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Short Communication

Ligand-exchange ion chromatographic determination of malic acid enantiomers in apple juice with photometric detection

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ABSTRACT

An ion chromatographic separation with photometric detection using a chiral copper(II) complex as the eluent has been developed for the resolution of enantiomers of malic acid in commercially available apple juices. The results obtained by this method were in good agreement with those by an enzymatic method with separation by high-performance liquid chromatography.

INTRODUCTION

Chiral organic compounds usually exist in nature in only one enantiomeric form. For example, malic acid in pure apple juices is wholly in the L-form [1,2]. In Japan, a synthetic racemic mixture of malic acid may be used as a food additive to reduce production costs, although concentrations of the respective enantiomers in the commercial product have rarely been reported.

A time-consuming enzymatic method with separation by high-performance liquid chromatography (HPLC) has been used for the determination of malic acid enantiomers [1,2]. Direct HPLC separation of these enantiomers has been also attempted [3–7]. However, complicated post-column reaction requirements still prevent this method from being applied to real samples. Direct detection after chromatographic separation has been reported [8], but the system could not simultaneously determine the enantiomers in actual samples as a result of the presence of interfering peaks.

A simple, selective system has been reported [9] for the direct separation and detection of malic acid enantiomers by ligand-exchange photometric ion chromatography (PIC). This paper reports the application of this system to the determination of these enantiomers in commercially available apple juices and sour drinks.

EXPERIMENTAL

The PIC apparatus and reagents were as described previously [9]. The enantiomeric separation

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was performed with a 5 cm × 4.6 mm I.D. anion-exchange column (TSK gel IC-Anion-PW, Tosoh, Tokyo, Japan) maintained at 40°C. An eluent containing 1.5 m*M* copper(II) hydroxide and 3 m*M* L-tartaric acid (adjusted to pH 4.8 with sodium hydroxide solution) was delivered at a flow-rate of 0.8 ml/min. The detection wavelength was 283 nm.

Eleven commercially available apple juices and five sour drinks were analysed. The only pretreatment of the sample was dilution with the eluent, followed by ultrafiltration through a Tosoh Ultracent-10 disposable cartridge. A 30-μl volume of the ultrafiltrate was injected into the PIC system.

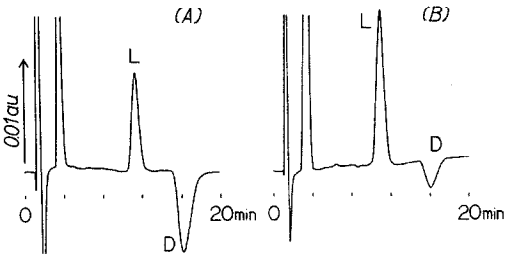

Fig. 1. Photometric ion chromatograms of (A) standard L- and D-malic acid (30 nmol each) and (B) a commercially available apple juice (No. 4 in Table I) diluted twenty-fold with eluent. Peaks: L = L-malic acid; D = D-malic acid.

RESULTS AND DISCUSSION

Previous studies [9] of the separation and resolution of malic acid enantiomers had shown that an increase for copper(II) hydroxide and L-tartaric acid in the eluent led to a reduction in the time required for the analysis, although the sensitivity was lower. An increase in the molar ratio of copper to tartrate or in the pH value of the eluent led to an improvement in the separation of the enantiomers, although the elution of the analyte was delayed. Consequently, an aqueous solution containing 1.5 m*M* copper hydroxide and 3 m*M* L-tartaric acid was chosen as the eluent. Under these conditions, L-malic acid eluted before the D-isomer and the D-isomer gave a negative peak at all pH values, although the L-isomer showed an inversion of the peak direction as the pH increased. When the pH of the eluent was 4.8, both the peak height of L-malic acid and the peak depth of D-malic acid were approximately equal and linear calibration graphs were obtained for a plot of the peak areas *versus* the amount injected in the range 3–100 nmol. Fig. 1A shows a typical chromatogram of a standard solution. The standard deviation for repeated injections of this mixture was less than 2% for each enantiomer. Care must be taken when performing successive injections as the system peak appears at about 50 min. However, this peak can be removed from the chromatogram by a column-switching procedure [10], in which the pretreatment column is joined to the anion-exchange column by a switching valve.

