# Direct chiral resolution of tartaric acid in food products by ligand exchange capillary electrophoresis using copper(II)–D-quinic acid as a chiral selector

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## Abstract

Chiral resolution of native DL-tartaric acid was performed by ligand-exchange capillary electrophoresis using copper(II)–D-quinic acid as a chiral selector. Factors affecting chiral resolution, migration time, and peak area of tartaric acid were studied. The running conditions for optimum separation of tartaric acid were found to be 1 m$M$ copper(II) sulfate–10 m$M$ D-quinic acid (pH 5.0) with an effective voltage of $-15$ kV at 30°C, using direct detection at 250 nm, and resolution of racemic tartaric acid was approximately 1.3. With this system, chiral resolution of DL-tartaric acid in food products was conducted successfully.   © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Enantiomer separation; Food analysis; Ligand-exchange capillary electrophoresis; Tartaric acid; Quinic acid

## 1. Introduction

Tartaric acid has two asymmetric carbons. L-Tartaric acid is distributed in plants, especially grapes. D- and *meso*-tartaric acids have not been found to occur in nature. In Japan, L- and racemic (DL-) tartaric acids can be legally used in food products provided that they are declared on the label. Tartaric acid is not restricted in quantity of use in the food manufacturing process, so its amount in the final product is not clear. Thus, the enantiomeric determination of tartaric acid as a food additive is very important for the safety and quality control of food products.

It is well known that L-tartrate is oxidized to oxaloacetate in the presence of L-tartrate dehydrase [1]. In the presence of divalent metal ions, oxaloacetate is partly decarboxylated to pyruvate. Both oxo acids react with 2,4-dinitrophenylhydrazine and form 2,4-dinitrophenylhydrazones, which can be spectrophotometrically measured at 540 nm. However, to our knowledge, there is no enzymatic method that can specifically measure D-tartrate. High-performance liquid chromatography (HPLC) techniques on the chiral resolution of tartaric acid using the chiral ligand-exchange principle have been reported [2–5]. However, most of these techniques were not used to enantioseparate tartaric acid in food products.

Capillary electrophoresis (CE) is a recently de-

veloped powerful analytical technique with a wide range of applications. Since Davankov and Roghozin in 1971 [6] first reported ligand-exchange chromatography, this separation principle has become a very useful method for the separation of amino acids by HPLC. Chiral resolution in CE was first introduced by Zare and co-workers [7,8]. Based on the chiral ligand-exchange principle, they used L-histidine– or aspartame–copper(II) complexes as chiral selectors for the resolution of dansylated amino acids. Further investigations with ligand-exchange CE were reported by many researchers [9–16]. In chiral ligand-exchange CE, copper(II) complexes with amino acids or their derivatives have been used as chiral selectors for the enantioseparation of amino acids, their derivatives and α-hydroxy acids. In aqueous solution, four water molecules are arranged differently, namely, in the form of a planar square. Amino acids are bidentate ligands with a high affinity for copper(II). In the presence of amino acids as ligands, water molecules located in the square planar position are replaced by either one or two molecules of amino acids, forming copper(II)–amino acid complexes as chiral selectors at ratios varying from 1:1 to 1:2 depending on the amino acid concentrations [10,16]. Based on the ligand-exchange principle, when DL-amino acids are introduced, they will exchange ligands to form a ternary copper(II) complex with mixed ligands. As described above, in ligand-exchange CE, amino acids and their derivatives have been used as chiral ligands so far. However, it seems that the selection of the ligand is the most important factor. Recently, we have reported that malic acid is enantioseparated by ligand-exchange CE using copper(II)–L-tartrate as a chiral selector [17]. Use of various selectors, other than a copper(II)–amino acid complex, may extend the applications of chiral ligand-exchange CE.

