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Resolution of (±)-β-Methylphenylethylamine by a Novel Chiral Stationary Phase for Pirkle-Type Column Chromatography

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ABSTRACT In this study, a new Pirkle-type chiral column stationary phase for resolution of β-methylphenylethyl amine was described by using activated Sepharose 4B as a matrix, L-tyrosine as a spacer arm, and an aromatic amine derivative of L-glutamic acid as a ligand. The binding capacities of the stationary phase were determined at different pH values (pH = 6, 7, and 8) using buffer solutions as mobile phase, and enantiomeric excess (ee) was determined by HPLC equipped with chiral column. The ee was found to be 47%. *Chirality 22:252–257, 2010.* © 2009 Wiley-Liss, Inc.

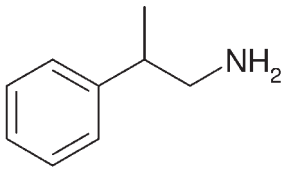
KEY WORDS: Sepharose 4B; resolution; chiral amines; HPLC; Pirkle-type column chromatography

INTRODUCTION

It is a well-known fact that two important features of chiral compounds make them distinctive from achiral compounds: Firstly, their behavior toward plane-polarized light and secondly, their interactions with other chiral compounds. Chiral compounds are separated into enantiomers through different chromatographic (HPLC, liquid chromatography and Clay column chromatography) methods.[1–3] In addition, the above-mentioned separation processes are also conducted through capillary electrophoresis method.[4] If amines with high enantiomeric purity are of biological importance, they can have wide medical applications. For this reason, the preparation of chiral compounds and their analysis is very important. High enantiomeric purity is always demanded in environmental chemistry, pharmaceutical industry, and clinical analyses. The main power behind the design enantioselective reactions is the pharmaceutical industry. Typically, only one enantiomer of a chiral drug is active. Ibuprofen (sold under the names Advil, Motrin, or Nuprin) which is anti-inflammatory is such an example. Only the (S) isomer of ibuprofen is active. The (R) isomer of ibuprofen does not show anti-inflammatory effect.[5]

(*S*,*S*)-Ethambutol is one of the enantiomers of a chiral tuberculostatic drug that especially shows effective therapy, but (*R*,*R*)-ethambutol is toxic to humans. (*S*)-2-Aminobutanol is the key intermediate in the manufacture of the (*S*,*S*)-ethambutol.[6]

β-Methylphenylethylamine belongs to the group of drugs related to amphetamines. It is known that it has stimulant effects in man, as the β-methyl group slows metabolism by monoamine oxidase enzymes. The structural formula is given below:



β-methylphenylethylamine

The separation of racemic mixtures of chiral compounds is conducted by using different techniques at present. One of these is chromatographic separations. The chiral stationary phases (CSPs) that are used for this process include chiral ligand-exchange columns, chiral affinity columns, helical polymer columns, cavity columns, and Pirkle-type columns.[7] Polysaccharide derivatives, cyclodextrin derivatives, macrocyclic chiral compounds, ligand exchange complex, crown ether, imprinted polymers, and some molecular selectors such as Pirkle-type compounds are used in the preparation of CSPs.[8–12] The chiral selectors used in CSPs include polysaccharide derivatives and studied enantioseparation of binaphthyl compounds[13] and imidazolinone herbicides.[14] Farkas et al.[15] reported the preparation and testing of a new pyridino-18-crown-6 ether

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based CSP and studied the enantioseparation of protonated primary arylalkylamines and aromatic amino acid derivatives. Enantiomers of some amino acid ester derivatives have been separated on brush-type CSPs derived from terpenoid compounds.[16]

Pirkle-type column chromatography is a technique developed by the use of a series of different stationary phases as a matrix formed by the amino acid derivatives getting connected to the silica with covalent or ionic attachment. Generally, *N*-(3,5-dinitrobenzoyl) phenylglicyne is used as stationary phase. As these columns can be obtained commercially, they can also be prepared for preparative or analytical purposes. Mechanisms of enantiomeric separation are explained with many combined factors such as interaction through charge transfer, hydrogen bonding, dipole–dipole interactions, and steric effects.[17,18]

