SHORT COMMUNICATION

Simultaneous Determination of Organic Acids, Inorganic Anions, and Alditols in Wine with Valve-Switching Ion Chromatography

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Abstract
A valve-switching ion chromatography method coupled with conductivity and pulsed amperometric detection has been set up for the simultaneous determination of organic acids, inorganic anions and alditols in wine. The system, based on valve-switching technique, consisted of two pumps, two analytical columns and a trap column connected in series via one six-port valve. In addition, the optimal valve-switching time and chromatographic conditions were explored to obtain a good linearity, precision, accuracy, and sensitivity with a mean correlation coefficient of > 0.99, repeatability of 0.62–6.18% for eight replicates, and the average spiked recovery of 96.66%. The new method was successfully applied in the analysis of six kinds of wine from different regions.

Keywords Valve-switching ion chromatography · Organic acids · Inorganic anions · Alditols · Wine

Introduction

Organic acids, inorganic anions, and alditols play a significant role in wine for its properties (e.g., taste, aroma, and colour), nutrition, and biological stability. Therefore, they are regarded as chemical markers of ripeness, bacterial activity, or storage conditions. The content and kinds of organic acids are the decisive factors on acidity which are related to the region and climate during the growth and ripening of grapes [1, 2]. Similarly, alditols are seen as an emerging functional sweetener with less thermal, indigestibility, as well as low sweetness, which can make the wine more mellow and aromatic [3]. In addition, the main anions such as chloride, sulfate, and phosphate must be controlled within a certain extent, because they can influence the flavor of wine. As a result, the accurate determination of organic acids, inorganic anions, and alditols is highly valuable to optimize the production process and control product quality.

Nowadays, chromatographic analysis [4–14] or tandem mass spectrometry [15–19] is the most used to identify and quantify ions, organic acids, sugars, and alditols. Nevertheless, the analysis of some organic acids and alditols needs to employ complicated derivation by high-performance liquid chromatography (HPLC) [7–10], or gas chromatography–mass spectrometry [5]. Ion chromatography as a sensitive and selective method without derivatisation was widely employed to determine these polar compounds [11–14]. However, simultaneous determination of organic acids, inorganic anions, and alditols has not been reported. One same sample needs to be tested many times, respectively, so that it will cost more time to obtain all information of target substances. In the present study, valve-switching technique has offered a good pathway for the elimination of matrices interference and enrichment of the target substance in the chromatographic system. It not only overcomes the limitation in resolution of one-dimensional ion chromatography,

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but also simplifies tedious operation and decreases a great deal of testing time [20–22].

Therefore, we developed and validated an efficient ion chromatography method by valve-switching technique for the simultaneous determination of organic acids, inorganic anions, and alditols in this paper. The system consisted of two pumps, a six-port valve, two ion-exchange columns, conductivity detector, and pulsed amperometric detector and automatically switched by only one six-port valve. The new method was successfully applied in the analysis of six kinds of wine from different regions. Furthermore, it saved much working time, so that greatly improved the efficiency during wine-making process monitor and quality control.

Experimental

Apparatus and Chromatography Conditions

Analyses were conducted using an ICS-5000 system (Thermo Fisher Scientific, Sunnyvale, CA, USA) equipped with two gradient pump, an eluent generator (EG40) with potassium hydroxide (KOH) cartridge and CR-TC, a pulsed amperometric detector (PAD), a conductivity detector (CD), a self-regenerating suppressor (AERS 500 4 mm), an AS40 auto-sampler with a 25 μL sample loop, and a six-port valve. System control and data acquisition were performed by Chromeleon 6.8 software.

CarboPac MA1 Guard column (4 × 50 mm, Thermo Fisher Scientific) was used as trap column. An IonPac AS11-HC Analytical column (4 × 250 mm, Thermo Fisher Scientific) with an IonPac AG11-HC Guard column (4 × 50 mm, Thermo Fisher Scientific) was employed for the separation of organic acids and inorganic anions. A CarboPac MA1 (4 × 250 mm, Thermo Fisher Scientific) analytical column was used to separate alditols.

