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Short communication

Simultaneous determination of organic acids and inorganic anions in tea by ion chromatography

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Abstract

An ion chromatographic method using an anion-exchange column for the simultaneous analysis of organic acids and inorganic anions in tea was developed. A mixed eluent of potassium hydrogenphthalate and phthalic acid was used. Six organic acids and three inorganic anions in tea were separated without any interference from other compounds. The method is simple (no special sample pretreatment) and sensitive with detection limits ($S/N=3$) of 0.044–0.19 mg/l for inorganic anions and 0.48–1.34 mg/l for organic acids.

Keywords: Tea; Food analysis; Organic acids; Inorganic anions

1. Introduction

Ion-exchange chromatography (IEC) is widely used for the separation of inorganic anions. The short chain carboxylic acids (organic acids) which can partly be dissociated to anionic forms in aqueous solution can also be separated by IEC. However, the usual high-performance liquid chromatography (HPLC) modes used for organic acids are ion-exclusion chromatography and reversed-phase (RP) HPLC, because suppressed IEC cannot properly separate several organic acids simultaneously, and because coexisting inorganic anions interfered in the separation of organic acids.

In recent years, several methods for the simultaneous separation of organic acids and inorganic anions were developed. The first method [1] is based on a mixed-mode stationary phase containing both RP and ion-exchange functions. The second [2–7] is based on a single anion-exchange column. Suppressed IEC [2,3] can be used for the separation of monocarboxylic acids and F^-, or dicarboxylic acids and inorganic anions, but is not suitable for food samples in which mono-, di- and tricarboxylic acids and inorganic anions are included. Non-suppressed IEC with conductivity detection, in which a low-conductivity eluent is used, is an obvious alternative for the simultaneous determination of organic acids and inorganic anions in food samples, because the main organic acids and inorganic anions can be separated simultaneously, and other organic compounds in the samples do not interfere [4]. We [5] reported the simultaneous analysis of organic acids and inorganic anions in wine and juice, but phosphate anion and ascorbic acid in food samples were not included.

Tea is rich in organic acids and inorganic anions. The simultaneous chromatographic analysis of these compounds has not yet been reported. In this work, the simultaneous separation and determination of

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organic acids and inorganic anions in tea on an anion-exchange column was studied.

2. Experimental

2.1. Apparatus

The ion chromatograph used in this study was HIC-6A (Shimadzu, Japan), consisting of an LP-6A liquid delivery pump, a CTO-6AS column oven and a CCD-6A conductivity detector. The anion-exchange column (Shim-pack IC-A1, 100 mm×4.6 mm I.D.) was from Shimadzu. The column oven was maintained at 40°C. Samples were injected using a 20 μl loop injector. Data acquisition and treatment were accomplished using a Shimadzu data system (C-R4A).

2.2. Chemicals

All chemicals used in this study were of analytical-reagent grade and deionized water was used to prepare all solutions. The eluent used in this study is a mixture of potassium hydrogenphthalate (KHPh) and phthalic acid (H_2Ph).

2.3. Sample preparation

The dried tea sample (0.5 g) was placed in approximately 80 ml of deionized water, and was heated at 90–100°C for 20 min. After cooling, it was filtered through a 0.45 μm membrane filter, and then the filtered solution was transferred to a 100 ml flask, and deionized water added to 100 ml. This sample solution was injected into the ion chromatograph directly. Each sample was run six times.

3. Results and discussion

3.1. Sample preparation

The heating time of tea sample was studied using jasmine tea. Three jasmine teas were heated for 15, 20, 25 min respectively. The results (peak areas) of all nine analytes were approximately the same for the three heating times with relative standard deviation (R.S.D.) below 5%.

The stability of tea sample solution was studied. Jasmine tea and green tea were stable for one week when kept in a refrigerator, but Japanese tea was stable for only four days under the same conditions. The concentration of ascorbic acid, lactic acid and malic acid decreased over four days for Japanese tea. Over one week, some floccus in Chinese tea (jasmine and green), and some precipitate in Japanese tea appeared.

