Comparison of Three Chromatographic Systems for Determination of Organic Acids in Wine

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Three chromatographic systems: ion exchange chromatography, ion exclusion chromatography and reversed phase chromatography, have been used for the simultaneous determination of organic acids in wine. The common organic acids were separated using all three chromatographic systems. When an ion exchange column (TSKgel IC Anion-PW) was used, organic acids and inorganic anions (Cl^- and SO_4^{2-}) in wine were determined simultaneously without interference. The sharp peaks were obtained when an ion exclusion column (TSKgel OApak-A) was employed. A rapid separation of organic acids has been achieved, within about 7 min, when a reversed phase column (Zorbax ODS) was used. In ion exclusion and reversed phase systems combined with UV detection, however, other organic compounds which have ultraviolet absorption at 210 nm interfered with the determination of organic acids when practical wine samples were analyzed. IC is an obvious alternative for determination of organic acids in wine.

Keywords Ion exchange, ion exclusion, reversed phase, organic acid, wine

The quantitative analysis of organic acids is important for the quality control of wine, because the classes and content of organic acids give a characteristic taste to wine.[1] Acetic acid, lactic acid, succinic acid, malic acid, citric acid and tartaric acid are the main organic acids in wine.[2] Colorimetry[3], thin layer chromatography (TLC)[4,5], gas chromatography (GC)[6,7], enzymic method[8] and high performance liquid chromatography (HPLC) have usually been used for analysis of organic acids. However, such methods (except HPLC) need a complex pretreatment, and the simultaneous determination of common organic acids has not been achieved. HPLC is the most proper method for simultaneous separation and determination of organic acids without special pretreatment. HPLC methods used for the analysis of organic acids involved ion exchange chromatography (IC)[9,10], ion exclusion chromatography (IEC)[11-13] and reversed phase chromatography (RPC)[14-17].

Over the years, some excellent columns have been developed for separation of organic acids, and the selection of eluent become easier. A new ion exclusion column, TSKgel OApak-S, was made for analysis of organic acids, and was applied to foods by Tosoh.[18,19] A reversed phase column, TSKgel ODS-80T_M, was used for analysis of organic acids with 5 mM ammonium dihydrogenphosphate as eluent[20], but the detection limits for organic acids were poor when UV detection was used. Hoshino reported the separation of organic acids using an ion exchange column, TSKgel IC Anion-SW, with 1 mM phthalic acid as eluent.[21] We reported the analysis of organic acids in beverages such as wine, Japanese sake and fruit juice using a chemically bonded hydrophilic anion exchange column, SAM3-075 (Yokogawa) and potassium biphthalate eluent.[22]

In recent years, the analysis of organic acids which used IEC and RPC have increased. The comparison of IC, IEC and RPC has not yet been made for simultaneous determination of organic acids. In this work, the three chromatographic systems have been compared with a view to the simultaneous separation and determination of organic acids in wine using IC column (TSKgel IC Anion-PW), IEC column (TSKgel OApak-A) and RPC column (Zorbax ODS).

Experimental

Chemicals and samples

The distilled water was deionized with a Millipore Milli QII; this deionized water was used throughout the experiments.

All chemicals used were of analytical grade. Organic acids were prepared as stock solutions with concentrations of 1000 - 4000 ppm (μg/ml). Standard mixtures of organic acids were prepared by mixing the stock solutions. Potassium biphthalate and sulfuric acid were purchased from Tokyo Kasei and Wako Pure Chemicals, respectively; 4.0 mM ammonium dihydrogenphosphate eluent was adjusted to pH 2.1 with phosphoric acid. Wine samples were diluted 5-fold using deionized water after filtering through a 0.45 μm membrane filter.

Apparatus

The chromatographs and columns used in this study

Table 1 Apparatus and analytical conditions of three chromatographic systems for separation of organic acids

	IC	IEC	RPC
Chromatograph	Ion chromatographic analyzer IC-100 (YOKOGAWA)	High pressure liquid chromatograph TRI ROTAR-III (JASCO)	
Detector	CD	UV, 210 nm	UV, 210 nm
Column	TSKgel IC Anion-PW	TSKgel OApak-A	Zorbax ODS
Column size	4.6 mm i.d.×50 mm	6.8 mm i.d.×300 mm	4.6 mm i.d.×150 mm
Packing	Polymethacryrate	Polystyrene	Silica
Eluent	2.5 mM potassium biphthalate	0.75 mM sulfuric acid	4.0 mM ammonium dihydrogenphosphate
pH of eluent	4.17	2.8	2.1
Flow rate	1.0 ml/min	1.0 ml/min	0.8 ml/min
Temperature	40°C	40°C	40°C
Injection volume	25 μl	50 μl	25 μl

