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Short communication

Determination of phenolic acids in fruit juices by isocratic column liquid chromatography

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Abstract

A simple and rapid analytical method of five phenolic acids, gallic, chlorogenic, caffeic, ellagic and ferulic acid, which are naturally occurring bioactives, were determined in fruit juices by isocratic LC using photodiode array UV detection. The sample was pre-treated by solid-phase extraction (a combination of Sep-Pak Plus tC_{18} and Bond Elut PSA).

Keywords: Solid-phase extraction; Phenolic acids; Gallic acid; Chlorogenic acid; Caffeic acid; Ellagic acid; Ferulic acid

1. Introduction

Phenolic acids are widely distributed in the plant kingdom and are present in, e.g. tea, red wine, fruits, beverages and various medicinal plants [1–4]. In particular some typical low molecular phenolic acids in foods and foodstuffs, namely gallic, chlorogenic, caffeic, ellagic and ferulic acids (Fig. 1), have been reported to exert potential health-promoting effects as antioxidant [5,6], antitumor [7], antimutagenic [8] and anticarcinogenic agents [9]. Gallic, ellagic and ferulic acids are included in the List of Existing Food Additives as natural antioxidants in Japan [10].

The determination of these compounds in fruit juices using LC with gradient elution has been reported [11–13]. The determination of phenolic acids expect for ellagic acid using LC with isocratic elution has also been reported [14,15]. However, analyses using the reported LC conditions tend to cause tailing and broadening of their peaks. In this paper, we report the results of a simple and rapid isocratic LC method with UV detection for all five phenolic acids in fruit juices after pretreatment by solid-phase extraction.

2. Experimental

2.1. Samples, chemicals and reagents

The fruit juices were purchased from local grocery stores. Gallic acid monohydrate, ellagic acid dihydrate and ferulic acid were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The caffeic and chlorogenic acids were obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Methanol and acetonitrile were of HPLC-grade from Wako Pure Chemical Industries, Ltd. Cartridges used for

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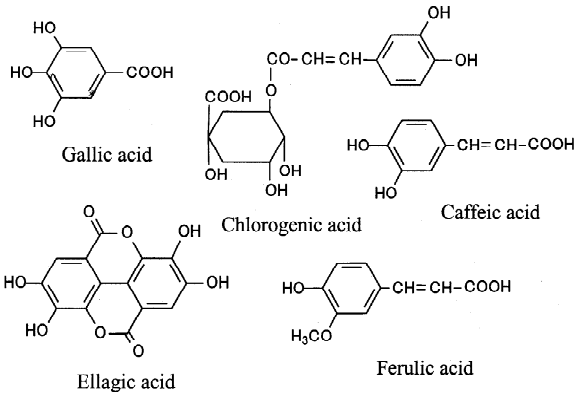

Fig. 1. Structures of phenolic acids.

the pretreatment were a Sep-Pak Plus tC_{18} cartridge (900 mg, Waters, MA, USA) and a Bond Elut PSA cartridge (500 mg, Varian Associates Inc., California, USA). The standards were dissolved in methanol or ethanol.

2.2. Apparatus

The HPLC analyses were carried out using a Shimadzu class LC-VP HPLC system with class LC-VP software, a pump (LC-10Advp), an auto-sampler (SIL-10AD), and a diode-array detector (SPD-M10Avp) (Shimadzu, Kyoto, Japan). An L-column ODS (5 μm, 250×4.6 mm I.D., Chemicals Inspection and Institute, Tokyo, Japan) was used for the analysis.

2.3. Sample preparation

For sample preparation, a juice (1–2 g) was weighed and then 0.1 mol/l HCl solution (100 μl)