Pirkle et al. developed Pirkle-type CSPs prepared from *N*-(3,5-dinitrobenzoyl) derivatives of (*S*)-leucine and (*R*)-phenylglycine and used them to separate the enantiomers of *N*-aryl-α-amino esters and 2-carboalkoxyindolines.[19] Hyun et al.[20] used Pirkle-type π-acidic CSP for the enantioseparation of amide derivatives of naproxen. The enantiomers of amino acid derivatives have been separated on new Pirkle-type CSP with long alkyl chains.[21] Hyun et al.[22] developed a new Pirkle-type CSP derived from *N*-(3,5-dinitrobenzoyl) leucine *N*-phenyl *N*-alkylamide and the resolution results were compared with those on various commercial π-acidic CSPs and enantiomers of racemic 2-hydroxycarboxylic acids were resolved as their *O*-ethoxycarbonyl π-basic anilide derivatives on a new CSP. In a study by Kraml et al., a series of carbobenzyloxy (cbz) derivatives of commercially available racemates was prepared and analyzed by enantioselective chromatography using a variety of mobile phases and polysaccharide and Pirkle-type CSPs. The cbz-derivatized product consistently demonstrated enhanced chiral resolution under HPLC and supercritical fluid chromatography conditions.[23]

We prepared a new CSP by using Sephorose 4B as a matrix, L-tyrosine as a spacer arm, and the aromatic amino derivative of L-glutamic acid as ligand. The spacer arm was placed between the matrix and the ligand. We tried to separate β-methylphenylethyl amine into its enantiomers, using buffer solutions at various pH values as mobile phase from this column. Our aim in this study is to determine the separation capacity of the Pirkle-type column we have prepared regardless of the characteristics of the amine used.

EXPERIMENTAL

Chemicals

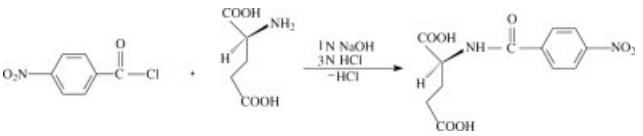
Sodium hydroxide, sodium acetate, L-glutamic acid, L-tyrosine, *p*-nitrobenzoyl chloride, hydrazine-hydrate solution, Pd/C, β-methylphenylethylamine, activated Sepharose 4B, perchloric acid, diethyl ether, and absolute ethanol were purchased from Sigma and Merck (analytical grade, Merck, Darmstadt, Germany) and Fluka (Switzerland). All other reagents were analytical grade and used without further purification. Activated Sepharose 4B was loaded as stationary phase. Acetate and phosphate buffer solutions were used as mobile phases at different pH values. In the determination of enantiomeric purity, perchloric acid was used as HPLC mobile phase.

Apparatus

A 1.0 cm × 20 cm (ID X L) Luer-lock, nonjacketed liquid chromatography column (Sigma-Aldrich) was used in the resolution of the chiral amine. Flow rate of eluates was adjusted with Watson Marlow-323 peristaltic pump. Amine concentrations in eluates were determined with a Perkin-Elmer-λ-35 UV-spectrophotometer. ^{1}H and ^{13}C NMR spectra of synthesized compounds were recorded by Bruker AC 400 MHz NMR spectrometer. Infrared (IR) spectra were recorded by Mattson 1000 FTIR-spectrophotometer. Enantiomeric purity of samples was determined with Bio-Der 2100 HPLC dual-pump system and a Shimadzu-3001 UV detector by using Crownpak CR-11 column (CR (+) 27014 chiral separation column and CR (+) 27011 guard column).

Synthesis of Ligand

N-p-nitrobenzoyl-L-glutamic acid ($C_{12}H_{12}N_2O_7$).



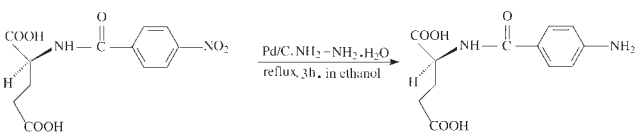
The L-amino acid was reacted with *p*-nitrobenzoylchloride and the product was reduced to the amine according to standard methods.[24,25]

0.73 g (0.5 mmol) of L-glutamic acid was dissolved in 15 ml 1 N NaOH and 1.5 g sodium acetate was added. Then, 0.93 g (0.5 mmol) powder *p*-nitrobenzoylchloride was added into this solution. The purple solution was stirred for 5 min and filtered. Then, 3 N HCl was added dropwise until the filtrate was yellow. The solution continued to be acidified with dilute hydrochloric acid by controlling pH. A white-yellow precipitate occurred. The mixture was kept for 1.5 h at room temperature and filtered several times in vacuum. The filtrate was kept for 15 min and precipitate was formed. Thus, *N-p*-nitrobenzoyl-L-glutamic acid was obtained. White product was filtered and crystallized from ethanol. Yield: 0.94 g (64%), mp 192°C, $[\alpha]_D^{30} = -15.7^{\circ}$ (C = 0.6, in ethanol).