Table 2 Concentrations (ppm) of standard samples of organic acids used in three chromatographic systems

No.	Solute	IC	IEC	RPC
1	Acetic	400	80	150
2	Lactic	400	120	200
3	Succinic	400	80	100
4	Cl^-	15		
5	Malonic	100		80
6	Malic	400	80	100
7	Maleic	200		2.5
8	Citric	400	80	100
9	Tartric	400	80	100
10	SO_4^{2-}	25		
11	Fumaric		4	2

are listed in Table 1. Eluents were degassed with Degasys DG-1200 (Uniflows). A pH meter (Toa, HM-60S) was used for pH adjustment of ammonium dihydrogenphosphate eluent. Data was handled with a Yokogawa Model LC-100 W/F PC workstation.

Results and Discussion

Elution behavior

The chromatographic conditions of three chromatographic systems have been studied. The optimum conditions selected are listed in Table 1.

The standard mixtures of organic acids used in three chromatographic systems were differrent. Chromatograms of the standard mixtures obtained under the optimum condition listed in Table 1 are shown in Fig. 1. The concentrations of organic acid standard mixtures in Fig. 1 were listed in Table 2. The common organic acids were separated by all three chromatographic systems.

In IC, an anion exchange column packed with hydrophilic anion exchange resin (Tosoh, TSKgel IC Anion-PW) was used. The elution behavior of organic acids was studied using 2.5 mM potassium biphthalate

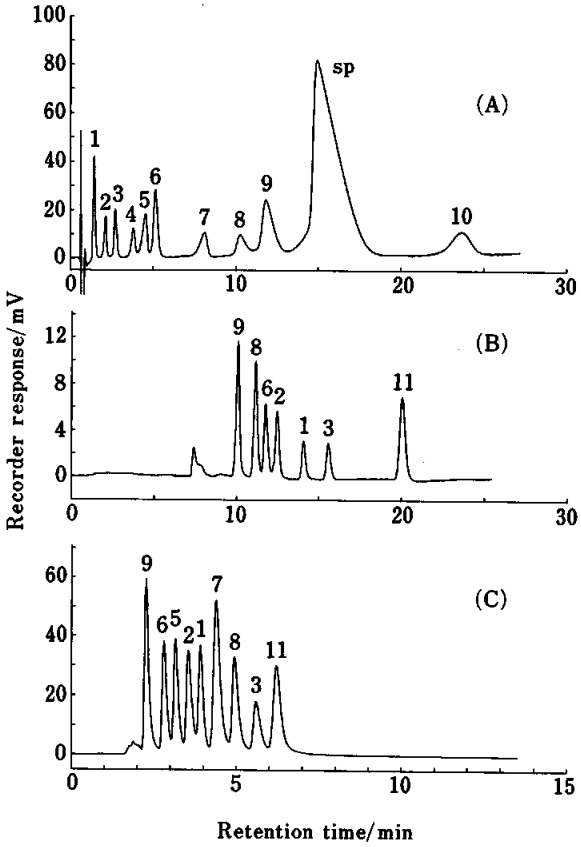

Fig. 1 Chromatograms of standard mixtures of organic acids obtained using (A) IC, (B) IEC and (C) RPC. Conditions refer to Table 1. Peaks: 1, acetic; 2, lactic; 3, succinic; 4, Cl^-; 5, malonic; 6, malic; 7, maleic; 8, citric; 9, tartaric; 10, SO_4^{2-}; 11, fumaric; sp, system peak.

(pH 4.17) as an eluent. As shown in Fig. 1A, organic acids and inorganic anions (Cl^- and SO_4^{2-}) were separated within 25 min. Fumaric acid (R_t: 13.67 min) was not included in this sample, because it overlapped with the system peak (R_t: 14.90 min). Organic acids were eluted mainly according to their dissociation constants, because the electrostatic interaction between organic acid

anions and ion exchange groups of the stationary phase plays the most important part. Acetic acid (pK_a=4.757) has the weakest interaction with ion exchange groups in the resin, because it has the smallest dissociation constant among the organic acids tested. Therefore, acetic acid was eluted faster than other organic acids. However, other factors such as ion charge and ion size of organic acid anions affect the ion exchange reaction rate between ion exchange groups and organic acid. In addition, secondary retention mechanisms such as partition between the two phases would made a contribution to retention of organic acids. Consequently, some reversals in elution order of organic acids were also observed. In this work, malonic acid (pK_{a1}=2.855) and maleic acid (pK_{a1}=1.921) were eluted faster than tartaric acid (pK_a=3.036).

