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Application of Liquid Chromatography – Polarimetric Detection to the Determination of Cyclodextrins in Mengkudu (Noni) Juice

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Abstract

Cyclodextrins (CDs) were separated and detected by liquid chromatography. The detection was based on optically polarimetric detection. The elution order of CDs on Develosil ODS-UG-5 column was γ-, α-, monoG_1-β-, and β-CD. The calibration graps of CDs were linear up to concentration 1 mM, with the concentration detection limit of α-CD, β-CD, γ-CD and mono-G_1-β-CD (S/N = 3) are 39, 52, 18 and 33 μM, respectively. Corresponding to the mass detection limit of α-CD, β-CD, γ-CD and mono-G_1-β-CD are 0.55, 0.96, 0.26 and 0.47 nmol.
The method has been applied to the determination of CDs present in Mengkudu (Noni) juice where the concentration of γ-CD found in Mengkudu (Noni) juice is 0.028 μM.

Keywords: Cyclodextrin, liquid chromatography, optically refractometry detector and mengkudu juice

Introduction

Mengkudu (Noni), *Morinda citrifolia Linn*, shown in Figure 1, known as a kind of plant that has many benefits for healthiness. The usage of Mengkudu has been studied well and set out to find the pharmacologically active ingredient of the mengkudu (Hiramatsu, et.al., 1993, Younos, et. Al., 1990). The mengkudu juice was shown to significantly increase the life span of the treated mice (from 105%-123%) with 9 of 22 mice surviving for more than 50 days (Hirazumi et al., 1994). Cyclodextrins (CDs) usually added to the Mengkudu (Noni) juice industries as stabilizer of the product in the form of emulsion.

CDs are cyclic oligosaccharides, produced by the action of the CD-trans-glycosidase enzyme on a medium of starch (Cramer, et al, 1967). CDs having six, seven and eight D-glucopyranose units called as α-, β- and γ-CD with internal diameter of 6, 7.5 and 9-10A", respectively. Dimension of CDs is shown in Figure 2.

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Due to their special molecular cavity structure, CDs can include guest molecules ranging from hydrophobic to ionic as hosts and form inclusion complexes in aqueous solution (Hamai, 1982; Wojcik and Rohrbach, 1975). The formation and typical structures of CD-complexes improves physical, chemical and biological properties of the guest molecules. This has lead to the wider application of CDs in the field of medicine, organic synthesis, enzyme, as well as food and juice industries as stabilizer of the product in the form of emulsion (Kodama *et al*., 1999; Frijlink *et al*, 1987, Jones *et al*, 1984a; Takeuchi and Miwa, 1994, Munaf *et al*, 2000).

Optically active detection such as polarimetry is one of the most prominent dection methods for LC because it can maintain a higher dynamic reserve defined as the ratio of the background to its noise level13. The larger the dynamic reserve, the better sensitivity can be achieved. The dynamic reserves for common detection such as UV and fluorimety are around 10^4 and 10^3, respectively.

The present paper describes the feasibility of polarimetric detection of cyclodextrins and its

application to the determination of cyclodextrins in noni juice.

Figure 1. Noni Fruit (*Morinda citrifolia* Linn)

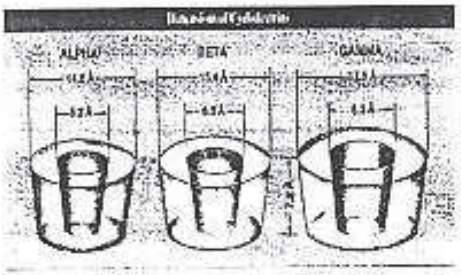

Figure 2. Dimension of CDs

Experimental

Reagents

The reagents used in this work were of analytical reagent grade and obtained from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. α-cyclodextrin (α-CD), β-CD, γ-CD, mono-G_1-β-CD and methanol were used as received. Purified water was prepared in the laboratory by using a GS-590 water distillation system (ADVANTEC, Tokyo, Japan).

Eluents were degassed under vacuum by using an ultrasonic vibration bath before use.

Apparatus

The chromatographic system consists of a model 880-PU intelligent HPLC pump, (Jasco, Tokyo, Japan), a model 7700 injection valve (Rheodyne, Cotati, CA, USA) coupled with a 21 µL loop, a 50 or 150×4.6 mm i.d. Develosil ODS-UG-5 packed column (5 µm; Nomura Chemical, Seto, Japan), a model OR-990 optically rotatory dispersion detector (ORD; Jasco), and a Chromatopac C-R7Ae plus data processor (Shimadzu, Kyoto, Japan). The separation column was immersed in a water bath to avoid variation in ambient temperature. The flow rate of the mobile phase was kept constant at 1.0 mL/min. The temperature of the water-bath was not regulated, but left ambient (ca. 20 °C).

Preparation of sample

Mengkudu (Noni) juice was purchased from Indonesia drug store, the liquid was then filtered through a 0.45-µm membrane filter (GL Science, Tokyo, Japan). The filtrates were then directly injected into the system.