The gradient elution program for organic acids and inorganic anions was: 1 mmol L^{-1} (1–15 min); 5 mmol L^{-1} (15–20 min); 20 mmol L^{-1} (20–31 min); 22.4 mmol L^{-1} (31–45 min); 40 mmol L^{-1} (45–55 min); 45 mmol L^{-1} (55–60 min); and 1 mmol L^{-1} (60–65 min). The isocratic 500 mmol L^{-1} NaOH was utilized for alditols at a flow rate of 0.4 mL min^{-1}. The pulsed amperometric detector was

equipped with a triple-electrode system that included a gold working electrode, an Ag/AgCl reference electrode, and a titanium cell body as the counter electrode. The waveforms are listed in Sect. 2.1 of Ref. [20].

Reagents and Samples

Organic acid standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). The anion standards (1000 mg L^{-1}) were purchased from the National Institute of Metrology. The alditol standards were supplied by Aladdin (Shanghai, China). Sodium hydroxide (50%, w/w, certified grade) was offered by Acros Organics (New Jersey, USA). All the solutions were prepared in 18.2 MΩ cm water (Milli-Q system, Millipore, Mosheim, France). The working standard solutions were prepared by diluting Stock standard solutions (1000 mg L^{-1}).

The wine samples of six brands were collected from different countries and regions including red wine from Italy Primitivo (R1), red wine from South Africa Remhoogte (R2), red wine from France La Tour Argent (R3), white wine from France Chateau Rievssec (W1), white wine from France Latour Laguens (W2), and white wine from Portugal AiQuan (W3). All samples were diluted 100 times and then filtered through 0.22 μm nylon membrane and were handled with Cleanert IC-RP column (Agela Technologies, Tianjin, China) for removing the macromolecular organic matters before analysis.

Valve-Switching Program

Four steps were operated to accomplish the valve-switching procedure (Fig. 1). First, the sample was loaded into the loop. Second, it was the extracting phase, in which alditols was eluted from the AS11-HC column and extracted on the trap column. Third, it was the analysis phase, where organic acids and inorganic anions were continually separated on the AS11-HC column and detected on conductivity detector, while alditols were eluted from the trap column, separated on MA-1 column, and analyzed by pulsed amperometric detector, respectively. Finally, it involves re-equilibration. Once the system reached re-equilibration, the cycle restarted

Fig. 1 Sketch map of the valve-switching program. Continuous black lines represent closed status of the path flow, and arrows indicate the flow direction
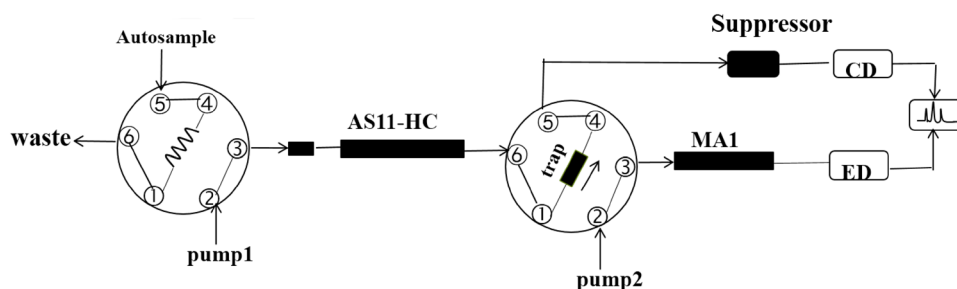
from the beginning. The time and the status of valves are listed in Table 1.

Method Validation

The method was validated for linearity, repeatability, reproducibility, limits of detection (LODs), and quantification (LOQs). The linearity was established by the injection of the standard mixtures at concentrations varying from 0.02 to 50 mg L^{-1}. The calibration curves were constructed by plotting peak areas versus different concentrations of standard mixtures. Besides, the repeatability was estimated using eight replicates of 2 mg L^{-1} standard mixtures within 1 day, whereas reproducibility was calculated by analyzing three replicates at three different concentrations levels (2, 5, and 10 mg L^{-1}) on three consecutive days. Furthermore, the LODs and LOQs were calculated as the amounts for which the signal-to-noise ratio (*S/N*) was 3 and 10, respectively. Eventually, the recoveries represented the mean of three levels (1, 5, and 10 mg L^{-1}) of spiked recoveries.

Results and Discussion

The Valve-Switching Time

At appropriate valve-switching time, alditols should be completely transferred to the trap column and the weak retain anion must not be eluted from AS11-HC column. Therefore, in this section, glycerol of 10 mg L^{-1} was selected as the standard to confirm the optimal valve-switching time as it was first eluted from the MA-1 column. Since glycerol did not appear before 3.2 min., the optimization of switching time started from 3.2 min and ended to 4.0 min (3.2, 3.4, 3.5, 3.6, 3.7, 3.8, and 4.0 min). The peak area of glycerol reached a maximum value at 3.5 min suggested that the optimal valve-switching time was 3.5 min.

