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Chiral separation and quantitation of pentazocine enantiomers in pharmaceuticals by capillary zone electrophoresis using maltodextrins

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Abstract

The chiral separation of pentazocine was achieved by capillary electrophoresis using oligosaccharides. Enantiomers were separated on 100 mM Tris/H_3PO_4 buffer (pH 2.5) with 5% maltodextrin as a chiral selector, and migration behavior was monitored at 200 nm. Under these conditions, (−)- and (+)-pentazocine and dextromethorphan (internal standard) migrated within 9 min, and the resolution of pentazocine enantiomers was 2.54. Linear calibration curves were obtained in the range 5–50 μg ml^{-1} for each enantiomer. The detection limit of pentazocine enantiomers was 29 pg, and the recoveries of (−)- and (+)-pentazocine were 98.9 (R.S.D., 3.4%) and 101.4% (R.S.D., 4.3%) with 10 μg ml^{-1}, respectively.

Keywords: Chiral separation; Pentazocine; Capillary electrophoresis (CE); Maltodextrin

1. Introduction

Pentazocine (1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methy-2-butenyl)-2,6-methano-3-benzazocine-8-ol) is a non-narcotic analgesic, structurally related to morphine, which is widely used in the management of patients with acute or chronic pain. The structures are shown in Fig. 1 [1]. The pentazocine currently used clinically is racemic, but enantiomers of pentazocine have different activities [1–4]. The main effect of pentazocine is due to the binding of (−)-pentazocine to κ- and μ-opioid receptors. On the other hand, (+)-pentazocine has little analgesic activity because of its high affinity for σ-receptor and low affinity for opioid receptors [3,4]. The activity of opioid analgesics regulated by σ-receptors has been reported [3]. It is planned to develop the

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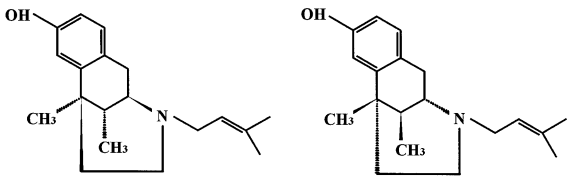

(-)-Pentazocine (+)-Pentazocine

Fig. 1. Structures of pentazocine enantiomers.

[pharmaceuticals of (−)-pentazocine in future. Therefore, a chiral separation method is necessary to evaluate the effects of these drugs.

Several methods have been reported for the chiral separation of pentazocine in pharmaceuticals. A HPLC method requires an expensive chiral column [6], and a large volume of organic solvent as the mobile phase [6,7]. Ameyibor and Stewart [7] reported the separation of pentazocine enantiomers without a chiral column, but this required a long separation time. On the other hand, A radioimmunoassay [5] is complicated and not useful for routine use. Recently, there have been numerous reports on chiral separation by capillary electrophoresis (CE) with high resolution [8–19]. Chiral separation can be easily performed by adding chiral selector to buffer solution without changing the capillary tube. The chiral selectors used in these reports have included cyclodextrin (CD), its derivatives [8–16], maltodextrin [17–19], other polysaccharides [20,21], antibiotics [22], and chiral surfactants [23]. The CE method offers several advantages over HPLC for chiral separation, including direct chiral resolution, high resolution and speed, and low reagent consumption. Thus, we investigated the chiral separation of racemic pentazocine in pharmaceuticals by the CE method using a chiral selector.

2. Experimental

2.1. Chemicals

Standard (±)-, (−)-, (+)-pentazocine and tablets of pentazocine hydrochloride (content: 25 mg of pentazocine) were provided by Grelan Pharmaceutical (Tokyo, Japan). β-CD, dimethyl-β-CD, trimethyl-β-CD and γ-CD were purchased from Tokyo Kasei (Tokyo, Japan). Methyl-β-CD, propyl-β-CD, ethyl-β-CD and three types of maltodextrin (DE 4.0–7.0, 13.0–17.0 and 16.5–19.5) were obtained from Aldrich (Milwaukee, WI). Tris (hydroxymethyl) aminomethane (Tris), phosphoric acid and hydrochloric acid were purchased from Wako (Osaka, Japan) and dextromethorphan was obtained from Sigma (St Louis, MO). Water was purified (> 17 MΩ) by a Milli-Q water purification system (Japan Millipore, Tokyo, Japan).

2.2. Apparatus

The instrument used was a P/ACE 5510 system equipped with a photodiode-array detector (Beckman, Fullerton, CA). An untreated fused-silica capillary tube [75 μm i.d. × 47 cm (effective length: 40 cm)] was used for the separation. The capillary tube was kept at 25°C with a liquid coolant. The applied voltage was held constant at 10–30 kV. The detection wavelength was adjusted to 200 nm. Analytes were injected into the capillary tube by pressure mode for 3 s. Instrument control and data collection were performed using a P/ACE station program.