Results and Discussion

Effect of methanol concentration in the mobile phases

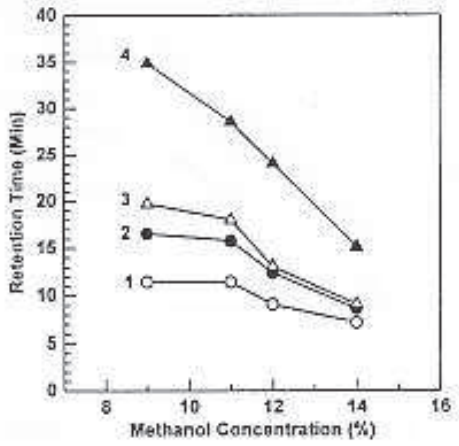

Figure 3.

CDs could be visualized by using aqueous methanol solution the mobile phase. The effect of methanol concentration in the mobile phases on the retention of α-, β-, γ- and mono-G_1-β-CD were examined and the results are shown in Figure 3. The results indicate that the retention of CDs decreases with increasing methanol concentration in the mobile phase.

Under the conditions in Figure 4 the background signal of the mobile phase is $+1.3 \times 10^{-2}$ degrees, whereas the noise level is 1.5×10^{-5} deg. The dynamic reserve is then calculated to be 880, which is lower than expected. If the dynamic reserve can be increased, the sensitivity of the present detection method can be improved. The dynamic reserve of the present detection system will be improved by increasing the concentration of optically active compounds or selecting a reagent with a higher specific optical rotation because the OR-990 detector can measure up to 0.5 degrees. The dynamic reserve is generally improved by increasing the background signal while keeping the noise level.

Linear dynamic range

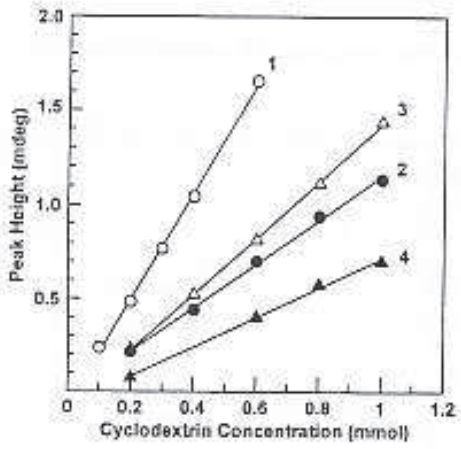

Figure 4.

Figure 4 demonstrates the separation of α-CD, β-CD, γ-CD and mono-G_1-β-CD on the ODS column using 11 % (v/v) methanol aqueous solution as the mobile phase. The analytes are completely separated within 30 min. The linearity of the signal was examined under the conditions in Figure 4, and the results are shown in Figure 5. The present methods can provide quantitative analysis of CDs, with the concentration detection limit of α-CD, β-CD, γ-CD and mono-G_1-β-CD (S/N = 3) are 39,52,18 and 33 μM, respectively. Corresponding to the mass detection limit of α-CD, β-CD, γ-CD and mono-G_1-β-CD are 0.55, 0.96, 0.26 and 0.47 nmol.

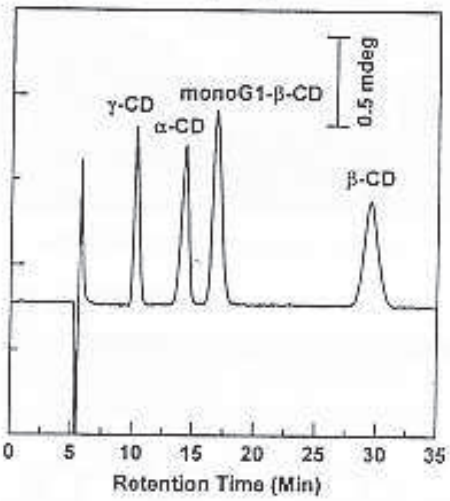

Figure 5.

Application to the determination of CDs present in Mengkudu juice

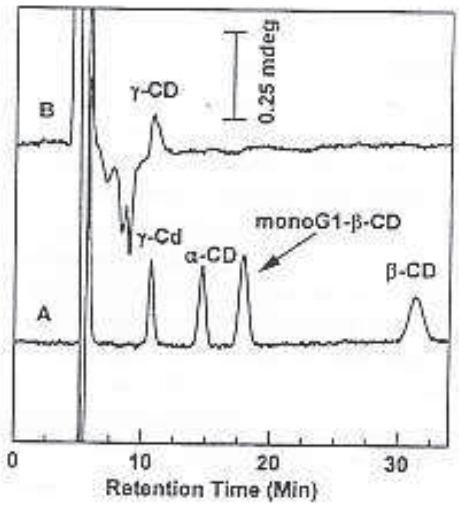

Figure 6.

The present system was applied to the determination of cyclodextrins contained in Indonesian Noni (Mengkudu) Juice. Sample was purchased in drug store and shaking before the determination. The chromatograms are demonstrated in Figure 6. From the figure it can be seen that CD, namely γ-CD present in Mengkudu Juice can be detected, with the concentration 0.028 μM.

In conclusion, the method could be applicable to the determination of CD contained in Mengkudu (Noni) juices. The sensitivity of the present detection system will be improved by selecting a reagent with a higher specific rotation.

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