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# Cyclodextrin-mediated micellar electrokinetic chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the enantiomer separation of racemorphan in human urine

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**Abstract**

Micellar electrokinetic chromatography (MEKC) was successfully and conveniently applied to the chiral separation with the addition of cyclodextrins (CDs) as chiral selector to the running buffer. Chiral separation depended on the type of CD; in particular, β-CD was effective for the chiral separation of racemorphan. We investigated the optimal conditions of type and concentration of CD as chiral selector for the routine enantiomeric separation of racemorphan with good reproducibility. The effects of other parameters such as buffer pH and detection wavelength were also investigated to obtain the optimum conditions for the enantiomeric separation of racemorphan. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was used for confirmation of racemorphan. The optimal conditions for enantiomeric separation of the racemorphan were as follows: 50 m*M* borate buffer at pH 9.4 with 50 m*M* SDS, 10 m*M* β-CD and 20% 1-propanol, 57 cm×50 μm fused-silica capillary column, and UV detection at 192 nm. Based on the developed method, racemorphan in human urine was also separated and determined using solid-phase extraction and MEKC. 

*Keywords:* Enantiomer separation; Doping analysis; Racemorphan

## 1. Introduction

Racemorphan consists of dextrorphan and levorphanol; the former is commonly used as an antitussive drug, the latter is a narcotic analgesic but has no antitussive properties. Due to its narcotic analgesic properties, levorphanol is classified as a banned drug by the International Olympic Committee (I.O.C.), thus separation of the two enantiomers is demanded.

In order to detect levorphanol-positive cases from suspected athletes for the purpose of doping control, we initially attempted to apply the separation conditions of a previous investigation [1]. However, we noticed that this method has disadvantages and needed to be optimized.

Dextromethorphan is an antitussive component of many cough medications, which is related chemically to a narcotic compound, but has few additive properties or drug-dependency tendency [2]. Dex-

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trorphan, one of the metabolite products of dextromethorphan, is the isomer of the codeine analog of levorphanol [3].

In a chiral compound the enantiomers may have different pharmacokinetic properties such as absorption, distribution, metabolism and excretion, and quantitatively or qualitatively different pharmacological or toxicological effects [4].

Therefore, it is important to study properties of individual drug enantiomers. For the investigation of chiral compounds mostly high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods [5,6] have been developed. While GC is limited to volatile compounds, chiral separations by HPLC are often inefficient. Also, chiral columns for HPLC are relatively expensive. For these reasons capillary electrophoresis (CE) is rapidly being developed for chiral separation because of its high separation efficiency, its relatively simple instrumental setup, and its lower operating costs.

In CE, when a proper chiral selector is used as a buffer additive, chiral separation is promising. Therefore, enantiomeric separation based on the differences in effective mobilities of enantiomers (since the complex stability between each enantiomer and chiral selector) is different [7].

Micellar electrokinetic chromatography (MEKC) is clearly one of the most powerful modes of CE, having been applied to a wide variety of compounds and sample matrices. To obtain chiral separations, a chiral selector must be part of the micellar system. Examples of chiral selectors used in MEKC are cyclodextrins (CDs), crown ethers, bile salts and metal–ion complexes [8]. CDs make extremely versatile chiral selectors because they have numerous chiral recognition centers. CDs are cyclic oligosaccharides consisting of six, seven or eight glucose units corresponding to α-, β-, γ-cyclodextrin [9]. CDs form a truncated cone with a rim of secondary hydroxyl groups at the opening with the larger diameter. The internal cavity contains no hydroxyl functions and exhibits a hydrophobic character. Owing to this hydrophobic nature of CDs, they are able to form inclusion complexes with aromatic or alkyl groups. The complex stability between a CD and its guest molecule is governed by factors like hydrophobic interactions, solvation effects and hydrogen bonds. Substantial differences in the complex formation constant are found even for structurally similar compounds like enantiomers. For this reason, CDs are successfully employed for the separation of enantiomers.

