Advance Journal of Food Science and Technology 9(2): 127-134, 2015
DOI: 10.19026/ajfst.9.1947
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: February 3, 2015 Accepted: March 1, 2015 Published: August 05, 2015

Research Article

Analysis of Organic Acids in Blueberry Juice and its Fermented Wine by High Performance Liquid Chromatography

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Abstract: A rapid analytical method for simultaneous separation and determination of organic acids is of the essence for quality control of blueberry juice and its fermented wine. In this present study, a High Performance Liquid Chromatography (HPLC) method for separation and determination of organic acids (oxalic acid, gluconic acid, tartaric acid, formic acid, pyruvic acid, malic acid, isocitric acid, shikimic acid, lactic acid, acetic acid, citric acid, succinic acid and propionic acid) in blueberry juice and wine has been developed. The chromatographic separation was performed at 35°C by using an ammonium hydrogen phosphate buffer (pH 2.8) as mobile phase and 0.6 mL/min as the column flow rate. A C_{18} analytical column and Ultraviolet Detection (UV) at λ = 210 nm were used for all acids above. The method was validated for linearity, limit of detection, limit of quantification, accuracy and precision. The applicability of the method was demonstrated by analyzing organic acids in real samples of six species of blueberry juices and wines. The results show that species significantly affect distribution of organic acids in samples but not the kinds of organic acids between six species. Oxalic acid, gluconic acid, malic acid, shikimic acid and citric acid are detected in blueberry juice. Citric acid, which accounts for a percentage >75% of the whole content of organic acids, is the major acid in four kinds of tested species (Sharpblue, Misty, Anna and Bluecrop). In the other two species (Britewell and Premier), malic acid, gluconic acid and citric acid own a mean percentage of 40, 32 and 25%, respectively. After yeast fermentation and aging, several new organic acids (pyruvic acid, isocitric acid, lactic acid, acetic acid, succinic acid and propionic acid) appear in wine.

Keywords: Analysis, blueberry juice, fermented blueberry wine, HPLC, organic acids

INTRODUCTION

Blueberry is suitable for wine fermentation because of its high sugar, acid and anthocyanin content. Not only does the blueberry wine possess a unique flavor, but it also has the function of nutrition and health care (Norberto *et al*., 2013; Shukitt-Hale, 2011). Organic acid is an important parameter of quality and freshness as they are widely distributed in fruit juice and wine. The content of organic acids in fruit juice and wine not only play important roles in balancing flavor, taste and color, but also influence the chemical equilibrium, pH value and microbial activity and ultimately affects the quality and acceptability (Kerem *et al*., 2004). Some organic acids originate from fruits (i.e., oxalic acid, tartaric acid, gluconic acid, malic acid, shikimic acid and citric acid), others are produced during the fermentation and aging of wine (i.e., formic acid, pyruvic acid, isocitric acid, lactic acid, acetic acid, succinic acid and propionic acid). The kind and content of organic acids varies between different fruits. For example, grapevine berries contain organic acids like tartaric, malic, citric and succinic acid (Eyéghé-Bickong *et al*., 2012), while apples mainly contain malic acid (Zhang *et al*., 2008). However, there has been limited research on organic acid of blueberry juice and its wine.

Several techniques have been used for qualitative and quantitative detection of organic acids, from a single organic acid test to several kinds of organic acids detection at the same time, which include enzyme (Mazzei *et al*., 2007), spectrophotometry (Shishehbore and Aghamiri, 2014), potentiometry (Yang *et al*., 2012), spectrofluorimetry (Mato *et al*., 2007), capillary electrophoresis (Turkia *et al*., 2013), gas chromatography (Lin *et al*., 2014) and ion-exchange chromatographic (Prusisz *et al*., 2008). However, most of these techniques have limitations. In recent years, HPLC methods have been developed for the determination of organic acids in wine and were gradually matured and widely used basing on its

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simplicity, high sensitivity, good selectivity and high accuracy (Igual *et al*., 2010; Liu *et al*., 2013).

Sample preparation of fruit juice and wine is required before HPLC in order to obtain a better separation effect in chromatogram. For example, Marconi *et al*. (2007) used an anion exchange resin to preprocess tomato juice samples; In addition, cellulose membrane and Solid Phase Extraction (SPE) were also combined to preprocess honey samples and grape wine samples (Davis *et al*., 1986; Suarez-Luque *et al*., 2002). However, most of these preparation methods are complicated and costly.

