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Bupropion hydrochloride: the development of a chiral separation using an ovomucoid column

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Abstract

The separation of bupropion enantiomers on an ovomucoid stationary phase was investigated. The mobile- and stationary-phase parameters that may influence the separation were identified. The parameters that were studied include: type and concentration of organic modifier, mobile phase pH, ionic strength, type of buffer, and column temperature, as well as the effect that the amount of sample injected had on the separation. The optimized chiral separation baseline-resolved the enantiomers in less than 10 min. Calibration curves for a standard were linear over a range of 0.27–53.0 μg/g (ppm) with a correlation coefficient of 0.999 for both enantiomers. A detection limit of 0.13 μg/g and a quantitation limit of 0.27 μg/g were also found. The system precision of the method was 0.2%.

Keywords: Enantiomer separation; Ovomucoid stationary phases; Stationary phases, LC; Bupropion; Antidepressants

1. Introduction

Bupropion, (*rac*)-2-*tert.*-butylamino-3′-chloropropiophenone, is a second generation clinically efficacious antidepressant agent that was first marketed in the USA in 1989 [1,2]. 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 in only one enantiomer [1].

In a policy guideline presented by the US Food and Drugs Administration (FDA), it was stated that it is essential that a stereospecific assay be used from the beginning of the development of chiral drugs [3,4]. For a drug substance, as well as a drug product, the FDA released a policy statement that requires the submission of either a stereochemically specific identity test and/or a stereochemically selective assay method [5]. If an identity test is to be a specific identity test for good manufacturing practice (GMP) purposes, it must distinguish between enantiomers, or between an enantiomer and the racemate [3,6]. Therefore, chiral chromatography has become an important analytical tool for the separation of enantiomers in a racemic mixture and for determining if the ''inactive'' enantiomer is present in an enantiomerically pure drug or finished product.

Many chiral stationary phases have been developed over the past several years specifically for the separation of racemic mixtures. Protein-bonded stationary phases have become popular for chiral separations due to their direct optical resolution and the wide chiral recognition for enantiomers. Several of the more common protein phases include bovine serum albumin (BSA) [7–10], human serum albumin (HSA) [11,12], α_1-acid glycoprotein (AGP) [13–21], ovomucoid [21–24], and α-chymotrypsin [25]. Re-

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cent publications discuss the retention mechanism of these chiral stationary phases [26,27]. Analyte retention on the protein bases stationary phases was attributed to a mixed mechanism: interactions due to nonselective sites and the interactions due to the enantioselective sites. The protein stationary phases, however, are not very efficient and generally give broad sample peaks with less than 3500 theoretical plates [28].

In this study, an ovomucoid (OVM) column was used for the separation of bupropion enantiomers. OVM has an isoelectric point of 4.5 and a molecular mass of 28 000. The molecule consists of a single 186-amino acid chain divided into three tandem homologous domains by nine disulfide bonds, carbohydrate moieties (four to five glycosylated asparagine residues) and sialic acid moieties which compose 0.5–1.0% of the total mass of the protein [23,29]. Research has shown that the retention mechanism for enantiomers on the OVM column consists of hydrophobic and ionic interactions between the enantiomers and the chiral stationary phase [30]. The OVM column has been shown to provide an effective separation of acidic and basic enantiomers without the need for derivatization. For many separations, small changes in mobile phase pH, column temperature and organic modifier concentration may dramatically influence analyte retention and resolution.

The mobile phase parameters that were found to influence the enantiomeric separation of bupropion on an OVM stationary phase were studied. The results that were found for each mobile phase parameter as well as the optimized enantiomeric separation are discussed.

2. Experimental

2.1. Reagents and instrumentation

Bupropion hydrochloride was purchased from Sigma (St. Louis, MO, USA). Citric acid, sodium hydroxide, methanol, ammonium acetate, and acetonitrile were purchased from Fisher Scientific (Fairlawn, NJ, USA). Ethanol was purchased from Quantum Chemical (Newark, NJ, USA). HPLC-grade water was obtained by passing de-ionized water through a Nanopure II water purification system (Barnstead, 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 150×4.6 mm, 5 μm Ultron ES-OVM chiral column was purchased from MacMod Analytical (Chadds Ford, PA, USA).