There are a couple of papers which demonstrated that CE can be used to investigate dextromethorphan [10] and racemorphan [1]. However, the former focused more on the separation of dextromethorphan and its metabolite dextrophan, and the latter focused on the sample clean-up procedures from urine rather than enantiomeric separation as was our aim in this paper. Also, in the latter case high concentrations of β-CD in the electrolyte buffer and a long capillary column were used. These were perhaps applied to analyze racemorphan and racemethorphan simultaneously. However, these conditions have some difficulties such as precipitation of β-CD and long migration times, which make them unsuitable for our goal which is to apply the separation method to detect levorphanol-positive cases from suspected athletes for doping control. Therefore, in this study we investigated the optimal conditions for enantiomeric separation of racemorphan (i.e., pH, concentration of CD, running buffer, organic solvent and micellar and column dimensions) in CD-modified MEKC using sodium dodecyl sulfate (SDS).

Also, we applied the developed method to analyze racemorphan in human urine by MEKC and confirmed the racemorphan using matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [11,12].

## 2. Experimental

### *2.1. Capillary electrophoresis*

A Beckman (Palo Alto, CA, USA) P/ACE System 5500 CE instrument equipped with a diode-array detector and operated at 192 nm was used. MEKC was performed on a 57cm×50 μm I.D. fused-silica capillary (Applied Beckman). Separations were carried out at the constant current of 50 μA and the observed voltage was always less than 22 kV. The samples were injected by pressure for 3 s at 0.5 p.s.i. (1 p.s.i.=6894.76 Pa). Electropherograms were monitored using Beckman Gold V810 software.

The running buffers were prepared with sodium

tetraborate and/or disodium phosphate and the pH was adjusted with 1 *M* NaOH or concentrated HCl to the value desired (pH 6.0–10). The buffers were filtered through 0.45-μm pore-size membrane filters and degassed with sonication prior to use. To obtain the reproducible results, the capillary was prepared prior to use by rinsing with 0.1 *M* NaOH, followed by water, and run buffer. For the determination of racemorphan in human urine, the sample preparation method developed by Aumatell and Well was used [1].

### 2.2. MALDI-TOF-MS

MALDI-TOF-MS was performed on a HP G2025A (Hewlett-Packard, Palo Alto, CA, USA) linear-type TOF-MS system operating in the positive-ion mode of detection. The ion-accelerating potential was 28 kV, and the length of the flight tube was 1 m. The operating pressure for studies with the HP G2025A MALDI-TOF-MS system were lower than $4 \cdot 10^{-6}$ Torr (1 Torr=133.322 Pa). A nitrogen laser was set to deliver 337-nm wavelength pluses (3 ns duration) onto the sample. The laser was operated at a rate of 5 Hz. The data were analyzed with the HP G2025A MALDI-TOF-MS system software version A.02.00. The matrix used was 2,5-dihydroxybenzoic acid (DHB) at a concentration of 10 m*M* in water–methanol (2:1, v/v).

### 2.3. Reagents

Phosphoric acid, 1-propanol, hydrochloric acid, and acetic acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). SDS, sodium hydroxide, sodium phosphate dibasic, sodium borate and heptakis(2,6-dimethyl)-β-cyclodextrin (DM-β-CD) were purchased from Sigma (St. Louis, MO, USA). α-, β- and γ-CDs were purchased from Merck (Darmstadt, Germany), and methyl-β-CD and hydroxypropyl-β-CD (HP-β-CD) from Aldrich (Milwaukee, WI, USA). Dextromethorphan and dextrorphan were purchased from Hoffman-La Roche (Basel, Switzerland). Levophanol was kindly provided by Doping Control Center (Seoul, South Korea). All other reagents were of analytical-reagent grade, as appropriate. Water was purified in a Milli-Q system (Milipore, Bedford, MA, USA).

## 3. Results and discussion

Since our ultimate goal was to apply the separation method to detect levorphanol-positive cases from suspected athletes for doping control, we first attempted to follow the method of the previous investigation [1]. However, soon after our initial attempts we realized that the previous method had some difficulties in the application as a routine analysis and needed to be optimized.

### 3.1. Enantiomer separation of CD-modified MEKC

In CD-MEKC, CDs cannot be solubilized in MEKC, and cannot interact with the micelle. And the CD behaves as another phase. The solutes are distributed among three phases: the aqueous, the micelle and the CD phase. Solutes form inclusion complexes with CDs based on their size, geometry and physicochemical properties, while interactions with micelles are based on solute hydrophobicity. The solute is partitioned between the micelle and the CD cavity, and it migrates with the micellar velocity [13,14]. Therefore in the CD-MEKC mode of CE, the main parameters affecting the chiral resolutions will be the nature and concentration of the CD and pH of the running buffer.

