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Enantiomeric purity determination by high-performance liquid chromatography with coupled polarized photometric/UV detection
Analysis of adulterative addition of synthetic malic and tartaric acids

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Abstract

A high-performance liquid chromatographic method for determining the enantiomeric purity of malic and tartaric acids with the combined use of UV and polarized photometric detectors in series has been established, in which no physical separation of enantiomers is required. Complexation with molybdate significantly increases the specific rotation of these acids. Thus, coupling polarized photometric detection with anion-exchange chromatography using a molybdate solution as the eluent makes possible the highly sensitive detection of optically active analytes with the conventional system. The proposed method can detect synthetic racemic acids in adulterated fruit juices at the 5% level.

1. Introduction

There are many optically active compounds in foodstuffs. In Japan, several synthetic racemates are approved for use as food additives. In particular, synthetic hydroxy carboxylic acids such as malic and tartaric acids are frequently used as acidulants to reduce production costs. Since each enantiomer might have different effects in biological systems and the safety of an unnatural form is open to question, it is important to determine the enantiomeric purity of the commercial products. Conventional enantiomeric resolution techniques involving liquid chromatography utilize interactions between an analyte and chiral solid and/or mobile phases resulting in diastereoisomeric products, and a racemate separates into two peaks [1–3]. The problem then arises as to how to resolve the racemates and avoid interference from other ingredients in the sample. So far, studies on the simultaneous chiral separation of these hydroxy acids for practical use have apparently not been published. It has been reported that the use of a detector based on measuring the rotation of polarized light combined with another type of

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detection such as UV or refractive index (RI) in series is ideal for the determination of the enantiomeric ratio without the separation of each enantiomer [4,5]. However, to determine the enantiomeric composition of low optically active acids using this method, derivatization is required to increase their optical rotations [6]. In general, besides introducing the chromophore into the chiral centre of the analyte, the formation of a metal complex significantly changes its optical rotation (Pfeiffer effect) [7]. It is known that the optical rotations of sugars [8] and hydroxy acids [9,10] are significantly increased by the formation of molybdate complexes.

Recently, we developed a novel detection method, polarized photometric detection (PPD), for detecting non-chromophoric, optically active analytes photometrically. In this system two polarizers are placed on either side of the UV–visible absorbance detector flow cell [11–13]. PPD can measure the optical rotation as the change in light intensity transmitted through the analyte placed between the polarizers. PPD has a sensitivity similar to a conventional polarimetric detector based on the Faraday effect. It is fortunate that a conventional and popular photometric detector is easily used as the PPD system. We report here the determination of synthetic acidulants based on the UV and PPD responses with the molybdate eluent system.

2. Experimental

All reagents were of guaranteed grade. D-Malic acid was purchased from Aldrich (Milwaukee, WI, USA), L-tartaric acid from Tokyo Kasei (Tokyo, Japan), ammonium molybdate, L-malic and D-tartaric acids from Wako (Osaka, Japan). Ammonium molybdate solution at 0.1 *M* concentration was prepared as a stock solution and serially diluted to obtain the desired concentration. All eluents were filtered through a 0.45-μm membrane filter.

Four readily available grape juices were used for the experiment. The only pretreatment of the sample was ultrafiltration through a Tosoh Ultracent-10 disposable cartridge, except that the highly coloured sample was decoloured by activated charcoal (recovery of malate and tartrate >90%) (Wako Darco G-60) prior to ultrafiltration.

The chromatographic separation was performed on a 250 × 4.6 mm I.D. anion-exchange column (TSK gel QAE-2SW, Tosoh, Tokyo, Japan) maintained at 45°C by a Tosoh CO-8011 column oven. An eluent containing 70 m*M* molybdate (adjusted to pH 5.9 with sodium hydroxide solution) was delivered by a Tosoh CCPD pump at a flow-rate of 0.7 ml/min. Injections were made through a Rheodyne Cotati, CA, USA) Model 7125 injector with a 100-μl loop.