^{1}H NMR ($CDCl_3$) δ: 1.93–2.16 (m, 2H), 2.36–2.50 (m, 2H), 4.40–4.46 (m, 1H), 8.12 (d, 2H), 8.33(d, 2H), 9.02 (d, 1H), 12.35(b.s, 2H).

^{13}C NMR ($CDCl_3$) δ: 26.3, 30.9, 52.7, 124.0, 129.5, 140.0, 149.6, 165.5, 173.5, 174.2.

N-p-aminobenzoyl-L-glutamic acid ($C_{12}H_{14}N_2O_5$).



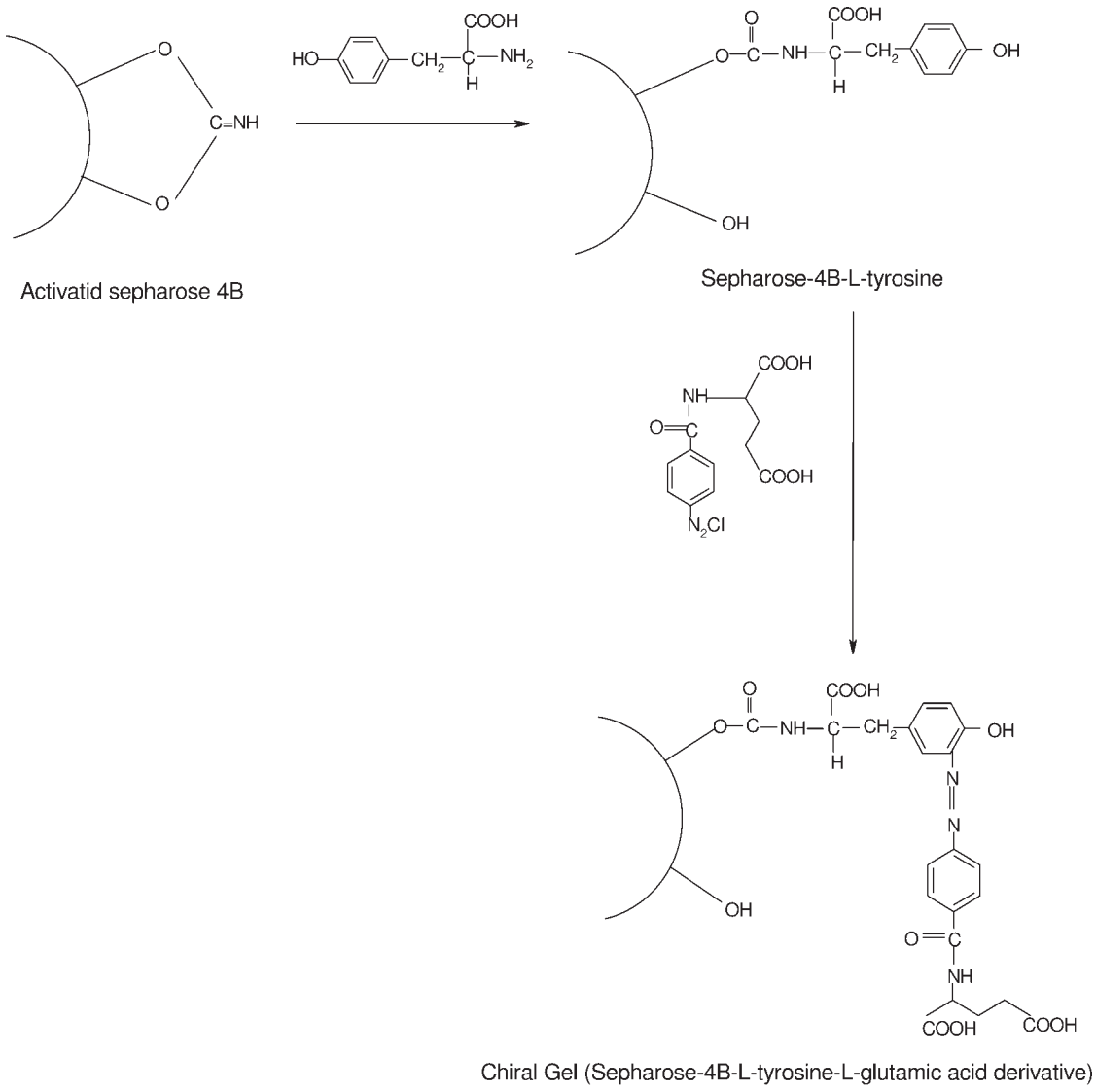


Fig. 1. Preparation of chiral gel (Sepharose-4B-L-tyrosine-L-glutamic acid derivative).