In order to enantioseparate tartaric acid, one of the α-hydroxy acids, by ligand-exchange CE, we used D-quinic acid (Fig. 1), another α-hydroxy acid, as a chiral ligand. This paper describes a direct ligand-exchange resolution method for the separation of tartrate enantiomers by CE using copper(II)–D-quinic acid as a chiral selector. Parameters influencing the migration time, peak area and resolution of tartaric acid were defined, and the proposed method was applied in the chiral resolution of tartaric acid in food products.
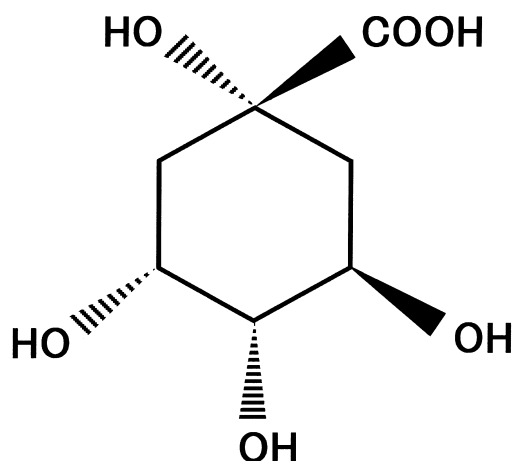


Fig. 1. Structure of D-quinic acid.

## 2. Materials and methods

### 2.1. Chemicals

D-, L- and DL-Tartaric acids, corresponding to $(S,S)$-, $(R,R)$- and racemic tartaric acids, respectively, and other chemicals (analytical grade) were purchased from Wako (Osaka, Japan).

### 2.2. Apparatus for CE

Electrophoretic experiments were carried out using a capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany). Samples were injected by pressure (25 mbar, for 2 s). The separations were performed in a poly(vinylalcohol) (PVA)-coated bubble cell capillary of 48.5 cm (effective length 40 cm)×50 µm I.D. (Agilent Technologies). The capillary was kept at 30°C. The analytes were detected at 250 nm. The power supply was operated in the constant-voltage mode, at −5 kV, and the substances migrated towards the positive pole.

### 2.3. Buffer and sample preparation

The background electrolyte (BGE) in the electrophoretic experiments, unless stated otherwise, was composed of 1 m$M$ copper(II) sulfate and 10 m$M$ D-quinic acid. The final pH of the BGE was adjusted to 5.0 by adding 0.1 $M$ sodium hydroxide solution and was filtered with a 0.22-µm filter before use.

Purified water was prepared using a Toray Ultra Pure Water System (Mishima, Japan).

Stock solutions of 100 m*M* D-, L- and DL-tartaric acids were individually prepared in purified water, stored at 4°C and diluted to 1 m*M* before use.

Three brands of grape juices, three wines, three soft drinks, three sakes, two cooking sakes, two jams, two types of candies, three types of tablet candies and three brands of pickles were purchased from a local market. These samples, except for the grape juices and the wines, were labeled as having added acidulants. Jams were diluted 10-fold with purified water. Candies and tablet candies were crushed and were diluted 20- and 50-fold, respectively. These diluted samples were centrifuged at 3000 rpm for 5 min and the supernatants were filtered with 0.22-μm filters. Grape juices, wines, soft drinks, sakes, cooking sakes, and the seasoning liquids of the pickles were diluted 10-, 30-, 3-, 3-, 3- and 4-fold with purified water, respectively, and were filtered with 0.22-μm filters. For recovery examination, 0.5 m*M* (75 mg l$^{-1}$) racemic tartaric acid was added to 20-fold diluted grape juice and 60-fold diluted wine samples.

## 2.4. Calculation of resolution

The resolution ($R_s$) of the enantiomer was calculated by using the following equation:

$$R_s = 2(t_2 - t_1)/(w_1 + w_2)$$

where $t$ is the migration time, and $w$ is the width of the peak at the baseline.