2.2. Procedures

Several standard solutions 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 50 μg/g (ppm) was typically used for all studies. A flow-rate of 1.0 ml/min was used for all separations with UV detection at 230 nm. A column temperature of 35°C was used with an injection volume of 25 μl.

3. Results and discussion

The mobile phase parameters that had a significant effect on the retention and resolution of bupropion (Fig. 1) enantiomers on the OVM column were studied. The parameters include: the type and concentration of organic modifier, mobile phase pH, ionic strength, buffer used, and column temperature. The amount of analyte injected into the chromatographic system was also evaluated to determine what effect analyte concentration would have on retention and resolution. Each of these parameters was studied

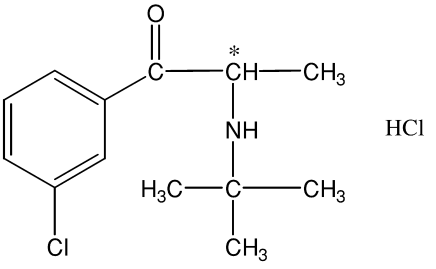

Fig. 1. Structure of bupropion, (*rac*)-2-*tert*.-butylamino-3′-chloropropiophenone hydrochloride.

to evaluate the influence on the separation of bupropion enantiomers.

3.1. Effect of organic modifier

The concentration of organic modifier was found to influence enantiomer retention and resolution. Fig. 2 shows the influence that the mobile phase concentration of ethanol had on bupropion retention. As the concentration of ethanol in the mobile phase was increased, a corresponding decrease in retention was observed. This is consistent with previous reports for other enantiomers separated on the OVM column [29]. Resolution was found to decrease between the enantiomers as the ethanol concentration was increased. When the concentration of ethanol was greater than 10%, little or no resolution was observed while the greatest resolution was when the mobile phase contained 6% ethanol.

The optimal concentration of ethanol was found to be 6% (mobile phase: 40 m*M* citric acid, pH 5.0). However, the bupropion enantiomers were not baseline resolved using ethanol. Two other organic modifiers, acetonitrile and methanol, were studied to determine if either would baseline resolve the enantiomers. Acetonitrile provided results similar to ethanol where the bupropion enantiomers were separated but not baseline resolved.

When methanol was used as the organic modifier, the bupropion enantiomers were baseline resolved. Methanol was the only organic modifier studied that provided baseline resolution for both enantiomers on the OVM column (mobile phase: 40 m*M* citric acid, pH 5.0, 15% methanol). When the concentration of methanol was decreased, resolution improved; however, band broadening and peak tailing were observed. At higher concentrations of methanol the two enantiomers were not baseline resolved. Since methanol was the only organic modifier that baseline resolved the enantiomers, only methanol was used for all additional studies.

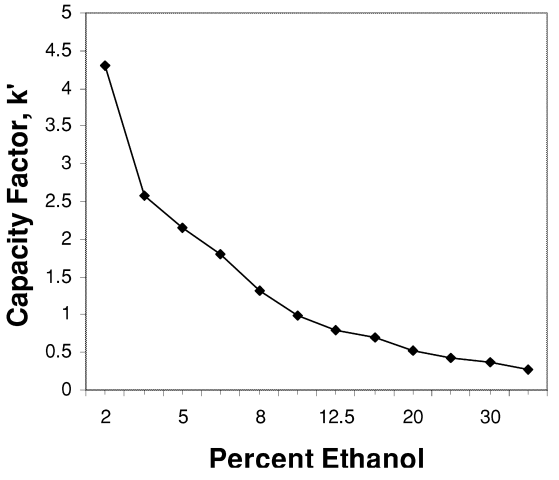

Fig. 2. The effect of ethanol concentration on bupropion retention.