The reaction was carried out in a 250 ml two-necked round-bottom flask equipped with a reflux condenser and a dropping funnel and placed in a magnetic stirrer. 0.8 g *N-p*-nitrobenzoyl-L-glutamic acid was placed in the flask and dissolved in 60 ml ethanol with stirring. 0.05 g Pd/C was added to the stirred solution, and 10 ml hydrazine hydrate solution was added dropwise to the mixture. The reaction mixture was refluxed at 80°C for 2.5 h. The hot dark solution was filtered immediately and the ethanol evaporated. Thus, reduced amine was obtained. Yield: 0.55 g (78%), $[\alpha]_D^{30} = -9.8^\circ$ (C = 0.8, in ethanol).

^{1}H NMR ($CDCl_3$) δ: 1.85 (d, 2H), 1.95–2.08 (m, 2H), 4.11 (m, 1H), 6.55 (d, 2H), 7.55 (d, 2H), 7.87 (d,2H). Carboxylic acid and amide protons were not observed.

^{13}C NMR ($CDCl_3$) δ: 29.4, 34.3, 55.1, 113.2, 121.8, 128.9, 151.9, 166.2, 177.0, 179.2.

Preparation of Chiral Column

Affinity chromatography columns for enzyme purification are prepared with Sepharose 4B matrix and different enzymes specific ligands are used.[26] By modifying this method, we prepared a new stationary phase with *N-p*-aminobenzoyl-L-glutamic acid as a ligand to interact with a chiral amine and activated Sepharose 4B as a matrix.

Five grams of activated Sepharose 4B was suspended in 20 ml distilled water and washed on a Buchner funnel several times with 200 ml distilled water to remove the impurities and 100 ml 0.2 M $NaHCO_3$ buffer (pH 10) at room temperature, respectively. This mixture was placed into a 100 ml beaker and L-tyrosine solution (80 mg/20 ml distilled water) was added by stirring with a magnet for 2 h at +4°C, and then left for 16 h without stirring at the same

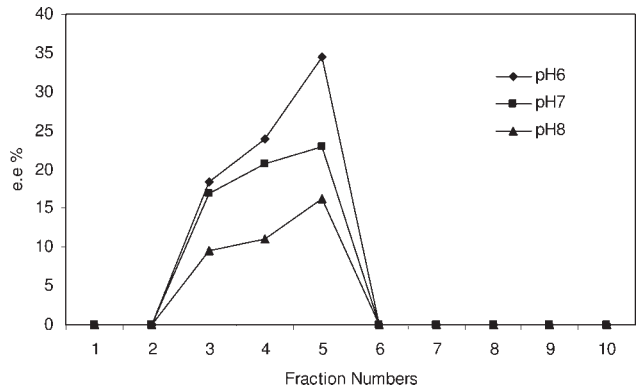


Fig. 2. Resolution of racemic 100 μl β-methylphenylethylamine mixture at different pHs by the Sepharose 4B chiral Pirkle-type column.

temperature. It was then washed again on a Buchner funnel with 100 ml 0.2 M $NaHCO_3$ buffer (pH 8.8). Thus, L-tyrosine was coupled to activated Sepharose 4B.

Fifty milligrams of *N-p*-aminobenzoyl-L-glutamic acid was dissolved in 10 ml 1 N HCl. Sodium nitrite solution (100 mg $NaNO_2$/5 ml distilled water) was added to this solution slowly with stirring at 0°C. The diazotized *N-p*-aminobenzoyl-L-glutamic acid was poured into 40 ml of the Sepharose-4B-L-tyrosine suspension. The pH was adjusted to 9.5 with 1 M NaOH and the mixture stirred for 3 h at room temperature. The yellow suspension was washed with 500 ml distilled water and 200 ml phosphate buffer (pH 6). The solution was packed into a column (1 cm × 20 cm) and, after precipitation of the gel, equilibrated with the same buffer using a peristaltic pump (flow rate: 30 ml/h). The reaction scheme is shown in Figure 1.