A Shimadzu (Kyoto, Japan) SPD-10AV UV–visible detector for PPD was equipped with two Polaroid (Norwood, MA, USA) type HN32 polarizers on either side of the flow cell, where the inclination of the transmitting axis of the polarizer on the transmitting light side relative to that on the incident light side is set at an angle of 1 radian to the left as one faces the light source. The PPD sensitivity of this detector was improved by maximizing the intensity of the tungsten lamp and by setting the time constant at the slowest value (10 s). Thus the sensitive detection of optically active analytes becomes possible. Ultraviolet detection at 333 nm (Shimadzu SPD-6AV) was used in series with PPD at 520 nm. Quantitative determinations and linearity plots were obtained by measuring the peak heights.

3. Results and discussion

Because PPD detects optical rotation as a change in absorbance, it is unsuitable for light-absorbing analytes. Molybdate provides colourless complexes with hydroxy acids in acidic solutions, whose ratios of metal to ligand are 1:1 and 1:2 [14,15]. Fig. 1 shows the relationship between the specific rotations of the complexes at 589 nm, determined by a flow-injection analysis of the PPD system [13], and the ratios of molybdate to hydroxy acid ligands at a 70 m*M* concentration of molybdate at a pH around 5.3. The specific rotations of both acids of L-form

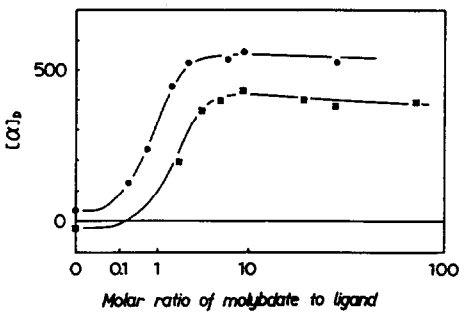

Fig. 1. Dependence of the specific rotation on the molar ratio variation of molybdate to L-tartrate (●) and L-malate (■).

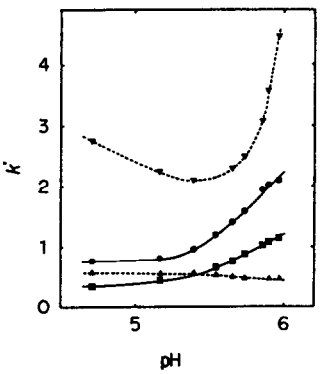

Fig. 2. Capacity factors of analytes and system peaks as a function of 70 m*M* molybdate eluent pH. Symbols are the same as in Fig. 1 except for the system peaks indicated by the open triangles.

showed positive (dextrorotatory) shifts as the molar ratio of molybdate to the acids increased, and their values plateaued in the presence of excess molybdate solution. Their specific rotations under conditions in which the molybdate existed sufficiently in excess increased as the measuring wavelength became shorter. Thus, highly sensitive PPD of the optically active hydroxy acids can be obtained using molybdate.

Recently, it has been reported that these complexes are retained in an anion-exchange column [16]. Investigations of the elution behaviour of these acids in the molybdate eluent system using various columns revealed that an increase in the exchange capacity of the column improved their separation and peak shapes. The choice of Tosoh QAE-2SW as an analytical column, whose ion-exchange capacity is 3.7 mequiv./g or more, made possible the sensitive determination of these acids polarized photometrically with the simplest apparatus without derivatization. In this system, an improvement in analyte peak shape was achieved by increases in both the column temperature and the eluent concentration. Increased molybdate concentration is desirable for the quantitative complexation with analyte acids, but excess addition exerts a negative influence on the stainless-steel tubing in HPLC or lowers the UV sensitivity as a result of increasing the baseline absorbance. Thus a molybdate concentration of 70 m*M* was chosen and a column temperature of 45°C, which is the upper limit for this column.

The influence of the eluent pH on the retention of the acids under the conditions stated above is shown in Fig. 2. They became strongly retained as the pH was increased. It seems that the negative charges of their complexes increase with an increase in the eluent pH, as suggested by Maruo *et al.* [16]. In this system, there are two extraneous peaks, called system peaks, arising from the component in the molybdate eluent. When the pH of the eluent was 5.9, both acids eluted within the capacity ratio of 2 and satisfactory separated from the system peaks.