In IC combined with a CD detection, inorganic anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^- and SO_4^{2-}) were also detected. Cl^-, Br^-, NO_3^- and SO_4^{2-} were separated with organic acids. PO_4^{3-} (or HPO_4^{2-}) were coeluted with the system peak. F^- was partly overlaped with the peak of lactic acid, and NO_2^- was overlaped with malonic acid. SO_4^{2-} was eluted last, because it has a stronger electrostatic interaction with ion exchange groups in the resin due to its valency of two.

In IEC, a cation exchange column TSKgel OApak-A (Tosoh) was used. The chromatogram of organic acids at the optimum condition is shown in Fig. 1B. Seven common organic acids were separated within about 20 min. Maleic acid (R_t: 9.25 min) was not included in this standard mixture. Malonic acid (R_t: 11.30 min) was overlaped with citric acid (R_t: 11.15 min).

In RPC, a Zorbax ODS column (Shimadzu) and 4.0 mM ammonium dihydrogenphosphate eluent (pH adjusted to 2.1 with phosphoric acid) were used. As shown in Fig. 1C, nine common organic acids were separated within about 7 min.

When the IC system was used, the smaller peak half-width was obtained for organic acids which eluated fast (peak 1 - 6 in Fig. 1A), while the wider peak half-width was obtained for others which eluated later, such as maleic acid, citric acid and tartaric acid, their peaks were also broadened. The sharp peaks were observed using the IEC and RPC columns.

A good chromatographic resolution with a smaller (0.016 - 0.026 mm) height equivalent to a theoretical plate (HETP) was obtained when the IEC column was used. The HETP of IC column was about two times that of the IEC column for monocarboxylic acids such as lactic acid, while almost the same HETP were obtained for dicarboxylic acids such as malic acid using the two columns.

The peaks in the IEC chromatograms had a good symmetry. The tailing factors (B/A) of organic acid peaks in this system were from 0.66 to 1.32. On the other hand, the peaks in RPC chromatogram were tailing with greater tailing factors from 1.48 for acetic acid to 2.04 for tartaric acid.

Table 3 Detection limits (ppm) and linear ranges[a] (ppm) obtained using three chromatographic systems

Solute	IC		IEC		RPC	
	Limit	Range	Limit	Range	Limit	Range
Acetic	2.9	10 - 4000	1.4	10 - 4000	1.3	10 - 2000
Lactic	4.4	10 - 4000	1.4	10 - 4000	1.9	10 - 2000
Succinic	5.1	10 - 2000	1.5	10 - 4000	2.0	10 - 2000
Cl^-	0.2	0.5 - 100	ND		ND	
Malonic	1.2	4 - 800	0.5	5 - 2000	0.4	5 - 2000
Malic	3.7	10 - 2000	0.8	5 - 2000	0.9	5 - 2000
Maleic	3.8	8 - 800	0.009	0.05 - 25	0.009	0.05 - 25
Citric	10.9	20 - 2000	0.5	5 - 2000	1.1	5 - 2000
Tartaric	3.8	10 - 2000	0.4	5 - 2000	0.6	2 - 2000
Fumaric	ND		0.011	0.05 - 25	0.008	0.05 - 25
SO_4^{2-}	0.8	2 - 200	ND		ND	

a. By peak area.
ND=not detected.

Detection limits and linear range

The detection limits (ppm) at S/N=3 and linear range of peak area calibration curves for organic acids are listed in Table 3. All three systems have enough sensitivity for the determination of the main organic acids in wine. IEC and RPC showed the same lower detection limit for most organic acids, while the lower detection limits in IC were several times greater than in the other two systems, due to a large amount of noise (base line width). Maleic acid and fumaric acid showed very high sensitivities with UV detection. The lower detection limits for these two organic acids were better by two orders of magnitude than for the other organic acids, because there are conjugated double bonds in the molecules so that they show very strong ultraviolet absorption at 210 nm.