Resolution of β-Methylphenylethylamine

A solution of racemic β-methylphenylethylamine (100 μl amine/5 ml 0.1 M phosphate buffer pH 6) was loaded onto the column and eluted with phosphate buffer (pH 6) flow rate 25 ml/h. Resolution studies were repeated with the same buffer at pH 7 and 8. Elutes were collected in 3 ml fractions, and absorbance values were determined at 256 nm by UV spectrophotometer. The amine was collected in the 3rd, 4th, and 5th tubes for each buffer (pH 6, 7, and 8). The pH of the tubes was adjusted to approximately pH 14 with 1 N NaOH solution and extracted with diethyl ether. The ether phase was evaporated and 2 ml $HClO_4$ (pH 1.5) was added to the residue in each tube.

This study was repeated for 50 and 25 μl racemic β-methylphenylethylamine at the same conditions. The amine was collected in the 7th, 8th, and 9th tubes with pH 6 buffer.

Determination of Enantiomeric Purity by HPLC

Enantiomeric purity of eluates (Preparation of Chiral Column section) was measured by chiral HPLC with a UV detector. The amines were diluted with distilled water (mobile phase: HPLC grade $HClO_4$ pH 1.5), flow rate: 1.0 ml/min, column pressure: 100 bar, wavelength: 200 nm).

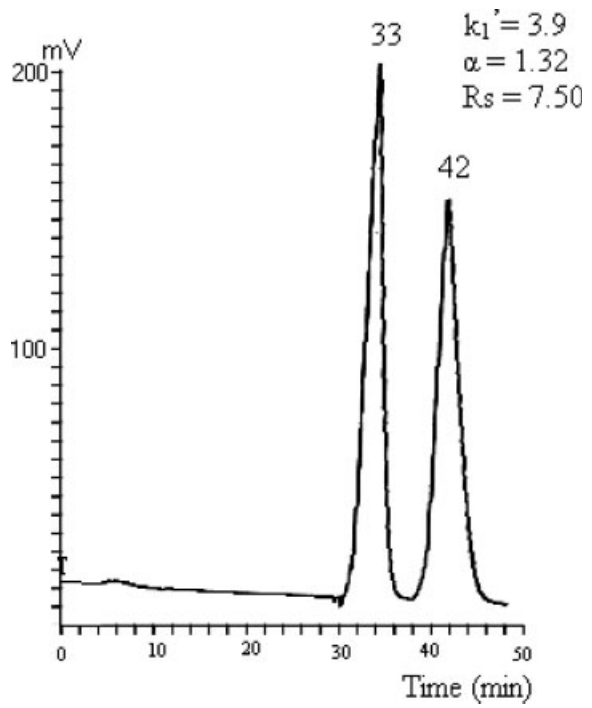


Fig. 3. The chromatogram of 100 μl racemic mixture of β-methylphenylethylamine on CHIRALPAK® CR-11 with 0.03 N $HClO_4$ for 5th fraction in pH 6 buffer (Flow rate: 1.0 ml/min, temperature: 25°C, detection: UV 200 nm).

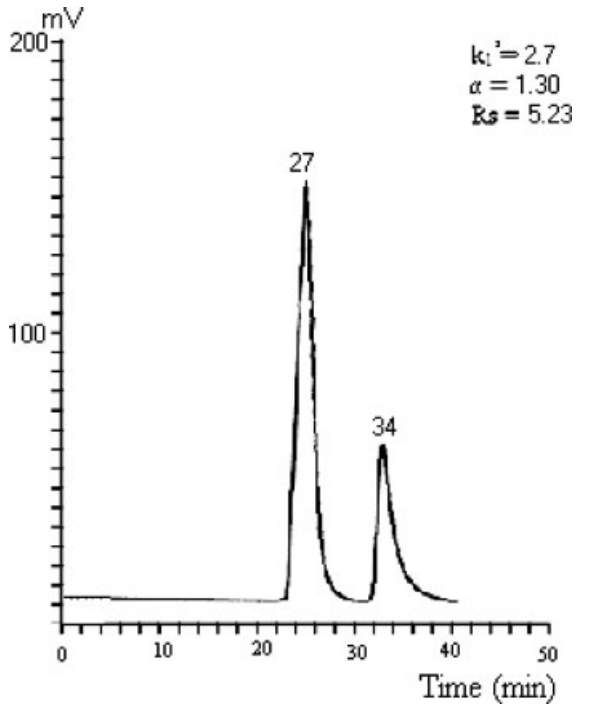


Fig. 4. The chromatogram of 50 μl racemic mixture of β-methylphenylethylamine on CHIRALPAK® CR-11 with 0.03 N $HClO_4$ for 8th fraction in pH 6 buffer (Flow rate: 1.0 ml/min, temperature: 25°C, detection: UV 200 nm).