2.3. Sample preparation

Tris/H_3PO_4 buffer (100 mM) was prepared by dissolving 1.21 g of Tris in water, and adjusting to pH 2.0 ~ 7.0 with phosphoric acid. The buffer was filtered through 0.45-μm membrane filters before use. CDs and maltodextrins were dissolved in this buffer. An internal standard solution was prepared by dissolving dextromethorphan in water. A standard solution of racemic pentazocine was dissolved in 0.01 M HCl solution.

Four tablets of pentazocine hydrochloride (weight: 0.108 g) were weighed accurately and ground into powder. The equivalent of one tablet of the powder and 0.167 or 0.0417 g of authentic pentazocine were mixed with 20 ml of 0.01 M HCl solution in a 25-ml volumetric flask. The mixture was sonicated for 30 min, diluted with 0.01 M HCl solution, and filtered through a 0.45-μm

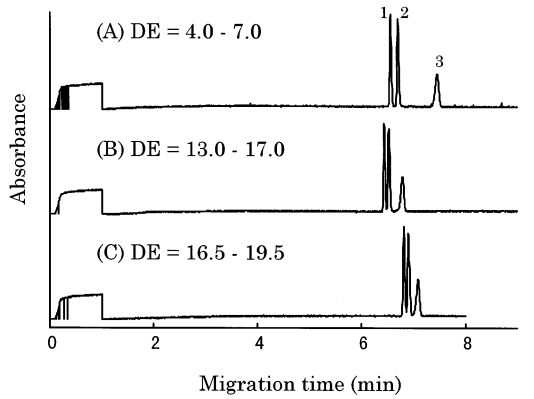

Fig. 2. Electropherograms of the three types by maltodextrin. Peak 1, (−)-pentazocine; 2, (+)-pentazocine; 3, dextromethorphan (internal standard). Conditions: fused-silica capillary (75 mm i.d. × 47 cm total length); temperature; 25°C, 100 mM Tris/H_3PO_4 (pH 2.5) containing 5% of each maltodextrin, applied voltage; 25 kV; 3 s of sample injection with pressure; detection, 200 nm.

membrane filter. Three hundred microlitres of this solution and 500 μl of 1000 μg ml^{-1} dextromethorphan as internal standard were mixed and diluted with 0.01 M HCl solution to 10 ml. This sample solution was then filtered through a 0.45-μm membrane filter.

The capillary was rinsed with the running buffer solution for 1 min before every run, and washed with 0.1 M NaOH solution for 1 min and water for 1 min after each run.

2.4. Calculations

The resolution (*Rs*) [24] values were calculated as

$$\text{Resolution } (Rs) = 1.18(t_2 - t_1)/(Wh_2 + Wh_1)$$

where t_1 and t_2 are the migration times, and Wh_1 and Wh_2 are the widths of the first and second peaks at 50% of the peak height, respectively.

The absolute injection volume [25] was calculated as

$$Vc(L) = \Delta P \pi r^4 t / 8\eta L$$

where Vc is injection volume, ΔP is the pressure across the capillary (3.44×10^4 dyne/cm^2), r and L are the inner radius and length of the capillary, respectively, η is the viscosity (0.890×10^{-2} dyne/s per cm^2), and t is injection time.

3. Results and discussion

3.1. Optimum conditions for the chiral separation of pentazocine enantiomers

In this study, three types of maltodextrins (DE 4.0–7.0, 13.0–17.0 and 16.5–19.5) were used as chiral selectors. Maltodextrins are mixtures of linear oligo- and polysaccharides, which consist of D-glucose units. The effect of maltodextrins on chiral recognition was investigated using 100 mM Tris/H_3PO_4 buffer solution (pH 2.5 and 7.0) containing 2% maltodextrins. Since average maltodextrin molecules are unknown, concentrations are given in percentages. A chiral recognition was achieved using maltodextrins. Maltodextrins have a helical structure with an inner hydrophobic region, much like CDs, that is important for chiral recognition. The change in conformation from a flexible coil to a helix in the presence of complexing molecules and buffer salts may play an important role in selective interactions. According to Soini et al. [18], maltodextrins are much more flexible than CDs, which leads to fewer restrictions. Therefore, pentazocine may be able to interact with the hydrophobic region of the maltodextrin helix, but not with the cavity of CDs, in this buffer system.