The tea samples were also injected onto an ion-exclusion column for the analysis of organic acids combined with UV detection. However, the analysis of organic acids could not be accomplished due to interferences resulting from the adsorption of other organic compounds.

3.2. Detection limit

The detection limits ($S/N=3$) and linear ranges of calibration for various anionic species are given in Table 1. The detection limits obtained by using the mixed eluent of 0.75 mmol/l KHPh and 0.25 mmol/l H_2Ph with pH 3.5 were several times lower than those obtained by using a single 1.0 mmol/l KHPh eluent with pH 4.0. The background conductivity of the mobile phase containing 0.75 mmol/l KHPh and 0.25 mmol/l H_2Ph is 162 μS/cm, while that of 1.0

Table 1
Detection limit ($S/N=3$) and linear range of organic acids and inorganic anions

Compound	Detection limit (mg/l)		Linearity range (mg/l)	r^2
	KHPh/H_2Ph	KHPh		
Acetic acid	0.58	1.04	4–1000	0.999
Ascorbic acid	0.96	2.90	4–1000	0.998
Succinic acid	1.04	2.08	4–1000	0.996
Formic acid	0.50	1.32	5–1000	1.000
Malic acid	0.58	2.18	5–1000	0.999
Citric acid	1.34	7.80	5–2000	0.997
Tartaric acid	0.48	3.38	5–1500	0.999
$H_2PO_4^-$	0.12	0.71	2–1000	1.000
Cl^-	0.044	0.063	1–1000	1.000
NO_2^-	0.13	0.22	1–1000	1.000
Br^-	0.14	0.19	2–1000	0.999
NO_3^-	0.11	0.18	2–1000	0.998
SO_4^{2-}	0.19	0.37	4–1000	0.998

mmol/l KHPh eluent is 197 μS/cm. A decrease in background conductivity will lead to an increase in detection sensitivity. On the other hand, the dissociation of organic acids is suppressed as the pH of the mobile phase decreases. This factor will give a negative contribution to the detection limit of organic acids. But the effect of decreasing dissociation to sensitivity drop is smaller than the effect of decreasing background conductivity to sensitivity rise.

As shown in Table 1, the calibration graphs of peak areas for all analytes were linear over two or three orders of magnitude with a regression coefficient (r^2) of 0.996–1.000.

3.3. Analysis of tea

KHPh eluent is used most widely in non-suppressed IEC. However, a negative peak influences the determination of acetic and lactic acids for alcoholic drinks [5], or ascorbic acid for tea samples. In addition, $H_2PO_4^-$ and succinic acid in tea cannot be separated. In order to resolve these problems, a mixed eluent of 0.75 mmol/l KHPh and 0.25 mmol/l H_2Ph with pH 3.5 was used in this work. The chromatogram of the standard mixture of organic acids and inorganic anions is shown in Fig. 1, and the chromatograms of tea samples are shown in Fig. 2. The main organic acids and inorganic anions in tea were separated without interference peaks. This is the greatest advantage of IEC when comparing IEC and RP-HPLC.

The analytical results of three tea samples (non-fermented tea) are listed in Table 2. Similar chromatograms were obtained for all three samples. Citric acid, tartaric acid and SO_4^{2-} contents in Japanese tea are smaller than in Chinese tea, while the contents of other compounds in Japanese tea are obviously greater than in Chinese tea.