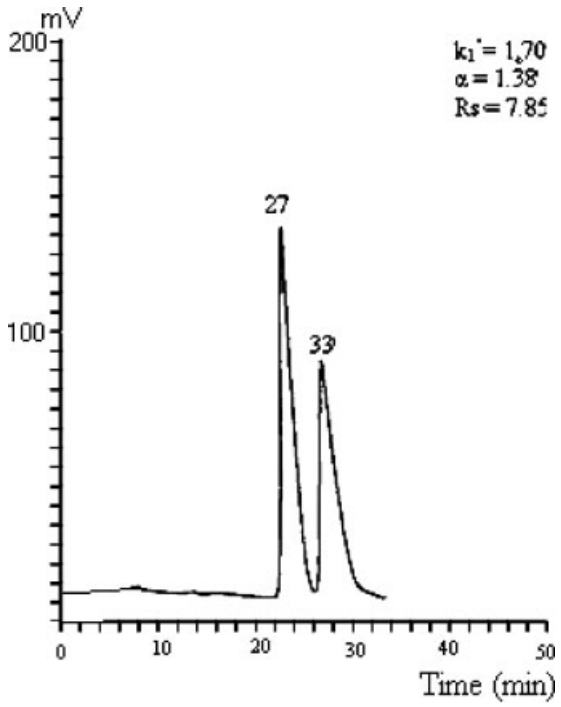


Fig. 5. The chromatogram of 25 μl racemic mixture of β-methylphenylethylamine on CHIRALPAK® CR-11 with 0.03 N $HClO_4$ for 9th fraction in pH 6 buffer (Flow rate: 1.0 ml/min, temperature: 25°C, detection: UV 200 nm).

Before the elutes were injected, a standard racemic mixture of the amine was injected and retention times were determined. (*S*)-β-methylphenylethylamine eluted before the (*R*) enantiomer. Enantiomeric excess (ee) and chromatographic parameters were calculated according to the literature.[27]

RESULTS AND DISCUSSION

N-p-Aminobenzoyl-L-glutamic acid, which is a new chiral ligand, has been synthesized in good yield. The synthesized compounds were screened with SciFinder Scholar program. The structures of ligand and nitro compound before reduction were characterized with IR, ^{1}H NMR, and ^{13}C NMR results. Spectroscopic data are given in Synthesis of Ligand section. The structures of synthesized compounds corresponded to spectroscopic data. *N-p*-Aminobenzoyl-L-glutamic acid ligand was diazotized and then coupled to the *o*-position of aromatic ring of L-tyrosine linked to Sepharose 4B. The gel color was changed light-yellow indicating that diazotization was successful.

The enantiomeric excess was calculated at pH 6, 7, and 8, and the maximum resolution was recorded at pH 6 for 100 μl β-methylphenylethylamine (Fig. 2). The racemic amine mixture was loaded to column three times at different amounts (100, 50, and 25 μl). Maximum enantiomeric purity was determined at 50 μl sample compared to the others. Thus, it was found that the column capacity is 50 μl sample and the optimum pH is 6 for β-methylphenylethylamine.

The ee values were 34.5, 47.1, and 24.2% for 100, 50, and 25 μl racemic mixtures, respectively. Enantioselectivity (α) for sample of 50 μl was 1.30, which is smaller than others under the same conditions. The chromatographic results obtained with various initial amounts of racemic β-methylphenylethylamine have been shown in Figures 3–5.

After each separation study conducted with different pH values and different amine amounts, the column was regenerated by washing with 1 N 100 ml HCl. Carboxyl groups of L-glutamic acid, bound to the L-tyrosine at stationary phase, were turned into nonionic form. Then, it was equilibrated with 100 ml 0.1 M phosphate buffer. Thus, the column became ready for use.

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