## 3. Results and discussion

### 3.1. Factors affecting chiral separation

A PVA-coated capillary, in which the electroosmotic flow is almost completely suppressed, was used. Since the carboxyl groups of tartaric acid, with a p$K_{a1}$ of 2.93 and a p$K_{a2}$ of 4.23, are dissociated in the BGE at pH 5.0, the analyte migrates electrophoretically to the anode. Fig. 2 shows the effect of D-quinic acid concentration on the resolution, migration time and peak area of tartaric acid under the conditions where the concentration of copper(II)
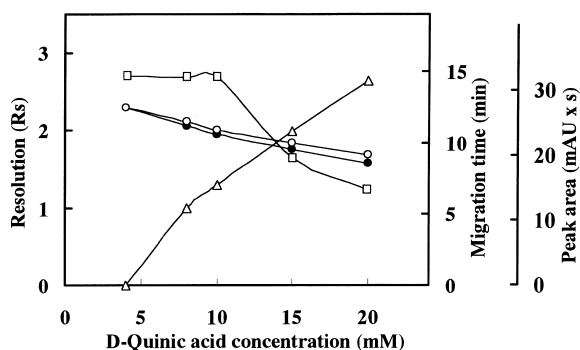


Fig. 2. Effect of the concentration of D-quinic acid on the enantiomeric resolution, migration time and total peak area of D- and L-tartaric acids. Racemic tartaric acid (1 m*M*) was analyzed by ligand-exchange CE. The BGE (pH 5.0) was composed of various concentrations of D-quinic acid containing 1 m*M* copper(II) sulfate. (△) Resolution ($R_s$); (○) migration time of L-tartrate; (●) migration time of D-tartrate; (□) total peak area of D- and L-tartrates.

sulfate was fixed at 1 m*M*. An increase in the concentration of D-quinic acid brought about an increase in the resolution of D- and L-tartaric acids, but caused a decrease in the migration time of the racemate. The total peak area of the D- and L-tartaric acids was kept constant with increasing D-quinic acid concentration up to 10 m*M* and then tended to decrease.

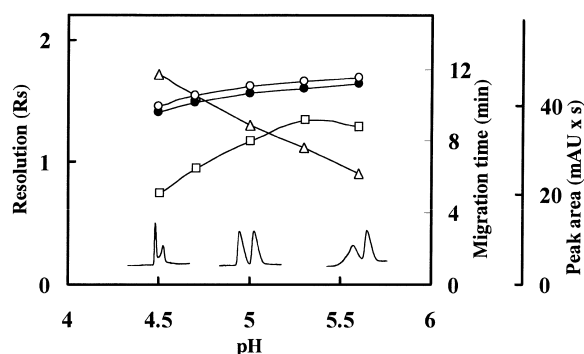Fig. 3 shows the effect of pH of BGE on the



Fig. 3. Effect of pH of the BGE on the enantiomeric resolution, migration time and total peak area of D- and L-tartaric acids. Racemic tartaric acid (1 m*M*) was analyzed by ligand-exchange CE. The BGE was composed of 1 m*M* copper(II) sulfate and 10 m*M* D-quinic acid. (△) Resolution ($R_s$); (○) migration time of L-tartrate; (●) migration time of D-tartrate; (□) total peak area of D- and L-tartrates. The inserted peak shapes of D- and L-tartaric acids were obtained by using BGE at pH 4.5 (left), 5.0 (middle) and 5.6 (right).