Maltodextrins are characterized by their dextrose equivalent value (DE, defined as the percentage of reducing sugars calculated as glucose on a dry substance basis); a high DE indicates that the oligomeric chains in a mixture are short. The effects of the DE value of maltodextrin on resolution are shown in Fig. 2. We compared three types of 5% maltodextrins with DE values of 4.0–7.0, 13.0–17.0 and 16.5–19.5, respectively. As shown in Fig. 2, a higher resolution was observed with a lower DE value. Also, Soini et al. [18] have reported that the higher oligomers are particularly effective as chiral selectors. Since maltodextrins with lower DE values have longer oligomeric chains, they should have more binding sites than those with higher DE values. On the

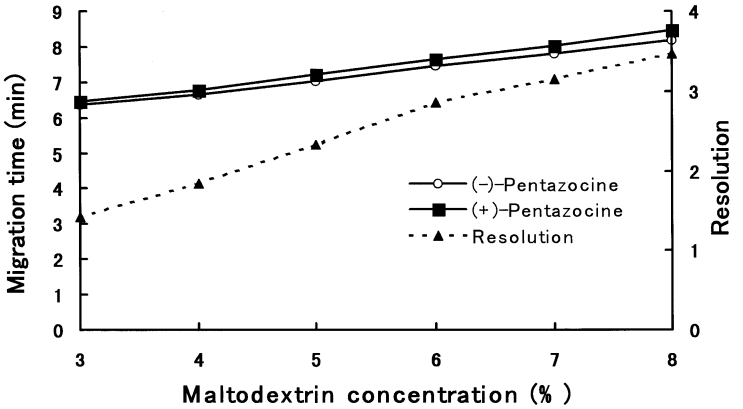

Fig. 3. Effect of maltodextrin concentration on migration time and resolution. Conditions: 100 mM Tris/H_3PO_4 buffer (pH 2.5).

other hand, the shortest migration time of pentazocine enantiomers was observed with DE values of 13.0–17.0. It is suggested that this finding is based not only on the differences of DE values and composition of maltodextrins, but also on the interaction of pentazocine on maltodextrins. However the detail of separation mechanism is not clear. The maltodextrin that showed the best resolution in this experiment (DE, 4.0–7.0) was selected as a chiral selector.

The effects of maltodextrin (DE, 4.0–7.0) concentration on the migration time and resolution were investigated using 100 mM Tris/H_3PO_4 buffer solution (pH 2.5) containing maltodextrin over a concentration range of 2–8%. The results are shown in Fig. 3. Resolution and migration time both increased with an increase in the maltodextrin concentration. This behavior seem to be due to the greater number of binding sites available to interact with solutes in the buffer, due to the increase in the maltodextrin concentration. A good resolution was obtained above 5%. A long time is needed for the separation of pentazocine enantiomers with a higher concentration of maltodextrin. In addition, the solute peaks become broad. Therefore, the optimum maltodextrin concentration was determined to be 5%.

The effects of buffer pH on the migration time and resolution are shown in Fig. 4. Tris/H_3PO_4 buffer solution (100 mM) with 5% maltodextrin in the pH range 2.0–7.0 was used. The migration times and resolution both decreased with an in-

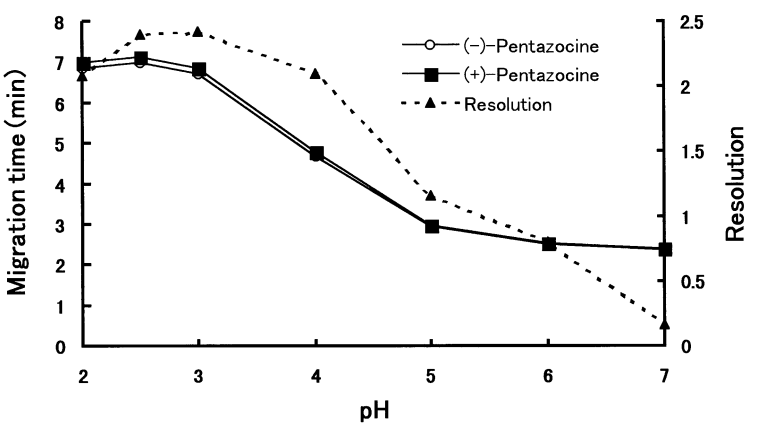

Fig. 4. Effect of pH on migration time and resolution. Conditions: 100 mM Tris/H_3PO_4 buffer containing 5% maltodextrin.