### 3.2. Optimization of separation conditions

As in the other CE modes, the type, pH and concentration of the running buffer are also important factors for the separation of analytes in CD-modified MEKC. We investigated the effect of pH on resolution using a 50 m*M* borate buffer of pH from 6.0 to 10.0. When the pH was lower than 8.5, racemorphan did not resolve in its enantiomers. However, at a pH higher than 8.5, an increase in resolution was observed upon increasing pH. At pH 9.4, fairly good resolution and sensitivity were obtained. Although the use of a pH buffer higher than 9.4 could improve the resolution to some point, the migration time is inadequate because of lengthened analysis time. The results are shown in Table 1. The effect of buffer concentration on resolution and migration time was also investigated by varying the concentration of borate from 10 to 70 m*M*, at pH 9.4. From this experiment, we observed

Table 1
Variation of migration time of the racemorphan as a function of buffer pH with borate buffer (50 m*M*)

| pH | Migration time (min) | | $R_s$ [a] |
|---|---|---|---|
| | Levorphanol | Dextrorphan | |
| 6.0 | 23.96 | 23.96 | 0.00 |
| 7.0 | 18.84 | 18.84 | 0.00 |
| 8.0 | 23.43 | 23.43 | 0.00 |
| 8.5 | 17.29 | 17.55 | 0.16 |
| 9.0 | 23.14 | 23.98 | 5.49 |
| 9.4 | 19.66 | 20.04 | 7.58 |
| 9.8 | 23.74 | 25.05 | 7.99 |

[a] $R_s$, resolution is calculated from $R_s = 1.171(T_2 \text{ to } T_1)/[W_{1(1/2)} + W_{2(1/2)}]$. Where $T_1$ and $T_2$ are the migration times of the two enantiomers and $W_{1(1/2)}$ and $W_{2(1/2)}$ are peak widths of each peak at half of the peak height.

an improvement of the enantiomeric resolution and a lengthening of the migration time which was obtained by increasing the borate concentration. A concentration higher than 50 m*M* borate buffer resulted in better resolution, but at the expense of significantly longer run times such as 30 min. Therefore, based on the results of our experiments, we decided that a 50 m*M* concentration of borate buffer was the optimal buffer concentration. When a 50 m*M* phosphate buffer at pH 9.4 was used instead of borate buffer in order to study the effect of the type of the running buffer, enantiomeric separation was not observed. It appears that a borate buffer is essential for chiral resolution. The borate anion may complex with the vicinal hydroxyl group of the analyte. This complex would have a greater net negative charge, and its motion would be retarded due to the increased electrophoretic attraction to the anode [15].

Another approach to the enantiomeric separation of racemorphan involves addition of chiral selector to the running buffer. CDs have been widely used as chiral selectors for enantioseparation in CE. Enantiomeric separation depends on the type of CD. The cavity diameter and hydrophobic nature of the internal portion of the CD probably influenced the effective or differential inclusion-complex formation of the enantiomers, leading to differential migration and chiral separation. In this study, α-, β- and γ-CDs, DM-β-CD, methyl-β-CD and HP-β-CD were investigated for the enantioseparation of the racemorphan using 50 m*M* borate buffer (pH 9.4) with 50 m*M* SDS containing 10 m*M* of each CD. As shown in Table 2, baseline separation was achieved using β-CD, partial separation was observed with γ-CD and HP-β-CD, but no separation was achieved with α-CD, DM-β-CD and methyl-β-CD. Also the effect of CD concentration on the migration time and enantioseparation was investigated using β-CD over the concentration range of 0 to 60 m*M*. When increasing the concentration of β-CD in the running buffer, the resolution was improved and the migration time shortened. A more stable inclusion complex of the solute with CD is formed at higher CD concentrations and therefore it migrates faster. At concentrations higher than 10 m*M* of β-CD, however, peak shapes were not symmetrical. Furthermore, when we used high concentrations of β-CD, we noticed that β-CD tended to be easily precipitated. This precipitation caused clogging of the capillary and therefore the reproducibility dropped dramatically. Therefore we chose 10 m*M* as optimal concentration of β-CD in our study.