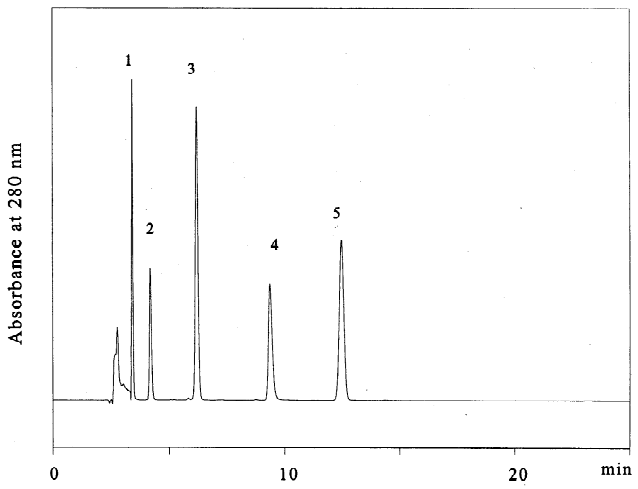

Fig. 2. LC chromatogram of standards (50 μg/ml, injected 10 μl). L-column ODS (4.6 mm I.D.×250 mm, 5 μm) column using 5 mmol/l potassium dihydrogenphosphate solution (pH 2.5)–acetonitrile (41:9, v/v) at 1.0 ml/min and 40°C. Peaks: 1, gallic acid; 2, chlorogenic acid; 3, caffeic acid; 4, ellagic acid; 5, ferulic acid.

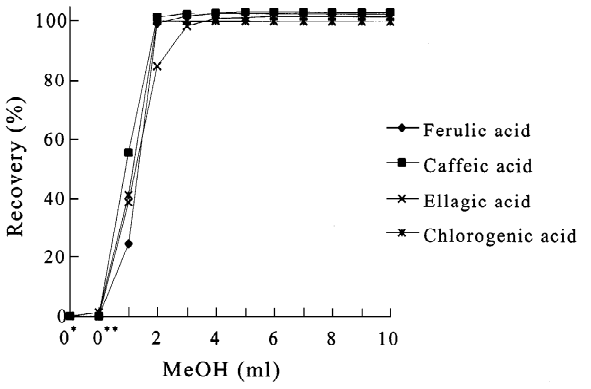

Fig. 3. Relationship between volume of methanol in eluting solution and recoveries of the five phenolic acids from Sep-Pak Plus tC_{18}. *applied to cartridge with sample solution; **eluted with 10 ml of H_2O.

was added. The sample was cleaned up using the Sep-Pak Plus tC_{18} and Bond Elut PSA cartridges. The tC_{18} cartridge was attached to the top of the PSA cartridge using an adapter. The cartridge assembly was conditioned with 10 ml of methanol, followed by 10 ml of distilled water. The sample was directly loaded onto the cartridge, and washed with 10 ml of distilled water. The attached cartridges were eluted with 10 ml of 0.1 mol/l HCl, and then continuously eluted with 10 ml of methanol. These eluates were collected in a flask and then evaporated under vacuum with a rotary evaporator at 40°C to dryness. Every extract sample was dissolved in 5 ml of methanol, and then filtered through a 0.5 μm filter before the HPLC.

3. Results and discussion

In this study, we tried to determine the optimum isocratic HPLC conditions for determination of the five phenolic acids. As a consequence, the sample solution was chromatographed on an L-column ODS with a mobile phase of 5 mmol/l potassium dihydrogenphospate solution (pH 2.5)–acetonitrile (41:9) at a flow-rate of 1.0 ml/min. Using these proposed HPLC conditions, a well resolved chromatogram between the different peaks detected for the standard by HPLC has been obtained. Fig. 2 shows a chromatogram of a mixture of the five standard phenolic compounds obtained with this method. For sample clean-up, Sep-Pak Plus tC_{18} and Bond Elut PSA cartridges were employed.

Fig. 3 shows the relationship between the volume of methanol used the desorption and recoveries of the five analytes from Sep-Pak Plus tC_{18}. These compounds can be eluted with 4 ml of methanol, but their complete elution was better when eluted with 10 ml. On the other hand, most of the gallic acid was not sorbed on the Sep-Pak Plus tC_{18} cartridge. We experimented with other solid-phases, and found that gallic acid was sorbed on a Bond Elut PSA, which is an ion-exchanger. Fig. 4 (a) shows the effect of the sample solution pH on the recovery of gallic acid