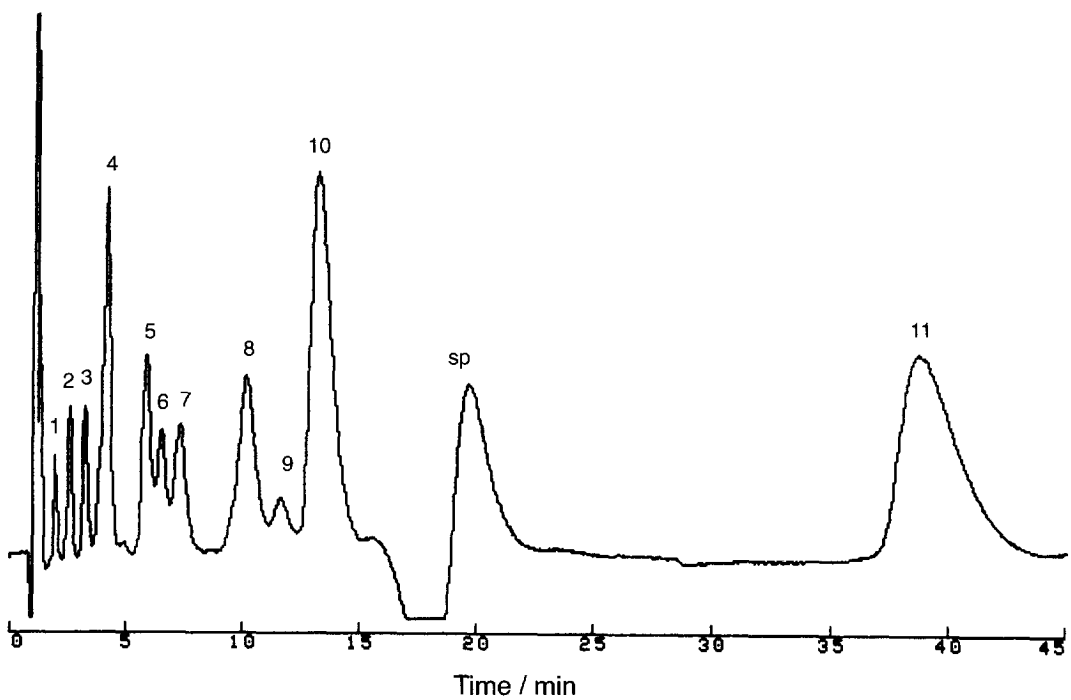

Fig. 1. Chromatogram of standard mixture of organic acids and inorganic anions. Column, Shim-pack IC A1; temperature, 40°C; eluent, mixture of 0.75 mmol/l potassium hydrogenphthalate and 0.25 mmol/l phthalate acid (pH 3.5); flow-rate, 1.0 ml/min; detector, conductance (1 μS/cm). Peaks: 1=acetic; 2=ascorbic; 3=succinic; 4=$H_2PO_4^-$; 5=malic; 6=Cl^-; 7=malonic and NO_2^-; 8=citric; 9=NO_3^-; 10=tartaric; 11=SO_4^{2-}; sp=system peak.