Analytical Properties

Table 2 lists the linear equation, range of linearity, and correlation coefficient (r^2) of calibration curves. Satisfyingly, the method showed good linearity, which the mean correlation coefficient was 0.996. As displayed in Fig. 2a, b,

all components were successfully separated without obvious interference under the optimal conditions. The relative standard deviation (RSD) of repeatability and reproducibility ranged from 0.62 to 6.18 and from 0.34 to 3.48%, respectively. The LODs were 0.01–0.03 mg L^{-1} while LOQs 0.01–0.10 mg L^{-1}. Table 3 summarizes the average recoveries of three levels with 83–113%.

Application to Wine Samples

The new method was applied to determine organic acids, inorganic anions, and alditols in wine samples of three kinds of red wines and three white wines from six regions. The chromatograms of six samples demonstrated a complete separation of organic acids, inorganic anions, and alditols (Fig. 2a, R$_1$, R$_2$, R$_3$, W$_1$, W$_2$, and W$_3$ and Fig. 2b, R$_1$, R$_2$, R$_3$, W$_1$, W$_2$, and W$_3$). Their contents are listed in Table 3.

First, 11 organic acids were found in these samples. The major acids were lactic acid, tartaric acid, succinic acid, citric acid, maleic acid, fumaric acid, and isocitric acid. Among them, lactic acid originates from the alcoholic fermentation and malolactic fermentation, in which the procedure gets the original wine more smooth. As the "wine diamond", tartaric acid has considerably higher concentration than other acids. In addition, the red wine had a larger amount of lactic acid than the white, because the white wine is lack of malolactic fermentation process [8]. Second, the data revealed all samples presented low concentration of chloride and phosphate. However, the content of sulfate was obviously higher than the other anions due to the addition of sulfur dioxide or sulfite for killing the bacteria and ensure the integrity of wine flavor. Finally, the amount of glycerol of sample R$_1$ was remarkably higher than others. In addition, three isomerides (sorbitol, mannitol, and dulcitol) are well separated and qualified. In brief, the content changes in organic acids, inorganic anions, and alditols of six samples demonstrated the diversities in process, quality, or geographical origin.

Conclusions

The work provided a reliable, sensitive, and effective method coupling with conductivity and pulsed amperometric detection for the simultaneous determination of organic acids, inorganic anions, and alditols in wine. It demonstrated that the method had a good linearity, precision, accuracy, and sensitivity with a mean correlation coefficient of > 0.99, repeatability of 0.62–6.18% for eight replicates, the average spiked recovery of 96.66%, and LODs of 0.01–0.03 mg L^{-1}. Results of real samples are satisfactory and highly valuable for wine-making process monitor and quality control.

Table 1 Timing and the status of valves

Procedure	Time (min)	Valve 1	Valve 2
Step 1	− 4.5	Load	Load
Step 2	0	Inject	Load
Step 3	3.5	Inject	Load
Step 4	3.5–65	Inject	Inject