Table 2
Enantiomer separation racemorphan with cyclodextrin (CD) type[a]

| CD type | Migration time (min) | | $R_s$ |
|---|---|---|---|
| | Levorphanol | Dextrorphan | |
| α-CD | 19.75 | 19.75 | 0.00 |
| β-CD | 19.26 | 19.73 | 7.90 |
| γ-CD | 11.56 | 11.97 | 6.89 |
| Methyl-β-CD | 16.66 | 16.66 | 0.00 |
| HP-β-CD | 16.75 | 16.92 | 4.00 |
| DM-β-CD | 15.87 | 15.87 | 0.00 |

[a] Separation solution, 50 m*M* borate buffer (pH 9.4) containing 50 m*M* SDS, 10 m*M* cyclodextrin, and 20% 1-propanol.

Although SDS was not required to attain enantiomer separation, enantioseparation was also affected by concentration of SDS, in the CD-MEKC mode. Especially enantioseparation of neutral hydrophobic compounds with neutral CDs requires the addition of surfactants, such as SDS, to the CD run buffer. SDS monomers can have their hydrophobic tails concluded in the CD cavity along with the solute. This could change the nature of the solute and CD interaction and consequently, the resolution. It is also significant that the increased fraction of the solute partitioning into the SDS micellar phase at higher SDS concentrations delays the migration time of the solute. We, therefore, tested the effect of SDS by

Table 3
Effect of SDS concentration on the resolution of racemorphan[a]

| SDS concentration (m*M*) | Migration time (min) | | $R_s$ |
|---|---|---|---|
| | Levorphanol | Dextrorphan | |
| 10 | 10.70 | 10.70 | 0.00 |
| 30 | 13.45 | 13.54 | 1.04 |
| 50 | 19.27 | 19.61 | 2.22 |
| 70 | 26.13 | 26.79 | 4.57 |

[a] Separation solution, 50 m*M* borate buffer (pH 9.4) containing 10 m*M* β-CD and 20% 1-propanol.

changing the concentration from 10 to 70 m*M*. As shown in Table 3, when the concentration of SDS was higher than 30 m*M*, the racemorphan started to separate. However, when the concentration of SDS was higher than 50 m*M*, the sensitivity diminished (data not shown) and the migration time increased although the resolution was improved. Based on these results, we decided to choose 50 m*M* concentration of SDS in the buffer system.

The resolution in MEKC can be improved by modifying the buffer by adding some short-chain alcohols, which decrease the electroosmotic flow (EOF) and affinity of the hydrophobic solute for the micellar phase [16,17]. Many organic solvents are useful to modify the buffer in MEKC. These include methanol, propanol, acetonitrile, and others. A review of the properties of CDs [18] reported a personal communication that claimed that β-CD was more soluble in aqueous systems containing up to 30% ethanol or 1-propanol than in water alone. Therefore we tested enantioseparation using the SDS–β-CD system at pH 9.4 modified by adding each of 1-propanol, methanol or acetonitrile at various concentrations. Enantiomer separation was obtained with addition of methanol, acetonitrile and 1-propanol at the concentration of 20%, but with methanol and acetonitrile, peaks were broad and the migration time was too long. The results is shown in Fig. 1 and the best enantiomer separation of racemorphan was observed with 20% of 1-propanol in the buffer system. Perhaps 1-propanol was included in the CD cavity causes the improvement of the selectivity. The addition of urea is also known to be effective for the improvement of selectivity in CD-MEKC [19]. However, in this study, the addition of urea was not effective for the separation.