In this system, peaks are only obtained with a compound such as malic acid which has a complex formation constant with copper similar to that of tartrate. This PIC method, highly specific for malic

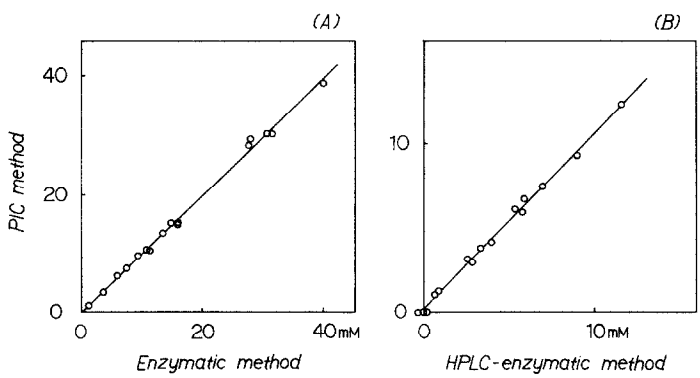

Fig. 2. Comparison of the results obtained for malate enantiomers by the proposed PIC and enzymatic methods. (A) L-malic acid, r = 0.998, n = 16; (B) D-malic acid, r = 0.997, n = 15.

TABLE I

CONCENTRATION OF MALIC ACID ENANTIOMERS IN COMMERCIAL FRUIT JUICES

Sample description	Sample No.	Malic acid concentration (g/100 g)			D-isomer (%)
		Total	L-isomer	D-isomer	
Fruit juice (juice = 100%)	1	0.460	0.405	0.055	11.9
	2	0.431	0.393	0.038	8.9
	3	0.517	0.517	nd	0
	4	0.495	0.404	0.091	18.3
	5	0.238	0.197	0.041	17.4
Fruit juice drink (50 ⩽ juice < 100%)	6	0.201	0.201	nd	0
	7	0.478	0.378	0.100	21.0
Fruit soft drink (10 ⩽ juice < 50%)	8	0.260	0.179	0.081	31.3
	9	0.098	0.098	nd	0
	10	0.142	0.128	0.014	9.8
	11	0.266	0.141	0.125	47.0
	12	0.138	0.138	nd	0
Soft drink (0 < juice < 10%)	13	0.366	0.201	0.165	45.1
Sour drink (juice = 0%)	14	0.031	0.015	0.016	51.7
	15	0.096	0.046	0.050	52.2
	16	0.162	0.083	0.079	48.9

acid, does not require any sample pretreatment other than dilution and ultrafiltration. Even with only this simple treatment, no interfering peaks are present, as shown in Fig. 1B.

The proportions of malic acid in commercially available apple juices and sour drinks were determined by both this method and the enzymatic method with HPLC separation. Fig. 2A compares the results for L-malic acid obtained by the PIC and enzymatic methods. Fig. 2B compares the results for the D-isomer obtained by the PIC method and results calculated from the difference between the analytical values obtained using HPLC and the enzymatic method. The results from the proposed PIC method agreed well with those obtained by other methods with correlation coefficients $\geqslant 0.997$.

Sixteen real samples were divided into five groups according to their content of pure fruit juice and the concentrations of malic acid enantiomers were determined (Table I). D-malic acid was detected in twelve samples. Four of the five fruit juices, in which pure apple juice alone was indicated, contained D-malic acid. Approximately the same amounts of malic acid enantiomers were observed in the sour drinks, which contain no fruit juice. Using the proposed method, it was possible to detect synthetic DL-malic acid in adulterated fruit juice at concentrations as low as 10%. The PIC method described here is simple and can be used for the routine detection of apple juice adulteration.

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