The objective of the present study is to establish a validated HPLC-UV method for simultaneous detection of 13 organic acids with a simple and inexpensive sample preparation method and then to detect the kinds and content of organic acids in blueberry juice and its fermented wine, so as to further reveal the organic acid composition rules of blueberry juice and wine.

MATERIALS AND METHODS

Reagents and chemicals: Oxalic acid, gluconic acid, tartaric acid, formic acid, pyruvic acid, malic acid, isocitric acid, shikimic acid, lactic acid, acetic acid, citric acid, succinic acid and propionic acid were purchased from Sigma-Aldrich Corp. in USA; Methanol (Fisher Scientific, USA) was of HPLC grade; $(NH_4)_2HPO_4$ and H_3PO_4 with analytical grade were obtained from Sinopharm Chemical Reagent Co., Ltd (SCRC) in China. Watsons distilled water was purchased from supermarkets for sample preparations. Pectinase and yeast used in fermentation were purchased from Laffort in French. Sugar and H_2SO_3 used in fermentation were food grade.

Standard solutions: Oxalic acid (40 mg), gluconic acid (250 mg), tartaric acid (100 mg), formic acid (500 mg), pyruvic acid (200 mg), malic acid (100 mg), isocitric acid (500 mg), shikimic acid (50 mg), lactic acid (300 mg), acetic acid (400 mg), citric acid (300 mg), succinic acid (300 mg) and propionic acid (500 mg) (accurate to 0.1 mg) were precisely weighed and dissolved in 10 mL mobile phase solution in a brown volumetric flask. The stock mixture was then gradually diluted to obtain six different concentrations and all standards were stored at -20°C until use.

Blueberry samples: Fresh blueberries of different species were obtained during a harvest in 2013 from Hefei blueberry plantation located in the central-eastern of China. In this article, blueberries from six blueberry cultivars were selected as samples, including three southern highbush blueberry (*Vaccinium corymbosum*): Sharpblue, Misty and Anna; two rabbiteye blueberry (*Vaccinium ashei*): Britewell and Premier; one northern highbush blueberry (*Vaccinium corymbosum*): Bluecrop. They were frozen and stored at -20°C for subsequent studies.

Fermentation process: Frozen blueberries were placed at room temperature for natural thaw. Then they were crushed into mash and then treated with 60 mg/L SO_2 and 0.03 g/kg pectinase. The fermentable sugar of samples were adjusted to 210.0 g/L of with sugar, then they were added with 0.4 g/L yeast, which was activated in a blueberry juice bath for about 20 min at 45°C in advance. Samples were subpackaged in triplicates. Then the main fermentation was carried out at 25°C and was finished when the sugar content dropped below 4.0 g/L. After siphoning, pressing, fining separation and sediment removal, wine samples were sealed and aged at 16°C for four weeks.

Sample preparation: Samples were injected directly for HPLC analysis after the following preparations. Blueberry juices (fermentated for 0 day) and its fermented wines (aged for 4 weeks) were centrifuged under freezing condition at the speed of 15000 r/min. And then they were diluted with mobile phase solution and filtered through 0.45 μm membranes.

Validation: The validation parameters consisted at linearity range, Limits of Detection (LOD), Limits of Quantification (LOQ), accuracy and precision.

Linearity range, LOD and LOQ: Linearity of the method was established by automatic injections of the standard mixture solutions in the investigated ranges from low to high concentrations, each concentration was repeated three times. LOD and LOQ were separately determined by diluting the standard solution.

Accuracy: The accuracy of the method was determined by spiking wine samples with known concentrations of thirteen standard references and comparing the increase in peak area with the expected increase calculated from the linear working range of the calibration curve. In this study, three known concentrations (low, medium and high) of the thirteen standard references were spiked into samples and every concentration were carried out in triplicate. Recovery of the organic acids in samples were detected to obtain accuracy data.

Precision: The precision of the method was determined by measuring the intra-day precision (repeatability, six successive injections on the same day) and the inter-day precision (intermediate precision, six injections on three different days), both expressed as RSD (%). The spiked blueberry wines used in accuracy analysis were also served in precision study.

