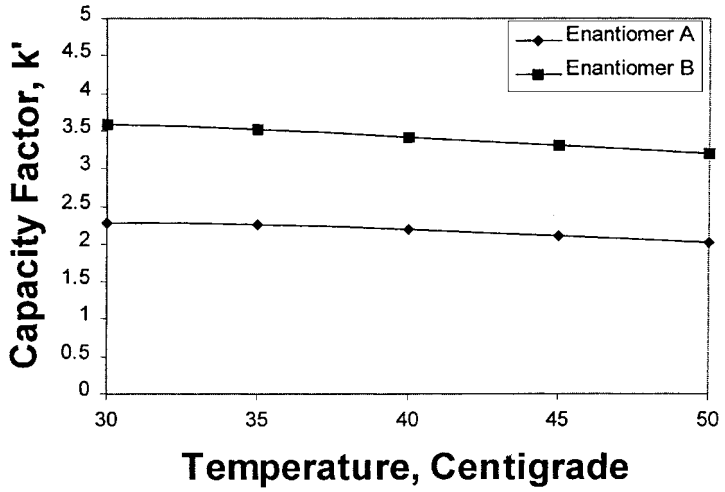


***Figure 7.*** The effect of column temperature on bupropion retention. (Mobile Phase: 20 mM citric acid, pH 5.5, 10% methanol).

***Table 4.*** The Effect of Column Temperature on Bupropion Enantiomer Resolution and Selectivity. (Mobile Phase: 20 mM Citric Acid, pH 5.5, 10% Methanol)

| | Column Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | 30 | 35 | 40 | 45 | 50 |
| Resolution | 1.66 | 1.75 | 1.80 | 1.88 | 1.95 |
| Selectivity | 1.55 | 1.57 | 1.59 | 1.61 | 1.61 |

of decreased peak tailing and improved peak shape due to faster transfer kinetics between the stationary phase and the enantiomers.

## Effect of Amount of Bupropion Injected

Table 5 shows the effect that the amount of bupropion injected had on enantiomeric resolution and selectivity, while Figure 8 shows how the amount of enantiomer injected affected retention. Resolution was fairly consistent over the range of 1.25 to 25 $\mu$g/g. Above 25 $\mu$g/g, resolution decreased significantly. Enantioselectivity showed a similar pattern as well. Resolution and selectivity, as well as peak tailing, were found to be the best at lower levels of bupropion. This indicates that the chiral AGP column is sensitive to the amount of sample injected and this should be taken into account when determining how much analyte may be chromatographed.

## Calibration Curves and System Precision

Calibration curves were established over the range of 0.50 to 100.0 $\mu$g/g (ppm) of each bupropion enantiomer: A correlation coefficient of greater than

***Table 5.*** The Effect of the Amount of Bupropion Injected on Enantiomeric Resolution and Selectivity. (Mobile Phase: 20 mM Citric Acid, pH 5.5, 10% Methanol)

| | Enantiomer Resolution and Selectivity ppm of Bupropion Injected | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.25 | 2.50 | 5.00 | 10.0 | 25.0 | 49.9 | 99.8 | 200 | 499 |
| Rs | 13.4 | 3.01 | 3.58 | 3.62 | 2.87 | 2.29 | 1.63 | 1.26 | 0.9 | 0.6 |
| $\alpha$ | 1.86 | 1.83 | 1.80 | 1.73 | 1.73 | 1.65 | 1.58 | 1.49 | 1.39 | 1.25 |

Rs-Resolution.
$\alpha$-Selectivity.





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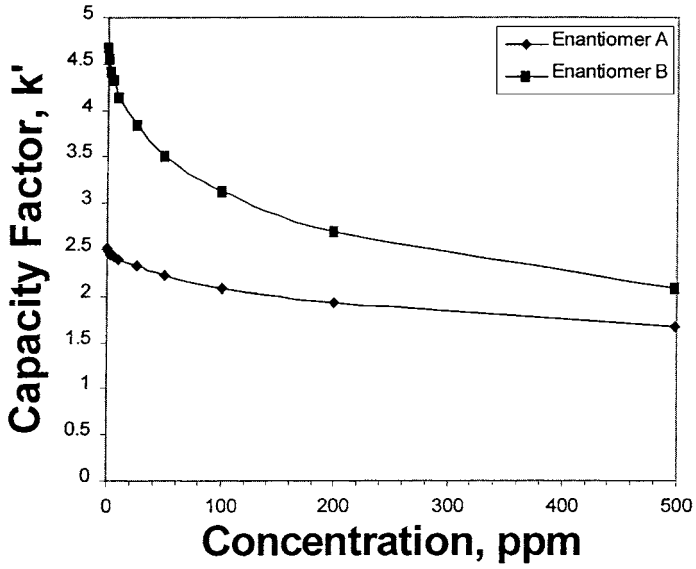


***Figure 8.*** The effect of the amount of bupropion injected on enantiomer retention. (Mobile Phase: 20 mM citric acid, pH 5.5, 10% methanol).

0.999 was observed for each enantiomer, indicating that the system is linear over this range. The limit of detection (3:1, signal: noise) was found to be 0.25 $\mu$g/g, while the limit of quantitation (10:1, signal: noise) was determined to be 0.50 $\mu$g/g.

Method and system precision were determined for the bupropion enantiomers. The system precision was determined by injecting a 53.0 $\mu$g/g standard six times. The %RSD for the peak areas of the six injections was found to be 0.5%. Six separate weighings were done to determine method precision. The %RSD was found to be 0.8 and 1.9% for the two enantiomers, respectively. The system was found to be precise.

## CONCLUSIONS

The chiral separation for the bupropion enantiomers was optimized. The mobile phase was composed of 20 mM citric acid, pH 5.5, that contained 10% methanol. A flow rate of 1.0 mL/min, a column temperature of 35°C, UV detection at 230 nm, and an injection volume of 50 $\mu$L were used. The bupropion enantiomers were baseline resolved with a runtime of less than ten minutes. The method was found to be linear, accurate, and precise.





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