3.2. The effect of mobile phase pH

The effect that the mobile phase pH had on bupropion retention and resolution was studied. The goal for this study was 3-fold: to determine how the mobile phase pH would influence enantiomer (1) retention, (2) resolution, and (3) peak tailing. Protein columns are known to show much greater degrees of peak tailing than achiral silica-based stationary phases; therefore when using the protein stationary phase, a compromise usually must be made between baseline resolution and peaks that have significant tailing [28]. In many cases, changing one or more mobile phase parameters (i.e., pH, ionic strength) may improve enantiomeric resolution, however, unacceptable peak shape may also be a result.

Iredale et al. [29] reported that as the pH of the mobile phase was decreased from 6.0 a corresponding decrease in retention was observed. The net negative charge of the protein was found to decrease as well as a corresponding change in the Coulombic interactions between the ovomucoid and charged analytes. A transition in the net charge of the protein takes place over the pH range of 3–6 since the isoelectric point of ovomucoid is about 4.5. At a mobile phase pH below 4.5 the stationary phase would be cationic, whereas above pH 4.5 the stationary phase would be anionic in nature. Cationic analytes were found to have lower retention at lower pH mobile phases due to cation–cation repulsion, whereas at mobile phases above pH 4.5 higher retention was observed due to favorable ionic interactions. This study indicated that retention and enantioselectivity on the immobilized ovomucoid stationary phase are a function of hydrophobic

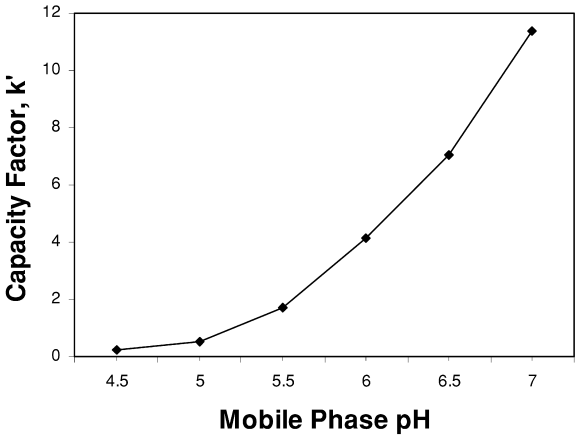

Fig. 3. The effect of mobile phase pH on bupropion retention.

interactions and Coulombic interactions between the analyte and the immobilized protein.

Fig. 3 shows the effect that the mobile phase pH had on analyte retention. The results that were obtained in this study were similar to those previously reported [29]. Retention was found to increase as the pH was raised from 4.5 to 7 using a sodium hydroxide solution (mobile phase: 10 m*M* citric acid, 25% methanol). At a mobile phase pH of 4.5, the enantiomers were separated but were not baseline resolved. Resolution increased over the entire range until pH 5.5 was reached at which point pH no longer had an effect on resolution. Even though bupropion continued to increase in retention at mobile phase pH values of 6, 6.5 and 7, resolution did not change when compared to pH 5.5. At the higher mobile phase pH values, peak tailing was found to increase significantly. Therefore, a mobile phase pH of 5.5 was chosen since this pH would provide good retention and resolution for the bupropion enantiomers.

3.3. The effect of citric acid concentration

Chiral columns may be affected by the concentration of buffers as well as the ionic strength of the mobile phase depending on the types of interaction that takes place between an enantiomer and the stationary phase. Oda et al. [31] found that the buffer concentration over a range of 10–300 m*M* phosphate did not affect analyte retention. This suggests that electrostatic interactions were not the driving force in the chiral separation for the compounds studied. The retention behavior was determined to be influenced by Coulombic interactions.

Peak shape was shown to improve for both acidic and basic compounds when a buffer was added to the mobile phase [32]. Resolution of the enantiomers were also shown to be unaffected by a buffer concentration over the range of 1.25 to 25 m*M*.

In this study, a citric acid buffer was used to determine how different buffer concentrations may influence retentivity and resolution of the two bupropion enantiomers. A concentration range of 1–100 m*M* citric acid was studied at a mobile phase pH of 5.5 using 25% methanol. In general, the enantiomers showed a slight decrease in retention as the buffer concentration was increased (Fig. 4). The change in retention, however, was not significant over the buffer concentration range of 15–100 m*M* citric acid. Resolution was not influenced by the buffer concentration over the range that was studied. These data conform to what was previously reported [31,32].