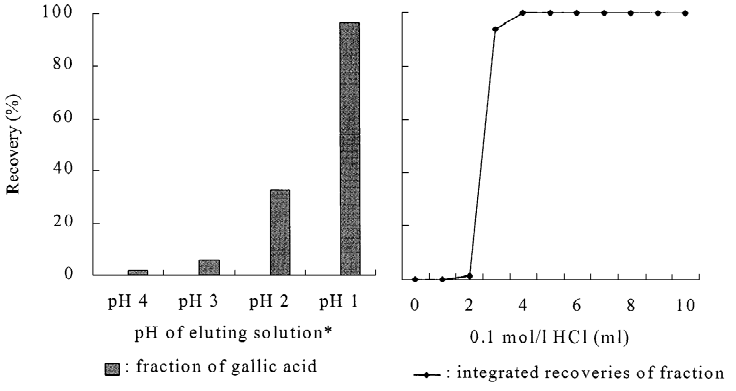

Fig. 4. Relationship between pH (a) [or volume (b)] of eluent and recovery of gallic acid from Bond Elut PAS. *eluted with 10 ml of each solution.

Table 1
Recoveries of phenolic acids from juices by the proposed method[a]

	Recovery(%)[b]			
	Apple juice	Grape juice	Pomegranate juice	Prune juice
Gallic acid	86	80	81	75
Chlorogenic acid	93	98	99	87
Caffeic acid	89	85	96	91
Ellagic acid	88	86	90	87
Ferulic acid	89	98	93	93

[a] The values are means of triplicate determinations.
[b] 50 μg of each compound was added to each of the juices per g.