The linear ranges of peak area calibration curves for the organic acids tested were over two orders of magnitude using all three chromatographic systems. The linear ranges of peak height calibration curves were several times narrower than that of peak area calibration curves, because the peaks of organic acids were broadened when the amounts of samples injected increased.

Application

The three chromatographic systems have been applied for determination of organic acids in wine. Chromatograms of white wine (Koshu 1989) and red wine (Muscat Bailey A 1989) are shown in Figs. 2 and 3, respectively. The results of organic acids in wine are listed in Table 4. As shown in Figs. 2 and 3, a lot of interference peaks were observed in IEC and RPC. This may be due to other organic compounds, such as phenols, which were detected by UV detection at 210 nm. The determination of organic acids was interfered with by those coeluted organic compounds. When the wine sample were injected onto the column, phenolics were more retained in the column at the tested conditions; the

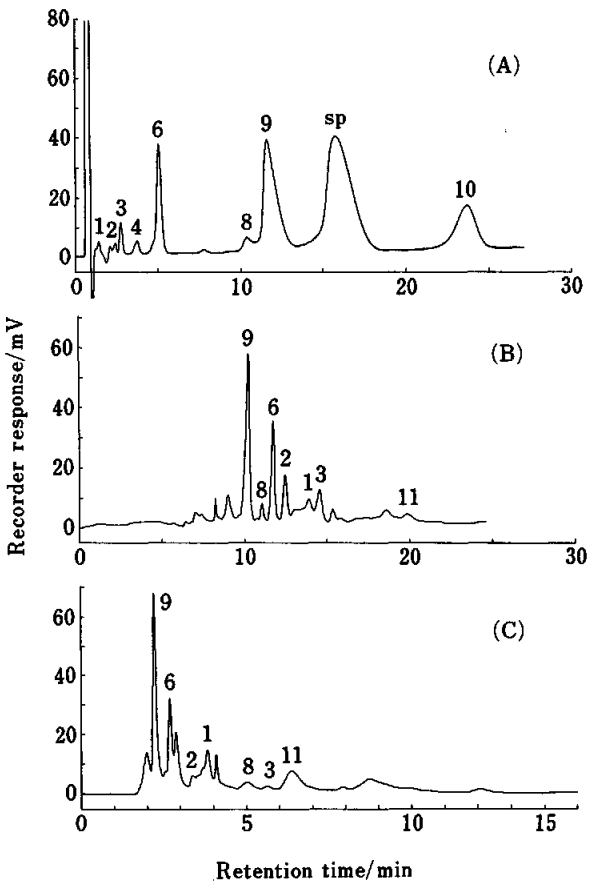

Fig. 2 Chromatograms of organic acids in white wine (Koshu 1989) obtained using (A) IC, (B) IEC and (C) RPC. Conditions refer to Table 1 and peak numbers refer to Fig. 1.

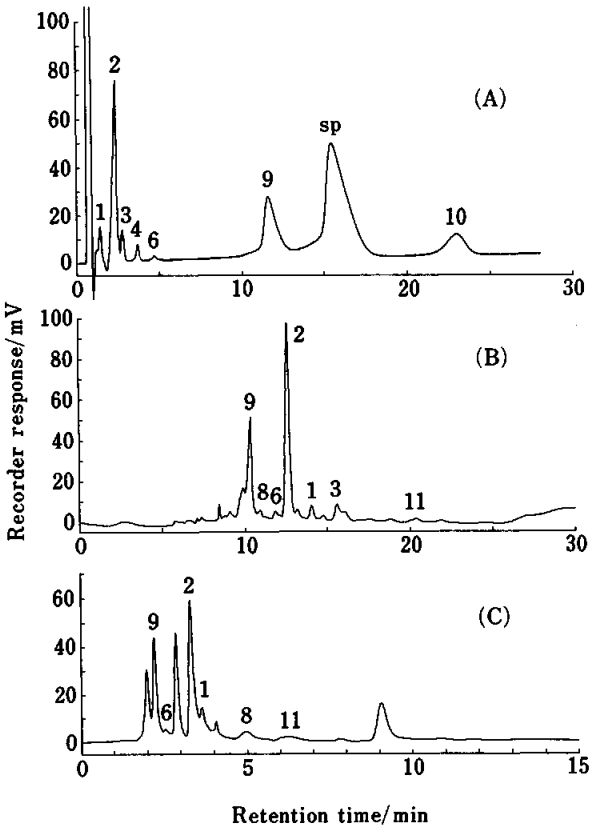

Fig. 3 Chromatograms of organic acids in red wine (Muscat Bailey A 1989) obtained using (A) IC, (B) IEC and (C) RPC. Conditions refer to Table 1 and peak numbers refer to Fig. 1.

last eluted compound was over 45 min. The interference from coeluted organic compounds was eliminated by using a CD detector. However, when the low pH eluents were used in IEC and RPC, the sensitivity of CD detection for organic acids was very low.