resolution, migration time and peak area of tartaric acid. The BGE was composed of 1 m$M$ copper(II) sulfate and 10 m$M$ D-quinic acid. In the pH range 4.5–5.6, the carboxyl groups of the analyte are dissociated. A higher pH resulted in a decrease in the resolution of tartaric acid, but brought about increases in both migration time and total peak area of the racemate. The electropherograms of DL-tartaric acid at pH 4.5, 5.0 and 5.6 are also shown in Fig. 3. The peak shapes of both D- and L-tartaric acids were significantly affected by pH of the BGE. The peaks of both D- and L-tartaric acids were relatively symmetrical at pH 5.0, but not pH 4.5 and 5.6. The peak shapes of both D- and L-tartaric acids may depend on the compositions of complexes formed by copper(II)–D-quinate with D- or L-tartrate at various pH values. Copper(II) can form a greater variety of complexes with L-tartaric acid (T:$C_4H_4O_6^{2-}$) than it can with an amino acid [18]. Such complexes include not only CuT, CuTH and $CuT_2$, but also $Cu_2T_2$, $Cu_2T_2H_{-1}$, $Cu_2T_2H_{-2}$, $Cu_4T_3H_{-5}$, $Cu_8T_6H_{-10}$. The composition of these complexes depends on the pH and the concentration of L-tartaric acid. Although the compositions of complexes formed by copper(II)–D-quinate with D-tartrate or L-tartrate are not known, it is likely that the composition of the complex formed by copper(II)–D-quinate–D-tartrate is different from that formed by copper(II)–D-quinate–L-tartrate.

Therefore, the optimum BGE conditions for both high resolution, short migration time and large peak area were found to be 1 m$M$ copper(II)–10 m$M$ D-quinate (pH 5.0) with an effective voltage of $-15$ kV at 30°C.

## 3.2. Analysis of DL-tartaric acids

Foods contain various ingredients including carbohydrates, free amino acids, organic acids and hydrophobic compounds. In the proposed CE method, only acidic substances migrated towards the positive pole and detector, because a PVA-coated capillary was used. We determined whether nine acidic substances, which are or might be included in food products, interfered with the chiral analysis of tartaric acid (Table 1). It was found that the migration times of both D- and L-tartaric acids were different from those of the nine substances tested and that none of the

Table 1
The migration times of D-, L-tartaric acids and nine acidic substances

| Substance | Migration time (min) |
|---|---|
| Fumaric acid | 4.33 |
| Pyruvic acid | 5.34 |
| Succinic acid | 6.64 |
| Citric acid | 6.90 |
| Acetic acid | 6.96 |
| DL-Glyceric acid | 7.34 |
| DL-Lactic acid | 7.58 |
| D-Tartaric acid | 10.63 |
| L-Tartaric acid | 10.98 |
| DL-Malic acid | 12.68 |
| meso-Tartaric acid | 13.14 |

chiral compounds in these nine substances (DL-lactic acid, DL-malic acid and DL-glyceric acid) were enantioseparated by the proposed method.

Racemic tartaric acid (0.03–2 m$M$) was subjected to the CE method under the above described optimum conditions. The calibration was performed with racemic compound and the calibration of the individual enantiomers was calculated from these data. Linearity ($r^2>0.999$) was demonstrated in the range 0.015–1 m$M$ (2.25–150 mg l$^{-1}$) by standard curves of each D- and L-tartaric acid. The precision of five consecutive determinations was evaluated at 1 m$M$ for racemic tartaric acid. Good reproducibilities of peak area (RSD<2.5%) and migration time (RSD<0.3%) of both D- and L-isomers were obtained. Good recoveries (97–100%) of D- and L-tartaric acids were attained.