Table 2 Results of method validation

No.	Analyte	Correlation coefficient	Regression equation	Linear range (mg L^{-1})	Repeatability (%RSD, $n = 8$)	Reproducibility (%RSD)	LOD (m L^{-1})	LOQ (mg L^{-1})
1	Quinic	0.9983	$Y = 5.18X - 0.95$	0.10–20.00	2.87	3.09	0.02	0.07
2	Lactic	0.9982	$Y = 7.80X - 1.32$	0.20–20.00	2.46	2.43	0.02	0.04
3	Acetic	0.9943	$Y = 10.44X + 0.54$	0.10–20.00	3.69	2.05	0.01	0.03
4	Propionic	0.9997	$Y = 90.20X + 1.22$	0.20–20.00	1.48	1.65	0.02	0.04
5	Formic	0.9999	$Y = 19.03X - 0.61$	0.20–20.00	3.52	2.94	0.02	0.02
6	Butyric	0.9918	$Y = 5.02X + 9.93$	0.50–20.00	0.62	1.50	0.02	0.06
7	Pyruvic	0.9993	$Y = 7.53X - 1.80$	0.20–20.00	3.71	3.30	0.02	0.05
8	Pentanoic	0.9827	$Y = 5.67X + 2.70$	0.50–20.00	1.88	2.16	0.02	0.04
9	Chloride	0.9999	$Y = 34.02X - 0.78$	0.02–20.00	0.45	0.34	0.01	0.01
10	Succinic	0.9974	$Y = 7.01X + 1.99$	0.02–50.00	0.89	1.64	0.01	0.02
11	Tartaric	0.9800	$Y = 8.07X + 35.16$	0.02–50.00	4.01	3.41	0.01	0.02
12	Maleic	0.9993	$Y = 8.72X - 1.30$	0.50–20.00	1.28	2.11	0.01	0.03
13	Sulfate	0.9978	$Y = 22.13X + 5.35$	0.02–50.00	0.86	0.67	0.01	0.01
14	Fumaric	0.9999	$Y = 4.81X + 0.61$	0.50–20.00	1.52	2.97	0.02	0.06
15	Phosphate	0.9968	$Y = 8.17X - 2.53$	0.20–20.00	2.47	1.84	0.02	0.05
16	Citric	0.9989	$Y = 5.93X - 0.01$	0.20–20.00	2.06	1.57	0.02	0.05
17	Isocitrate	0.9980	$Y = 4.00X - 0.55$	0.20–20.00	0.85	1.28	0.02	0.07
18	Aconitic	0.9994	$Y = 3.07X - 0.63$	0.20—20.00	6.18	3.39	0.03	0.10
A	Glycerol	0.9973	$Y = 8.85X - 0.96$	0.05–20.00	1.50	1.31	0.01	0.02
B	Erythritol	1.0000	$Y = 12.85X + 0.27$	0.05–20.00	1.58	1.67	0.01	0.02
C	Xylitol	0.9996	$Y = 11.24X - 0.58$	0.05–20.00	3.61	1.9	0.01	0.02
D	Arabitol	0.9990	$Y = 9.50X - 0.08$	0.05–20.00	3.29	1.95	0.01	0.03
E	Sorbitol	0.9951	$Y = 9.07X - 0.61$	0.05–20.00	1.54	1.51	0.01	0.03
F	Dulcitol	0.9976	$Y = 9.49X - 0.69$	0.05–20.00	2.11	1.63	0.01	0.03
G	Mannitol	0.9967	$Y = 7.84X + 1.91$	0.10–20.00	2.20	1.43	0.01	0.03
H	Maltitol	0.9998	$Y = 7.07X + 1.03$	0.20–20.00	5.63	3.48	0.02	0.07
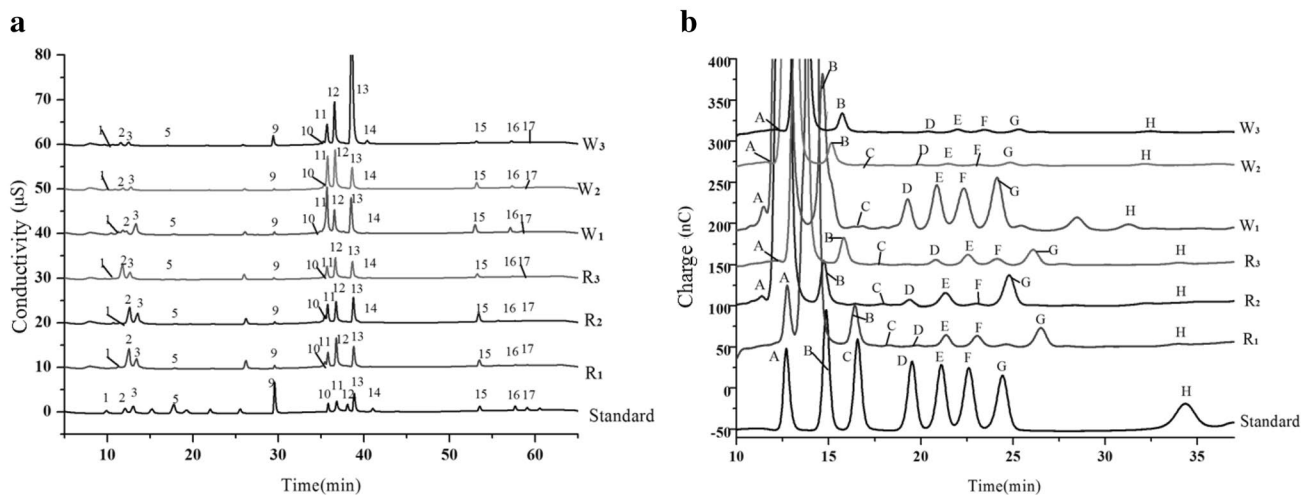
Fig. 2 **a** Chromatogram of organic acids and inorganic anions of the six samples and standard mixtures of 5 mg L^{-1}. **b** Chromatogram of alditols of the six samples and standard mixtures of 5 mg L^{-1}. Peak identities are given in Tables 2 and 3