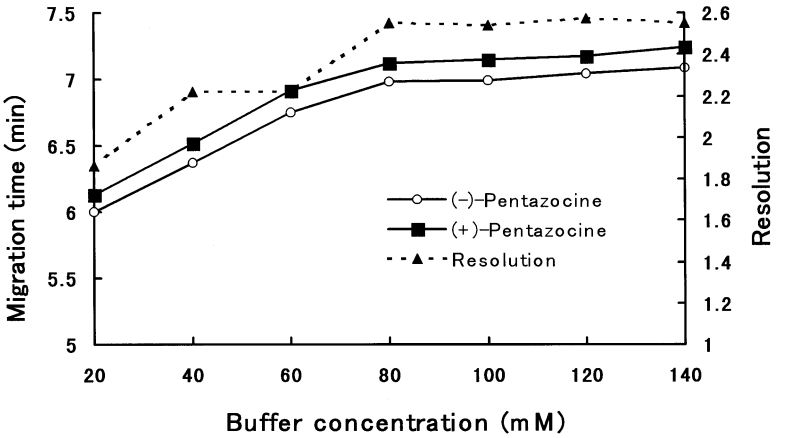

Fig. 5. Effect of buffer concentration on migration time and resolution. Conditions: Tris/H_3PO_4 buffer (pH 2.5) containing 5% maltodextrin.

crease in pH. This result may be due to an increase in the velocity of electroosmotic flow (EOF) at high pH, i.e. reduced chiral separation was caused by the shorter time available to interact with maltodextrin. Complete resolution was obtained at pH 2.0 and 2.5, which produced little EOF. Considering the migration time and peak shapes, a buffer solution of pH 2.5 was used in the following experiments.

The effects of buffer concentration on the migration time and resolution in the range 10–100 mM Tris/H_3PO_4 buffer solution (pH 2.5, 5% maltodextrin) are shown in Fig. 5. Migration times of pentazocine enantiomers increased with an increase in the buffer concentration, and plateaued above 80 mM. Baseline resolution was found above 40 mM and there were ca. 120 000 and ca. 200 000 theoretical plates at 40 and 100 mM, respectively. Therefore, all of the following studies were carried out with 100 mM buffer solution.

We also investigate the effect of the applied voltage on the migration time and resolution using 100 mM Tris/H_3PO_4 buffer solution (pH 2.5, 5% MD) in the range 10–30 kV. A good separation of enantiomers was obtained at all voltages except 30 kV. Considering the good resolution and fast migration time, a voltage of 25 kV was used in the following experiments.

The optimum conditions for the chiral separation of pentazocine enantiomers are as follows: buffer solution, 100 mM Tris/H_3PO_4 (pH 2.5, 5% maltodextrin); applied voltage, 25 kV. Under these conditions, the migration times of (−)- and (+)-pentazocine and dextromethorphan (internal standard) were 6.99, 7.15 and 8.02 min, respectively, and their relative standard deviations (R.S.D.) were 0.08, 0.10 and 0.10%, respectively ($n = 6$). Resolution of the (−)- and (+)-forms was 2.54 ($n = 6$), which is better than that obtained by HPLC (*Rs*, 1.8) [6,7].

3.2. Quantitation of pentazocine enantiomers in pharmaceuticals

Linear calibration curves were obtained in the range 5–50 μg ml^{-1} for each enantiomer. Typical regression slope, intercept and correlation coefficient were calculated to be 0.0292, 0.0388 and 0.9998 for (−)-pentazocine and 0.0286, 0.0328 and 0.9996 for (+)-pentazocine, respectively. The detection limit was 29 pg (S/N = 3), based on the concentration (1.5 μg ml^{-1}) and absolute amount of the injection (19.1 nl).

Fig. 6 shows a typical electropherogram of pentazocine enantiomers in pharmaceuticals and 50 μg ml^{-1} dextromethorphan (internal standard). Although the preparation method was very simple, there is no interference in the electro-

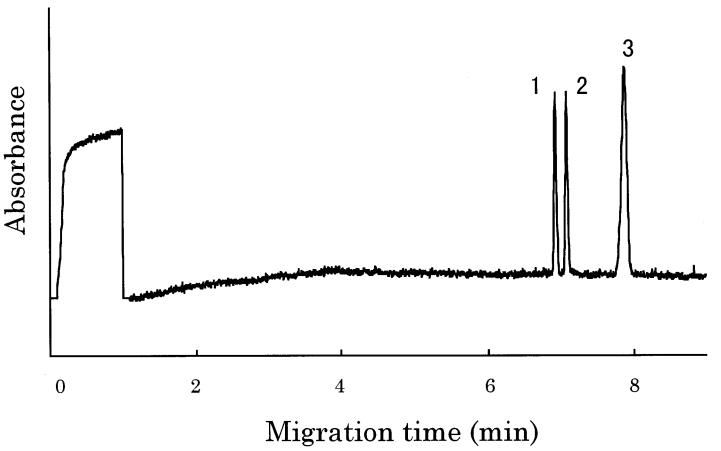

Fig. 6. Electropherograms of the pentazocine enantiomers in pharmaceuticals. Peak 1, (−)-pentazocine; 2, (+)-pentazocine; 3, dextromethorphan (internal standard). Conditions: 100 mM Tris/H_3PO_4 buffer (pH 2.5) containing 5% maltodextrin.