3.4. The effect of column temperature

Kirkland and McCombs [27] studied the effect

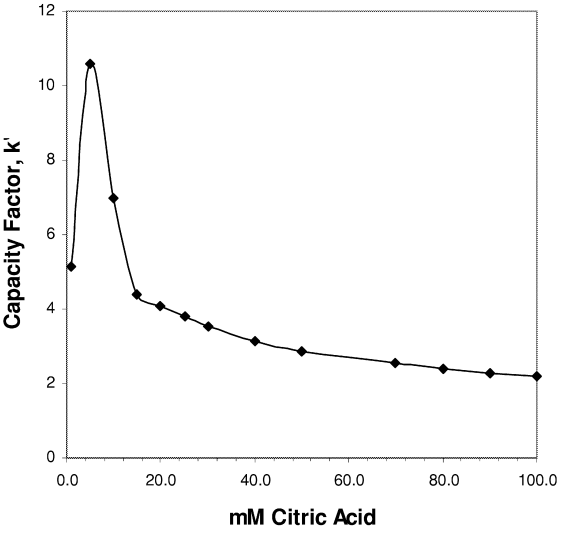

Fig. 4. The effect of citric acid concentration on bupropion retention.

that column temperature would have on enantiomer retention and resolution. This study indicated that selectivity and resolution might be improved by temperature changes. Chiral resolution of acidic drugs was found to decrease with increasing temperature while the retention of basic drugs was found to increase as the temperature was increased until a maxima was reached. This data showed that the column temperature should be carefully controlled for optimum reproducibility of enantiomer retention as well as for quantitative data.

The effect that column temperature had on bupropion retention and resolution was studied over the range of 25–50°C where a mobile phase of 40 m*M* citric acid, pH 5.5, and 25% methanol was used. The results that were found over this temperature range are shown in Fig. 5. As the column temperature was increased a corresponding decrease in retention was observed. This would be expected since the mass transfer kinetics are faster at higher temperatures, which results in lower enantiomeric retention.

Resolution was found to decrease from 25 to 30°C, then leveled off and remained constant from 35 to 50°C. From this data, it was determined that a column temperature of 35°C should provide a good separation for the bupropion enantiomers.

3.5. Effect of different buffer

Different buffers were studied to determine what effect this may have on the enantiomeric separation.

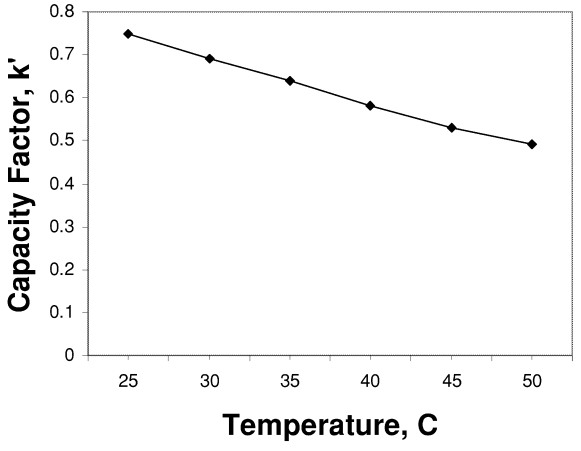

Fig. 5. The effect of column temperature on bupropion retention.

Ammonium acetate was used for the chiral separation and then compared to the separation using citric acid. The mobile phases were the same for both separations except for the buffer that was used. Fig. 6A shows the separation using 40 m*M* ammonium acetate, while Fig. 6B shows the separation where 40 m*M* citric acid was used (pH 5.5, 25% methanol). The ammonium acetate buffer showed higher retention and better resolution then did the citric acid mobile phase. This data indicated that the ammonium acetate buffer would provide a better separation than would the citric acid buffer.