Using the proposed CE method, D- and L-tartaric acids in the three grape juices and three red wines were determined and the representative results are shown in Fig. 4. L-Tartaric acid was detected at the level of 786–806 mg l$^{-1}$ for grape juices and at 1240–3000 mg l$^{-1}$ for red wines, but D-tartaric acid was not detected in these samples. D- and L-Tartaric acids in three soft drinks, three sakes, two cooking sakes, two jams, two candies, three tablet candies and three pickles, which were labeled as having added acidulants, were also analyzed. L-Tartaric acid, but not D-tartaric acid, was detected in a cooking sake at 53 mg l$^{-1}$ and in a tablet candy at 5540 mg kg$^{-1}$. Neither D- nor L-tartaric acids were detected in the other food products.
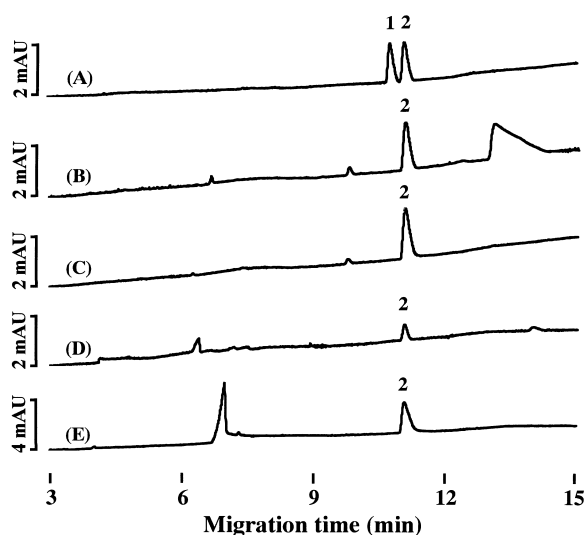
Fig. 4. Electropherograms of grape juice, red wine, cooking sake and tablet candy. (A) Standard solution (1 m*M* racemic tartaric acid); (B) grape juice diluted 10-fold; (C) red wine diluted 30-fold; (D) cooking sake diluted threefold; (E) tablet candy diluted 50-fold. (1) D-Tartaric acid, (2) L-tartaric acid.

In conclusion, direct chiral resolution of DL-tartaric acid was conducted by ligand-exchange capillary electrophoresis using copper(II)–D-quinic acid as a chiral selector. With this system, D- and L-tartaric acids in food products were analyzed successfully. Use of such a selector, other than a copper(II)–amino acid complex, may extend the applications of chiral ligand-exchange capillary electrophoresis.

## References

[1] L.D. Kohn, R.E. Hurlbert, W.B. Jakoby, in: H.U. Bergmeyer (Ed.), 2nd ed., Methods of Enzymatic Analysis, Vol. 3, Verlag Chemie–Academic Press, Weinheim–New York, 1974, p. 1397.
[2] R. Hirokawa, H. Sakamoto, T. Tanimura, J. Liq. Chromatogr. 9 (1986) 537.
[3] H. Katoh, T. Ishida, Y. Baba, H. Kiniwa, J. Chromatogr. 473 (1989) 241.
[4] N. Oi, H. Kitahara, R. Kira, J. Chromatogr. 592 (1992) 21.
[5] G. Galaverna, F. Panto, A. Dossena, R. Marchelli, F. Bigi, Chirality 7 (1995) 331.
[6] V.A. Davankov, S.V. Rogozhin, J. Chromatogr. 60 (1971) 280.
[7] E. Gassmann, J.E. Kuo, R.N. Zare, Science 230 (1985) 813.
[8] P. Gozel, E. Gassmann, H. Michelsen, R.N. Zare, Anal. Chem. 59 (1987) 44.
[9] A. Desiderio, Z. Aturki, S. Fanali, Electrophoresis 15 (1994) 864.
[10] N.G. Sundin, T.M. Dowling, N. Grinberg, G. Bicker, J. Microcol. Sep. 8 (1996) 323.
[11] M.G. Schmid, G. Gubitz, Enantiomer 1 (1996) 23.
[12] T. Horimai, M. Ohara, M. Ichinose, J. Chromatogr. A 760 (1997) 235.
[13] Z. Chen, J.-M. Lin, K. Uchiyama, T. Hobo, J. Chromatogr. A 813 (1998) 369.
[14] M.G. Schmid, O. Lecnik, U. Sitte, G. Gubitz, J. Chromatogr. A 875 (2000) 307.
[15] A. Karbaum, T. Jira, J. Chromatogr. A 874 (2000) 285.
[16] Z. Chen, K. Uchiyama, T. Hobo, Anal. Sci. 16 (2000) 837.
[17] S. Kodama, A. Yamamoto, A. Matsunaga, T. Soga, K. Hayakawa, Electrophoresis 22 (2001) 3286.
[18] L. Johansson, Acta Chem. Scand. A34 (1980) 495.