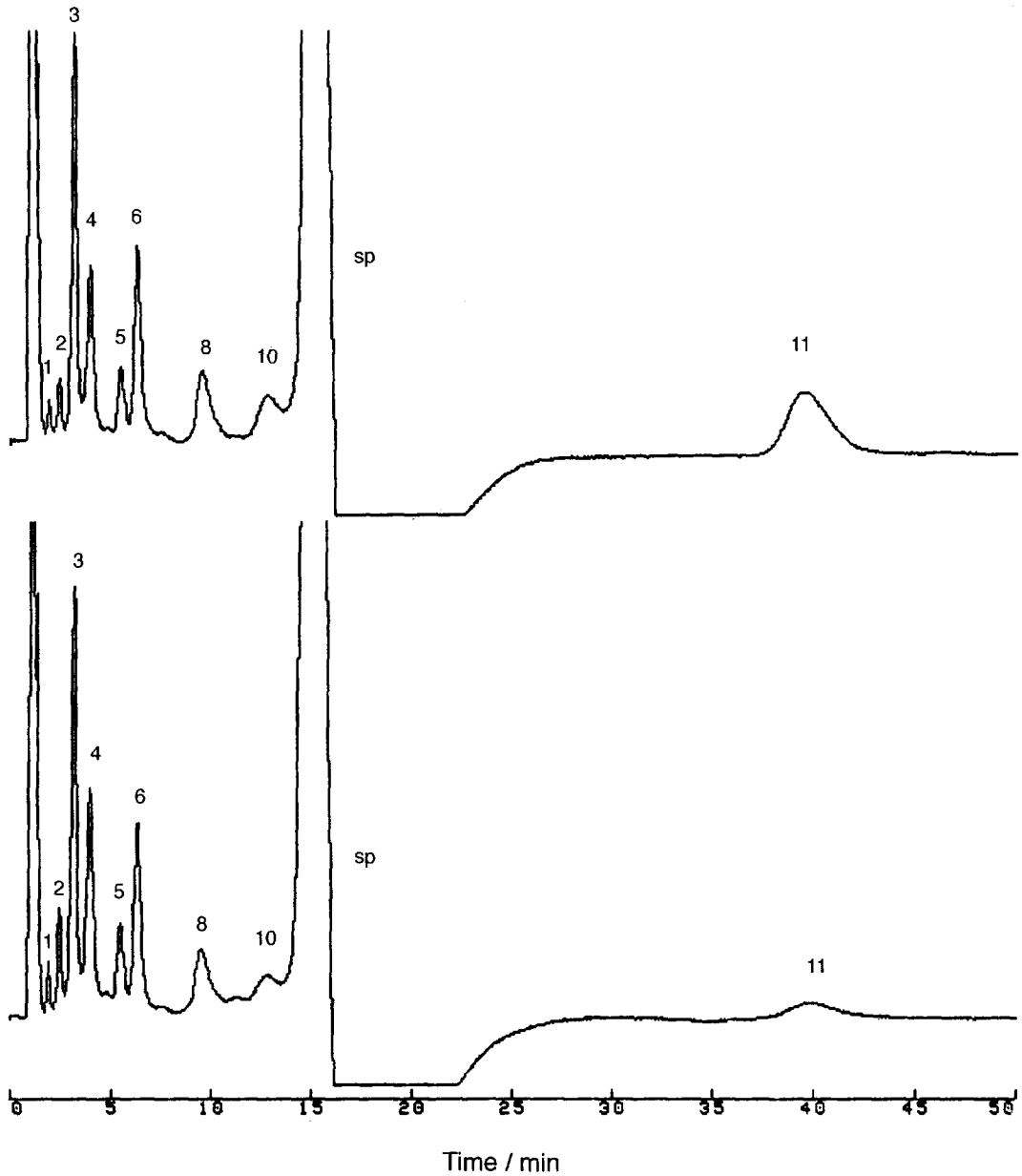

Fig. 2. Chromatograms of green tea (top) and Japanese tea (bottom). Conditions and peak numbers as in Fig. 1.

4. Conclusion

In this work, a mixed eluent of KHPh and H_2Ph was used for the simultaneous analysis of organic acids and inorganic anions. Compared with an eluent of KHPh only, the resolution and detection sensitivity of these analytes was improved when the mixed eluent was used. The simultaneous analysis of the main organic acids and inorganic anions in tea was completed without any interference from coexisting

Table 2
Analytical results ($n=6$) of organic acids and inorganic anions in tea

Compound	Jasmine tea		Green tea		Japanese tea	
	Mean (mg/g)	R.S.D. (%)	Mean (mg/g)	R.S.D. (%)	Mean (mg/g)	R.S.D. (%)
Acetic acid	6.70	3.98	5.84	2.57	10.56	2.73
Ascorbic acid	10.54	3.49	9.52	3.41	22.34	3.78
Succinic acid	49.44	1.23	65.44	2.04	77.88	2.27
Malic acid	3.60	3.22	4.02	2.46	5.52	1.54
Citric acid	8.54	3.73	7.64	2.35	7.42	2.17
Tartaric acid	1.24	3.27	0.80	3.55	0.46	3.98
$H_2PO_4^-$	8.00	2.95	7.88	1.74	11.84	0.62
Cl^-	1.04	2.37	1.78	1.58	1.94	2.49
SO_4^{2-}	3.58	1.17	4.58	3.25	1.54	3.50

compounds. The solution of tea samples can be injected on the column directly (without a special pre-treatment procedure). The method is very useful and practical for tea samples.

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