3.6. Optimized separation

After the ammonium acetate was chosen as the preferred buffer, the chiral separation was optimized based on the data collected when the different mobile phase variables were studied using the citric acid buffer. The optimized separation for the bupropion enantiomers is shown in Fig. 7 with a mobile phase composition of 40 m*M* ammonium acetate, pH 5.5, and 12.5% methanol. A flow-rate of 1.0 ml/min, a column temperature of 35°C and an injection volume of 25 μl were used. The bupropion enantiomers were baseline resolved with a runtime of less than 10 min.

3.7. Effect of amount of bupropion injected

The effect that the amount of bupropion injected had on enantiomeric resolution was studied. Resolution was not affected over a concentration range of 0.27–5.30 μg/g. When the bupropion concentration was equal to or greater then 10.6 μg/g, resolution started to decrease quickly. Resolution, as well as peak tailing, was found to be the best at lower concentration levels of bupropion. This indicates that the OVM column is sensitive to the amount of sample injected and this should be taken into account when determining how much analyte may be chromatographed.

3.8. Calibration curves and system precision

Calibration curves were established over the range of 0.27–53.0 μg/g (ppm) of each bupropion enantiomer. A correlation coefficient of greater than 0.999 was observed for each enantiomer indicating that the

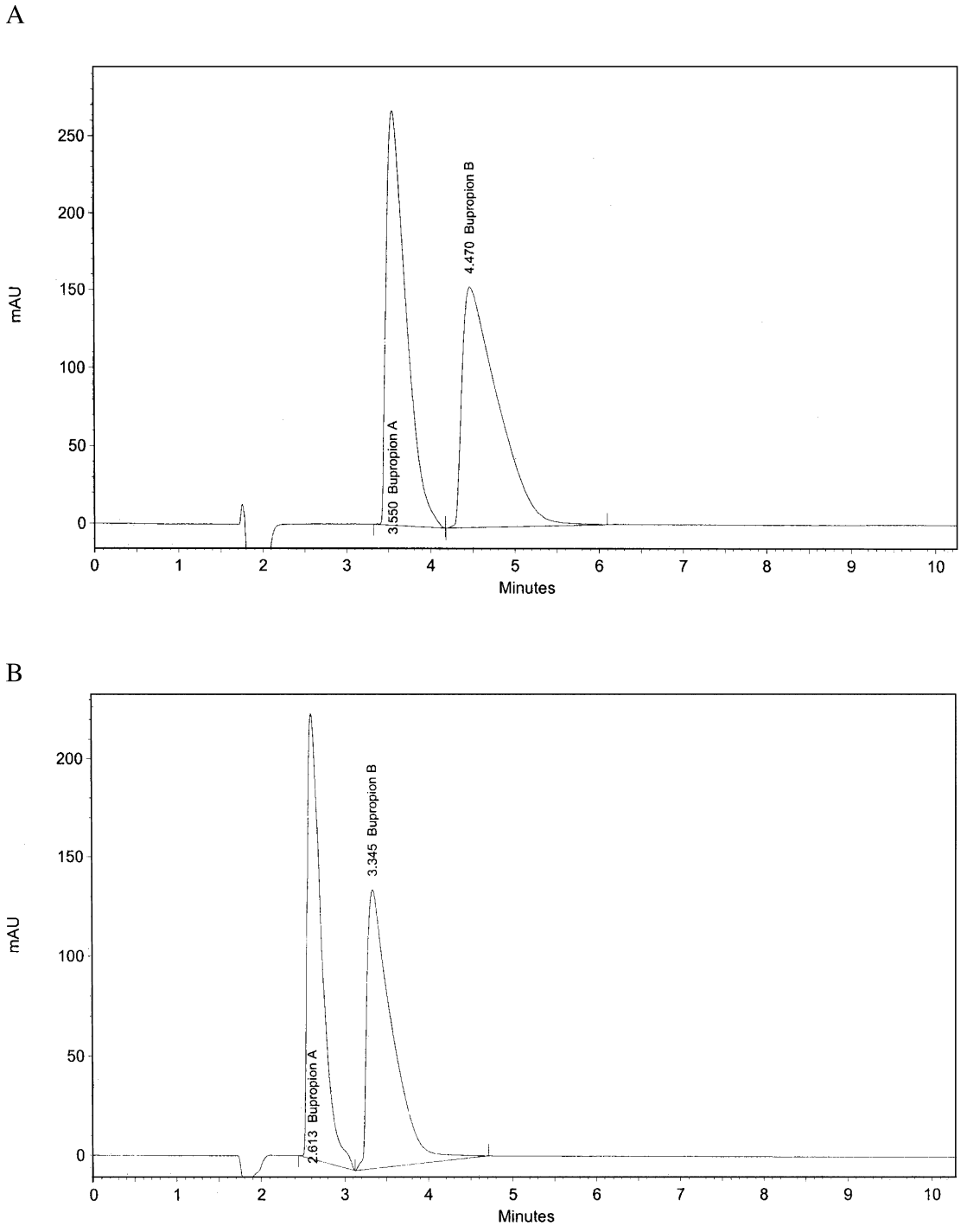

Fig. 6. Comparison of citric acid and ammonium acetate buffers on bupropion enantiomer retention and resolution: pH 5.5, 25% methanol; (A) 40 m*M* ammonium acetate; (B) 40 m*M* citric acid.

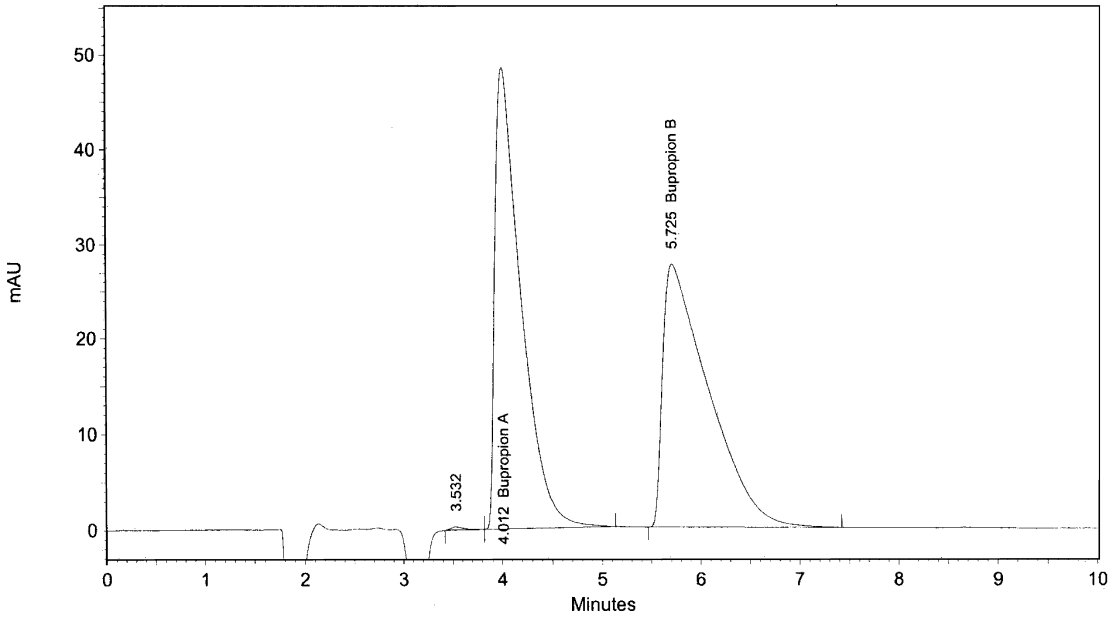

Fig. 7. The optimized bupropion enantiomeric separation.

system is linear over this range. The limit of detection (3:1, signal:noise) was found to be 0.13 μg/g while the limit of quantitation was determined to be 0.27 μg/g.

The system precision was determined for the bupropion enantiomers by injecting the 53.0 μg/g standard 6 times. The %RSD for the peak areas of the six injections was found to be 0.2%. The system was found to be precise.

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