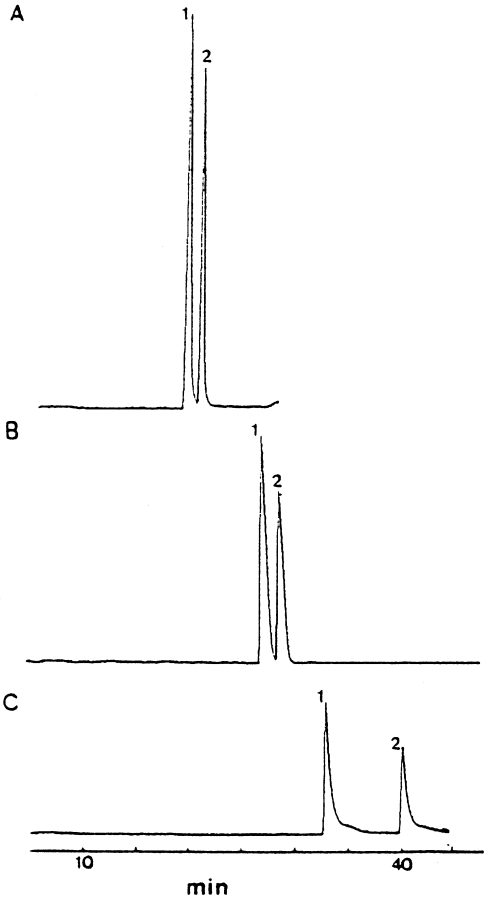


Fig. 1. Influence of different organic solvents on the resolution of racemorphan. The running buffer contained 20% (v/v) organic solvent. Buffer: 50 m*M* borate buffer (pH 9.4) containing 50 m*M* SDS and 10 m*M* β-CD; capillary: 57 cm×50 μm I.D.; UV detection at 192 nm. (A) 1-Propanol, (B) methanol, (C) acetonitrile. Peaks: 1=levorphanol, 2=dextrorphan.

The effect of temperature on enantioseparation was investigated. A rise in temperature causes an increase in EOF because it decreases the viscosity of the buffer. Thus, we studied the influence of the temperature on enantioseparation from 20 to 35°C. When the temperature was increased from 20 to 30°C, the resolution was improved and the migration time was also decreased. However, when the temperature was higher than 30°C, the resolution and sensitivity was dropped as shown in Fig. 2. Therefore, we chose 30°C as optimal temperature in this study.

We also studied peak sensitivity by changing UV

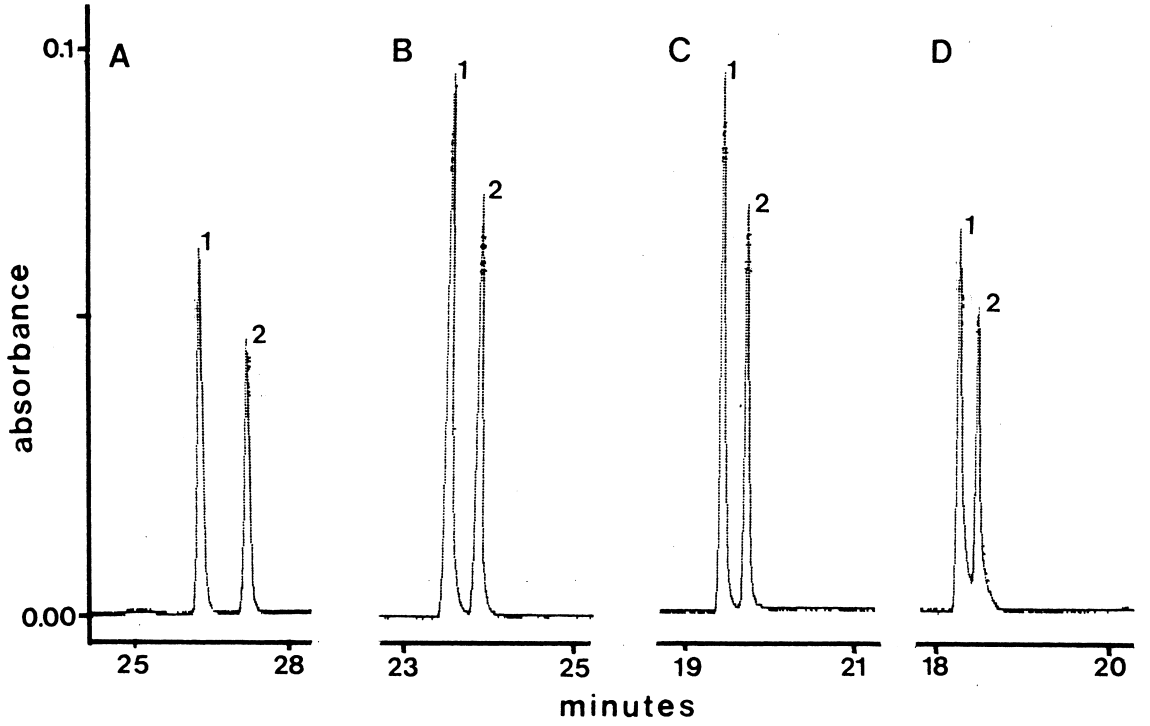


Fig. 2. Effects of temperature on the separation of racemorphan. (A) 20°C, (B) 25°C, (C) 30°C, (D) 35°C. Buffer: 50 m*M* borate buffer (pH 9.4) containing 50 m*M* SDS, 10 m*M* β-CD and 20% 1-propanol; capillary: 57 cm×50 μm I.D.; UV detection at 192 nm. Peaks: 1=levorphanol, 2=dextrorphan.