Table 1
Accuracy and precision for recovery test of pentazocine enantiomers in pharmaceuticals ($n = 9$)

Analytes	Concentration added (μg/ml)	Recovery (%)	R.S.D. (%)
(−)-Pentazocine	10	98.9	3.4
	25	101.4	1.9
(+)-Pentazocine	10	101.4	4.3
	25	102.3	1.8

pherogram and complete baseline resolution was obtained. The precision and accuracy (%) of this method were assessed using a non-spiked sample and samples spiked with 10 and 25 μg ml^{-1} pentazocine. The results of the recovery test are shown in Table 1. The mean recoveries of (−)- and (+)-pentazocine from pharmaceuticals were 98.9 and 101.4% with 10 μg ml^{-1} and 101.5 and 102.3% with 25 μg ml^{-1}, respectively.

4. Conclusion

We developed and validated a CE method for determining (−)- and (+)-pentazocine enantiomers in pharmaceuticals. Using this method, pentazocine enantiomers can be determined much more rapidly and at a higher resolution than with HPLC. The present method can be used for the separation and quantification of enantiomers in pharmaceuticals.

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References

[1] B.F. Tullar, L.S. Harris, R.L. Perry, A.K. Pierson, A.E. Soria, W.F. Wetterau, N.F. Albertson, J. Med. Chem. 10 (1967) 383–386.
[2] B. Berkowitz, Adv. Biochem. Psychopharm. 8 (1974) 495–501.
[3] C.-C. Chien, G.W. Pasternak, Eur. J. Pharmacol. 94 (1995) 303–308.
[4] B.A. Berkowitz, E.L. Way, J. Pharmacol. Exp. Ther. 177 (1971) 500–508.
[5] T. Nambara, T. Tanaka, M. Numazawa, J. Pharm. Dyn. 2 (1979) 151–156.
[6] J.W. Kelly, J.T. Stewart, C.D. Blanton, Biomed. Chromatogr. 8 (1994) 255–257.
[7] E. Ameyibor, J.T. Stewart, J. Chromatogr. B 703 (1997) 273–278.

[8] T.E. Peterson, J. Chromatogr. 630 (1993) 353–361.
[9] É. Szökó, K. Magyar, J. Chromatogr. A 709 (1995) 157–162.
[10] Z. Wang, Y. Sun, Z. Sun, J. Chromatogr. A 735 (1996) 295–301.
[11] H. Soini, M.-L. Riekkola, M.V. Novotny, J. Chromatogr. 608 (1992) 265–274.
[12] M.W.F. Nielen, Anal. Chem. 65 (1993) 885–893.
[13] H. Nishi, Y. Kokusenya, T. Miyamoto, T. Sato, J. Chromatogr. 659 (1994) 449–457.
[14] H. Nishi, S. Terabe, J. Chromatogr. 694 (1995) 245–276.
[15] S. Fanali, J. Chromatogr. 474 (1989) 441–446.
[16] Y.Y. Rawjee, D.U. Staerk, G. Vigh, J. Chromatogr. 635 (1993) 291–306.
[17] A. D'Hulst, N. Verbeke, J. Chromatogr. 608 (1992) 275–287.
[18] H. Soini, M. Stefansson, M.-L. Riekkola, M.V. Novotny, Anal. Chem. 66 (1994) 3477–3484.
[19] A. D'Hulst, N. Verbeke, J. Chromatogr. 735 (1996) 283–293.
[20] B. Chankvetadze, M. Saito, E. Yashima, Y. Okamoto, J. Chromatogr. A 773 (1997) 331–338.
[21] H. Nishi, J. Chromatogr. A 735 (1996) 345–351.
[22] V.S. Sharp, D.S. Risley, S. McCarthy, B.E. Huff, M.A. Strege, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 887–898.
[23] A. Amini, I. Beijersten, C. Pettersson, D. Westerlund, J. Chromatogr. A 737 (1996) 301–313.
[24] The Ministry of Health and Welfare, The Japanese Pharmacopoeia, 13th edn., 1996, p. 47.
[25] D.R. Baker, Capillary Electrophoresis, Wiley, New York, 1995, p. 96.