The detection wavelength was set at 520 nm for PPD, where the signal-to-noise (S/N) ratio is best, and the flow-rate was decreased to 0.7 ml/min to prevent the peak width from being broadened at the largest time constant of this detector. Typical chromatograms of authentic malic and tartaric acids solutions obtained under the above-described conditions are shown in Fig. 3, in which the top figure is the trace from the UV detector and the bottom that from the PPD. In sequence are injections of L-isomers (A) followed by the racemic mixture (B) and D-isomers (C) at 20-min intervals. In the UV trace, the peak around 7 min is malic acid, the one around 11 min is tartaric acid and the negative ones both in the front and in the rear are the system peaks. Although UV detection always gave positive peaks for each acid, PPD gave a positive one for each L-isomer, a negative one

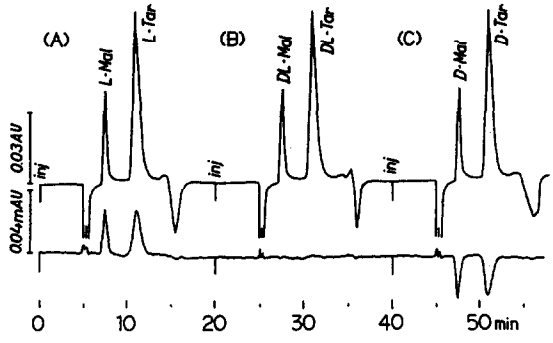

Fig. 3. Comparison of the chromatograms obtained by UV at 333 nm and PPD at 520 nm of mixtures of malic and tartaric acids on a QAE-2SW column. Sample amounts injected were 20 μg of malate and 12 μg of tartrate of each.

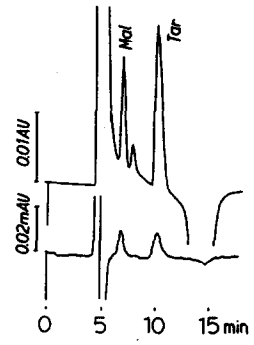

Fig. 5. UV and PPD chromatograms of a commercially available grape juice.

for the D-isomer and none for the racemate. Plots of peak-height units for PPD *versus* amounts of optically active samples showed good linearities up to 60 μg for malic acid and up to 30 μg for tartaric acid, and their detection limits are about 2 μg and 1 μg, respectively, at the $S/N =$ 3 level. Various enantiomer ratios were injected, with the total amounts of both acids kept constant at 20 μg for malic acid and at 12 μg for tartaric acid. The results are plotted in Fig. 4 as the peak-height ratio of PPD and UV detection *versus* the enantiomeric ratio in the sample. Both acids showed good linear relationships with a standard deviation (σ) of less than 1%. This means that the enantiomeric purities of both acids can be determined with an accuracy of $\pm$2.5% at 3σ, *i.e.*, a food to which the addition of synthetic acids over the 5% level can be classified as adulterated at 20 μg of malate and/or 12 μg of tartrate injection level.

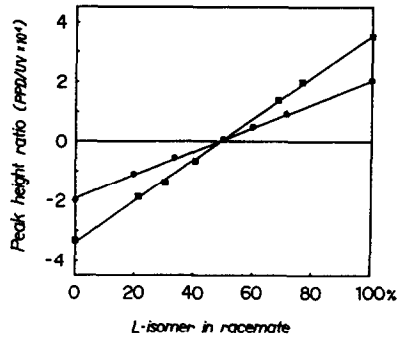

Fig. 4. Peak-height ratios *versus* composition of enantiomers for tartrate (●) and malate (■).

As an application of the proposed method, synthetic malic and tartaric acids in commercially available grape juices were determined. In this system, peaks are only obtained with the species complexed with molybdate. Other chelating compounds, such as citric or glutamic acid, eluted immediately after the dead volume of the column and did not interfere with the measurement of synthetic acidulants. However, PPD is subject to interference from the colouring matter. Although Fig. 5 illustrates actual chromatograms of highly coloured Concord grape juice, after pretreatment with activated charcoal prior to ultrafiltration no interference was observed in the PPD (bottom trace). Addition of synthetic racemates was not observed in all of the samples analysed by the proposed method.

In conclusion, we have successfully coupled a molybdate eluent HPLC system with PPD for the detection of synthetic malic and tartaric acids. The coupled PPD/UV detection system does not require the physical separation of L- and D-isomers. The primary advantage of PPD is the diversion of a conventional photometric detector to this purpose. The proposed method is suitable for the routine examination of grape juice adulteration.

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