detection wavelength from 254 to 192 nm. As the wavelength was changed from 254 to 192 nm, the peak sensitivity increased. As shown in Table 4, the maximum peak sensitivity was obtained at 192 nm and that showed about a three-fold improvement compared to the previous papers [1,10].

Under optimized separation conditions, the reproducibility and the detection limit were tested. Precision of the method using an injection of 3 s at a pressure of 0.5 p.s.i. was studied at concentrations of 200 μg/ml. Pseudoephedrin (200 μg/ml) was added as an internal standard. The relative standard deviations (RSDs) for migration time and peak area were 0.2% and 4.0%, respectively. Therefore the reproducibility can be considered to be satisfactory for quantitative and qualitative analysis.

The detection limit for the racemorphan was calculated from electropherograms obtained after sample preparation of human urine spiked with racemorphan. Taking a signal-to-noise ratio of 3 as a

Table 4
Impact of wavelength on the sensitivity of racemorphan

| Wavelength (nm) | Levorphanol ($H/W_{1/2}$)[a] | Dextrorphan ($H/W_{1/2}$) |
|---|---|---|
| 192 | 80 | 103.75 |
| 200 | 47.0 | 45.6 |
| 214 | 42.0 | 41.1 |
| 230 | 16.7 | 15.0 |
| 254 | 3.0 | 2.2 |

[a] $H$ is the peak height of each peak, $W_{1/2}$ is the peak width of each peak at the half-height. Conditions: 50 m*M* borate buffer (pH 9.4) containing 50 m*M* SDS, 10 m*M* β-CD, and 20% 1-propanol.

criterion, the limit of detection of this method was found to be 30 ng/ml for both dextrorphan and levorphanol.

### 3.3. Determination of racemorphan in urine

In CD-MEKC, we used solid-phase extraction with a $C_{18}$ Sep-Pak cartridge for sample concentration and clean-up. Pretreatment of the acid-hydrolyzed urine sample was accomplished by extraction with diethyl ether, and the clean-up process was done through a solid-phase extraction [10,20]. The final product was analyzed by CD-MEKC after it was dissolved in water.

First, to distinguish whether levorphanol or dextrophan is present in the urine sample from an athlete for doping control, we examined the sample by applying the optimal separation condition of CD-MEKC after sample pretreatment. Fig. 3A shows the electropherogram of CD-MEKC analysis. From increased peak by spiking with dextrophan and levorphanol standard, we could confirm dextrophan in the urine sample from the athlete (Fig. 3B). The dextrophan fraction was collected between 19 and 24 min after injection and the collection was performed several times repeatedly into 10 μl of buffer in collection vials of the CE system. The collected sample was mixed to 5 μl of the matrix solution. After crystallization, MALDI-TOF-MS analysis of the sample was performed for dextrophan confirmation. Fig. 3C shows the MALDI mass spectrum of the off-line MALDI-TOF-MS analysis. The molecular ion peak consisted of $[M+H]^+$. From this result, we confirmed the peak with the exact molecular mass of dextrophan ($m/z$ 257.3).

To determine the racemorphan from human urine, a blank human urine sample was examined by spiking the racemorphan standard with a concentration of 30 μg/ml. The separation of racemorphan in human urine is illustrated in Fig. 4A. By using the developed CD-MEKC method, enantiomeric separation of racemorphan from a human urine sample is possible. And the method is sensitive enough to use for routine doping control analysis. Also, a volunteer was administered cough syrup which contained 12.5 mg of dextromethorphan hydrobromide, a urine sample from the volunteer was collected, and then