Table 3 Results of determination in wine samples

No.	Analyte	R1 Content (mg L^{-1})	R1 Average recovery (%)	R2 Content (mg L^{-1})	R2 Average recovery (%)	R3 Content (mg L^{-1})	R3 Average recovery (%)	W1 Content (mg L^{-1})	W1 Average recovery (%)	W2 Content (mg L^{-1})	W2 Average recovery (%)	W3 Content (mg L^{-1})	W3 Average recovery (%)
1	Quinic	ND	93.90	98.72	98.47	96.59	91.70	217.19	87.85	161.47	84.89	85.26	98.35
2	Lactic	1920.74	94.67	1714.62	89.94	1552.08	88.52	108.68	88.72	188.23	101.03	243.25	98.02
3	Acetic	567.93	97.67	865.06	84.70	521.38	88.00	1071.38	86.42	292.73	84.41	410.36	100.21
4	Propionic	ND	91.12	ND	83.41	ND	91.30	ND	88.79	ND	99.22	ND	91.18
5	Formic	25.16	103.67	23.94	95.00	23.35	92.04	47.53	91.60	13.99	93.99	9.16	98.11
6	Butyric	ND	92.11	ND	96.10	ND	96.19	ND	95.91	ND	103.58	ND	103.17
7	Pyruvic	ND	84.65	ND	102.48	ND	96.08	55.75	92.90	50.03	93.15	59.20	96.02
8	Pentanoic	ND	83.18	ND	88.39	ND	85.87	ND	83.95	ND	95.78	ND	83.62
9	Chloride	39.44	96.94	33.21	106.05	32.57	100.50	49.25	98.71	25.37	99.83	164.73	102.70
10	Succinic	787.83	87.68	935.35	91.53	683.70	90.44	3883.21	86.11	2228.41	98.52	1503.13	93.16
11	Tartaric	1474.81	103.86	1017.46	103.04	998.26	104.96	975.32	99.75	2020.64	113.63	2452.09	95.65
12	Maleic	36.94	92.27	42.51	94.34	30.26	94.45	63.34	99.06	13.34	97.53	24.64	97.89
13	Sulfate	583.48	93.30	716.16	104.80	464.20	104.02	1000.05	93.04	590.53	87.91	3411.87	87.77
14	Fumaric	54.82	101.01	46.58	107.83	43.57	98.62	62.98	96.56	46.87	97.82	ND	99.41
15	Phosphate	588.06	106.42	833.84	97.47	380.15	101.76	802.35	103.04	493.78	106.15	184.99	97.07
16	Citric	83.64	92.80	13.88	99.26	55.30	101.04	518.71	102.19	176.61	102.68	189.99	100.81
17	Isocitrate	91.82	96.61	65.28	103.04	59.96	103.00	146.31	103.40	176.61	95.52	58.35	98.41
18	Aconitic	ND	99.37	ND	101.10	ND	101.70	ND	102.71	ND	98.70	ND	99.36
A	Glycerol	230.20	95.17	35.25	97.57	16.14	92.28	90.22	100.03	14.30	90.64	13.18	87.24
B	Erythritol	162.22	99.03	190.33	102.14	124.97	99.13	813.30	102.09	103.86	99.78	70.66	95.66
C	Xylitol	19.38	93.42	24.05	93.64	11.21	100.15	223.52	97.86	10.85	101.47	ND	103.09
D	Arabitol	63.91	100.59	42.27	102.82	36.14	102.70	221.20	98.93	5.02	102.44	20.27	103.15
E	Sorbitol	75.01	95.84	141.64	103.30	88.06	96.97	356.53	96.99	15.22	95.23	23.54	101.56
F	Dulcitol	14.63	99.12	13.68	98.57	52.01	99.86	359.05	99.48	7.10	99.76	9.14	101.68
G	Mannitol	153.71	99.63	323.34	94.14	157.18	97.76	504.73	100.85	29.30	97.07	28.79	96.82
H	Maltitol	83.83	94.14	33.58	91.85	18.47	96.11	53.85	83.51	21.56	99.69	30.42	94.39

ND not detected

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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