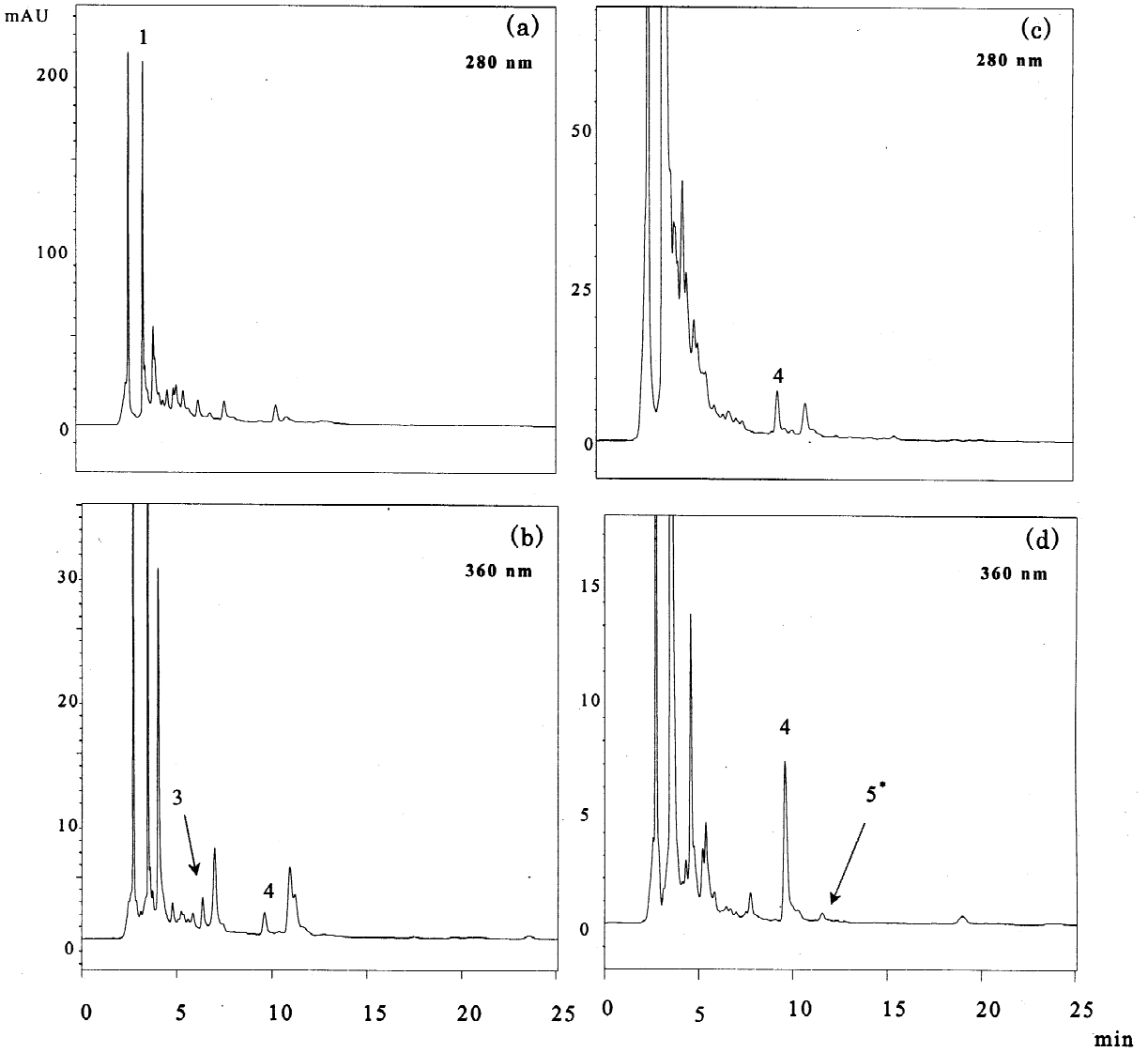

Fig. 5. LC chromatograms of juices. Chromatograms: (a), grape juice at 280 nm; (b), grape juice at 360 nm; (c), pomegranate juice at 280 nm; (d), pomegranate juice at 360 nm. Peak numbers are given in Fig. 2. *trace.

Table 2
Phenolic acid contents of commercial juices

	Content (μg/g, mean±SD, n=3)			
	Apple juice	Grape juice	Pomegranate juice	Prune juice
Gallic acid	ND[a]	12.5±0.1	ND[a]	20.9±0.2
Chlorogenic acid	16.6±1.1	ND[b]	ND[b]	190.2±0.1
Caffeic acid	Tr	9.5±0.5	ND[b]	Tr
Ellagic acid	0.6±0.3	2.5±1.8	14.3±0.5	ND[c]
Ferulic acid	ND[d]	ND[d]	Tr[e]	ND[d]

[a] Not determined (<5.0 μg/g).
[b] Not detected (<0.03 μg/g).
[c] Not detected (<0.015 μg/g).
[d] Not detected (<0.05 μg/g).
[e] Tr=Trace.

from the Bond Elut PSA. The pH should be about 1 to ensure complete recovery. Consequently, the solvent used in the elution was 0.1 mol/l HCl solution. Fig. 4 (b) shows the elution pattern of gallic acid from the Bond Elut PSA cartridge. Most of the gallic acid was eluted with 5 ml of 0.1 mol/l HCl, but its complete elution was better when eluted with 10 ml. Fortunately, all five phenolic acids were stable at 0.1 mol/l HCl. Therefore, the tC_{18} cartridge attached to the top of the PSA cartridge using an adapter, previously conditioned with 10 ml of methanol, followed by 10 ml of distilled water, was directly loaded with the sample solution and washed with 10 ml of distilled water. After washing with water, it was eluted with 10 ml of methanol, followed by 10 ml of 0.1 mol/l HCl. The eluates were evaporated under vacuum, and re-dissolved in methanol. The proposed method was applied to four kinds of juices spiked with the phenolic acids at a level of 50 μg/g. The data of Table 1 show that the recoveries of the five analytes were 75–99%.

The determination of the five phenolic acids could be monitored at 280 nm. However, monitoring should better be concurrently carried out at both 280 (gallic acid) and 360 nm (the others), because detection at 280 nm was occasionally adversely affected by impurities in the sample. Relevant examples and results are given in Fig. 5 and Table 2. The limits of detection were 0.015–0.03 μg/g.

4. Conclusions

A simple and rapid method for the determination of five phenolic acids in fruit juices by HPLC with photodiode array UV detection using isocratic elution has been developed for the first time. The sample was pretreated by passage through the cartridge assembly (a combination of Sep-Pak Plus tC_{18} and Bond Elut PSA) before the HPLC. We analyzed five juices by this method and the experimental result indicated that prune juice contains an especially high concentration of chlorogenic acid (190.2 μg/g) in their phenolic acids. It was also shown that the main acid of the other juices were ellagic acid (14.3 μg/g) in the pomegranate, chlorogenic acid (16.6 μg/g) in the apple and gallic acid (12.5 μg/g) in the grape juices.

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