In IC, a large system peak was eluted between tartaric acid and SO_4^{2-}, but it did not interfere with the two neighboring components. A small negative peak appeared between acetic acid and lactic acid when wine samples were analyzed. The precise determination of lactic acid was interfered with by this negative peak.

As shown in Table 4, the results of succinic acid and citric acid obtained using IEC were smaller than using IC and RPC. The results of lactic acid in wine obtained using IEC were several times greater than those obtained using IC and RPC. This is perhaps due to coeluation of other organic compounds with lactic acid in IEC. The chromatograms in Figs. 2 and 3, and Table 4, indicate that results for IC would be reliable, because no interference peak was observed from this system.

It is thus necessary to select an optimum chromatographic system according to the constituents of the samples, although separations of common organic acids were carried out using all three chromatographic systems. In IEC and RPC with UV detection, other organic compounds which have ultraviolet absorptions at 210 nm interfered with the determination of organic acids when practical wine samples were analyzed. IC is an obvious alternative for simultaneous determination of organic acids in wine, because organic acids were not interfered with by other organic compounds, and inorganic anions (Cl^- and SO_4^{2-}) in wine were determined simultaneously with organic acids. IEC and RPC are suitable for simultaneous determination of trace maleic acid and fumaric acid with about 10 ppb detection limits.

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Table 4 Determination results (ppm) of organic acids in wines using three chromatographic systems

Wine	System	Acetic	Lactic	Succinic	Malic	Citric	Tartaric	Fumaric	Cl^-	SO_4^{2-}
Riesling	IC	599	2554	943	152	ND	1493	ND	15.7	159
	IEC	656	2790	642	266	ND	2016	2.1	ND	ND
	RPC	774	2568	855	82	ND	1600	1.4	ND	ND
Chardonnay	IC	863	3806	965	138	ND	1224	ND	12.8	171
	IEC	952	6020	809	254	16	2192	4.6	ND	ND
	RPC	903	3584	1045	249	ND	1432	7.5	ND	ND
E.F.	IC	151	205	310	9287	237	2094	ND	ND	123
	IEC	449	3265	281	9067	183	2542	8.3	ND	ND
	RPC	352	113	460	8824	229	2425	5.7	ND	ND
C.S.	IC	346	3289	973	ND	ND	1125	ND	29.6	180
	IEC	428	9092	738	124	ND	1354	3.2	ND	ND
	RPC	391	2532	752	241	ND	1474	5.0	ND	ND
C.F.	IC	294	2010	1052	ND	ND	921	ND	67.6	239
	IEC	318	5902	745	166	ND	1489	2.5	ND	ND
	RPC	262	2126	1066	506	ND	1433	1.6	ND	ND
P.N.	IC	561	2204	786	ND	ND	1117	ND	29.4	146
	IEC	622	2635	496	279	ND	1464	2.6	ND	ND
	RPC	533	2289	844	190	ND	1522	3.5	ND	ND
Merlot	IC	160	ND	995	1745	274	1564	ND	23.6	141
	IEC	742	828	902	1822	122	1978	5.5	ND	ND
	RPC	ND	103	1082	1963	ND	1731	3.0	ND	ND
Koshu 1989	IC	146	139	688	1780	200	2208	ND	15.0	186
	IEC	520	153	437	2079	165	2312	3.2	ND	ND
	RPC	401	162	591	1749	152	2330	4.5	ND	ND
M.B.A 1989	IC	588	7852	845	153	ND	1394	ND	16.2	110
	IEC	659	9303	916	149	108	1655	3.4	ND	ND
	RPC	418	6139	ND	107	194	1554	3.7	ND	ND

ND: not detected. E.F., Ezer Furtu; C.S., Cabernet Sauvignon; C.F., Cabernet Franc; P.N., Pinot Noir; M.B.A, Muscat Bailey A.

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