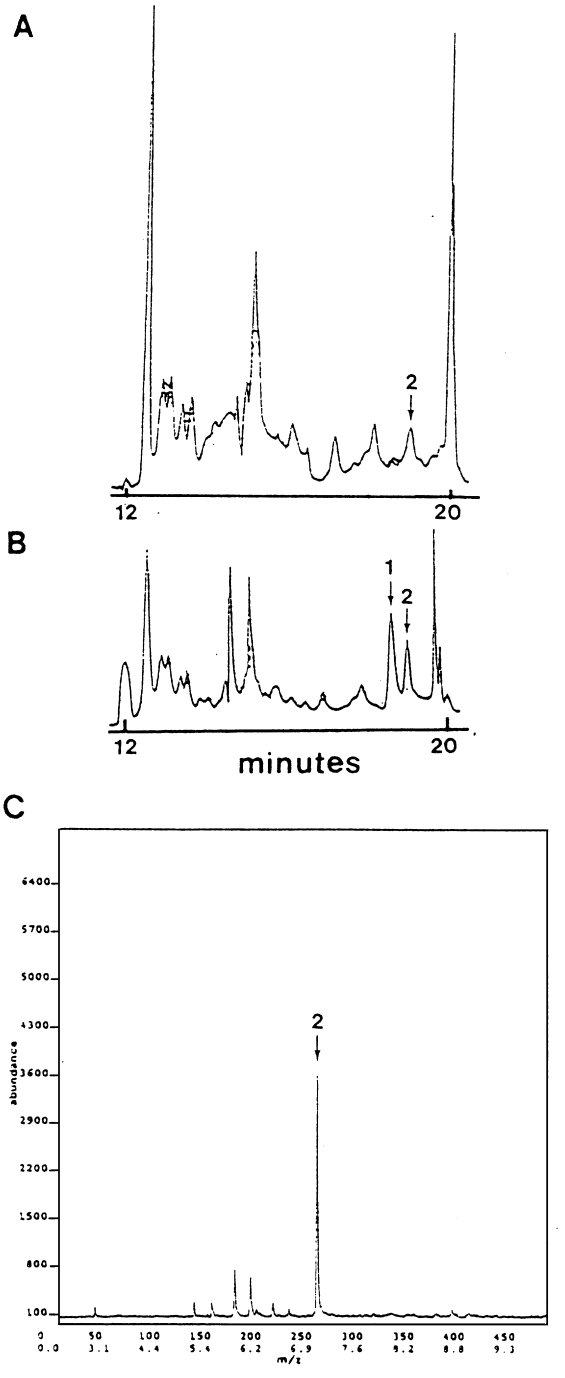


Fig. 3. Electropherograms. (A) Urine sample from an athlete. (B) Urine sample spiked with racemorphan. Conditions as in Fig. 2. (C) MALDI-TOF mass spectra of dextrorphan. Peaks: 1= levorphanol, 2=dextrorphan.

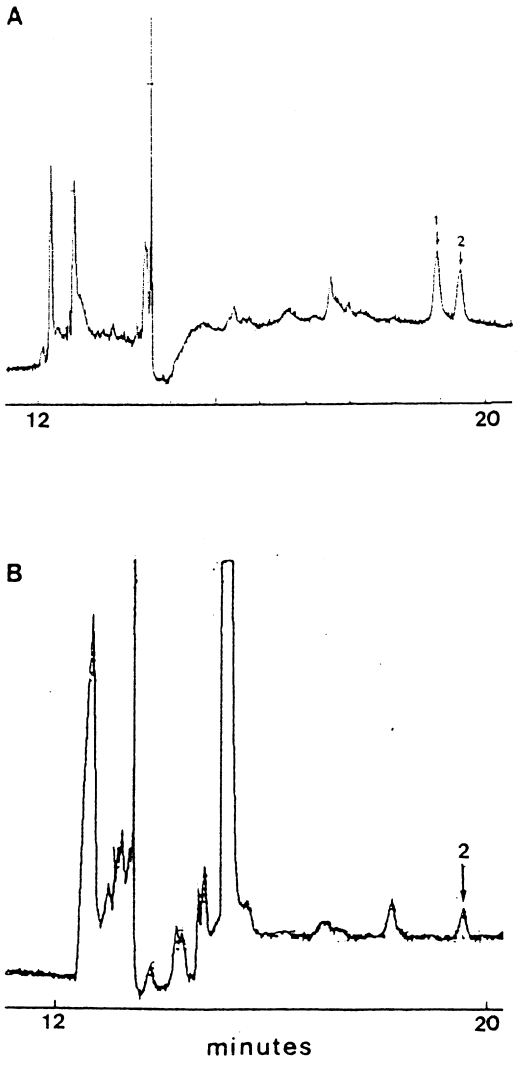


Fig. 4. Electropherograms. (A) Urine sample spiked with 0.3 μg/ml of levorphanol and dextrorphan. (B) Urine sample from volunteer administered cough syrup which contains dextromethorphan hydrobromide. Conditions as in Fig. 2. Peaks: 1=levorphanol, 2=dextrorphan.

analyzed by using the developed CD-MEKC method (Fig. 4B).

## 4. Conclusions

We studied the optimal conditions for enantiomeric separation of racemorphan using CD-MEKC as a routine analysis method. Because the previous separation method of Awmatell and Well [1] gave problems such as clogging of the capillary column due to a high concentration of β-CD and lengthening the analysis time by a long capillary column, this method could not be applied to a routine analysis and needed to be optimized. The investigations to optimize the conditions in this study involved changing CD type and concentration, buffer type and concentration, concentration of organic solvent, temperature and detector wavelength. The results of our study showed that racemorphan was successfully separated without clogging the capillary under the developed analysis conditions which used 10 m*M* β-CD. We obtained a reasonable analysis time of less than 20 min using a 50 cm capillary column, which is appropriate for a routine analysis of a lot of samples. Also, by the adjustment of buffer pH to 9.4 and changing the wavelength to 192 nm, we obtained some increase of sensitivity for about one-and-a-half- and three-times, respectively. On the basis of the above results, the optimal conditions for enantiomeric separation of racemorphan using CD-MEKC were developed as follows with high sensitivity, good reproducibility, and short analysis time: 50 m*M* borate buffer containing 50 m*M* SDS, 10 m*M* β-CD, and 20% 1-propanol at pH 9.4; 57 cm×50 μm I.D. fused-silica capillary; 192 nm wavelength UV detection; 50 μA (22 kV) power; and a running temperature of 30°C. By using the above conditions the enantiomeric separation of racemorphan was successfully performed. The method was applied to human urine samples, and confirmed by MALDI-TOF-MS.

## Acknowledgements

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## References

[1] A. Aumatell, R.J. Well, J. Chromatogr. Sci. 31 (1993) 502.
[2] B. Calensnik, J.A. Chistensen, Clin. Phamacol. Ther. 8 (1967) 374.

[3] T. Zysset, T. Zeugin, A. Kupfer, Biochem. Phamacol. 37 (1988) 3155.
[4] T.J. Ward, Anal. Chem. 66 (1994) 633A.
[5] F.A. Maris, R.J.M. Vervoort, H. Hindriks, J. Chromatogr. 547 (1991) 45.
[6] A. Venema, H. Henderiks, J. High Resolut. Chromatogr. 14 (1991) 676.
[7] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 603 (1992) 235.
[8] R. Weiberger, in: Practical Capillary Electrophoresis, Academic Press, New York, 1993, Ch. 7.
[9] J.P. Landes, in: Handbook of Capillary Electrophoresis, 2nd ed, CRC Press, Boca Raton, FL, 1997, Ch. 3.
[10] S. Li, K. Fried, I.W. Wainer, D.K. Lloyd, Chromatographia 35 (1993) 216.
[11] Y.S. Yoo, Y.S. Han, M.J. Suh, J.S. Park, J. Chromatogr. A 763 (1997) 285.
[12] M.J. Suh, Y.S. Kim, Y.S. Yoo, J. Chromatogr. A 781 (1997) 263.
[13] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barhart, L.R. Alexander, D.J. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23.
[14] H. Nishi, T. Fukuyama, S. Terabe, J. Chromatogr. 553 (1991) 503.
[15] J.E. Noroski, D.J. Mayo, M. Moran, J. Pharm. Biomed. Anal. 13 (1995) 45.
[16] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 609 (1992) 363.
[17] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[18] M.L. Bender, M. Komyama, Cyclodextrin Chemistry, Springer, Berlin, 1978.
[19] S. Terabe, Y. Ishihama, H. Nishi, F. Fukuyama, K. Otska, J. Chromatogr. 545 (1991) 359.
[20] M. Wenk, B. Keller, F. Fellath, J. Pharm. Biomed. Anal. 9 (1991) 341.