Statistic analysis: The experimental data were organized by Microsoft Excel 2010.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions: For method optimization, pH of the mobile phase,

Table 1: Regression equation, correlation coefficient (R^2), Linear range (mg/L), LOD (μg/L), LOQ (μg/L) of 13 organic acids

Organic acid	Regression equation	R^2	Linear range (mg/L)	LOD (μg/L)	LOQ (μg/L)
Oxalic acid	$C = 2.00*10^{-4}A+1.14$	0.9997	5.01-250.27	37.39	124.63
Gluconic acid	$C = 2.70*10^{-3}A-16.80$	0.9991	50.19-1750.57	515.11	1717.03
Tartaric acid	$C =1.30*10^{-3}A+46.80$	0.9996	63.92-2390.24	474.00	1580.00
Formic acid	$C = 2.10*10^{-3}A –0.33$	1.0000	22.21-549.93	373.68	1245.60
Pyruvic acid	$C = 1.00*10^{-4}A+0.09$	0.9999	1.44-93.62	73.67	245.57
Malic acid	$C = 2.10*10^{-3}A+3.98$	0.9994	5.31-74.10	503.53	1678.43
Isocitric acid	$C = 1.20*10^{-2}A– 17.40$	0.9995	18.17-1728.35	564.59	1881.97
Shikimic acid	$C = 7.00*10^{-5}A+0.13$	0.9997	0.62-16.20	5.37	17.90
Lactic acid	$C = 2.20*10^{-3}A+2.28$	0.9994	17.85- 608.38	452.22	1507.40
Acetic acid	$C = 3.30*10^{-3}A+ 27.70$	0.9997	68.37-1703.29	630.93	2103.10
Citric acid	$C = 4.10*10^{-3}A+ 24.10$	0.9996	44.20-917.89	2865.17	9550.57
Succinic acid	$C = 8.40*10^{-3}A+10.80$	0.9996	102.01-1315.98	1440.55	4801.83
Propionic acid	$C = 4.10*10^{-3}A+ 79.70$	0.9998	139.86-2599.78	3186.65	10611.17

column temperature, flow rate of the mobile phase and the concentration of samples were tested respectively to achieve good separation of as many peaks as possible within a short analysis time.

Chemicals that could be used as mobile phase to separate organic acids with the method of HPLC vary. Metaphosphoric acid (pH 2.2), 3 mM phosphoric acid and water with a 0.1% (v/v) of formic acid were once used as mobile phase to separate organic acids (De Quirós *et al*., 2009; Suarez-Luque *et al*., 2002; Uckoo *et al*., 2011). The ammonium hydrogen phosphate buffer which had a good separation effect on short-chain carboxylic acid was used in this study. The effect of pH on separation of the organic acids in standard samples was studied by adjusting pH of the mobile phase [0.01 M $(NH_4)_2HPO_4$] with H_3PO_4 to 2.4, 2.6, 2.8 and 3.0. Results showed that retention time shortened for all organic acids with pH increasing. At pH 3.0 and pH 2.6, malic acid and isocitric acid could not reach baseline separation; At pH 2.4, malic acid, isocitric acid and shikimic acid almost merged into a single peak. A good separation effect of 13 organic acids only at pH 2.8 was found existed. Therefore, the optimum pH of the mobile phase was 2.8 in this study.

Three sets of temperature (25, 30 and 35°C) and two sets of flow rate (0.6 mL/min and 1.0 mL/min) were used to test the effect of column temperature and flow rate of the mobile phase on separation of organic acids. Results showed that the higher temperature and faster flow rate, the more shortened the retention time organic acids have. However, the effect of temperature was not as significant as the flow rate. For fast and accurate detection, a final column temperature of 35°C and a flow rate of 0.6 mL/min were chosen for this study.

Samples were diluted in 10-fold, 16-fold and 20-fold with mobile phase solution to analyze the effect of concentration of samples. Results showed that the stability of baseline decreased with dilution ratio increasing. When the dilution ratio is 10, baseline is stable and peak shape is good. So the optimum dilution ratio of samples was 10.

Method validation: The validation parameters consisted at linearity range, LOD, LOQ, accuracy and precision, which accorded to the Pharmacopoeia of the People's Republic of China guidelines for bioanalytical method validation (Pharmacopoeia Commission of People's Republic of China, 2010).

Linearity of the method was established by automatic injections of the standard mixture solutions in the investigated ranges from low to high concentrations, each concentration was repeated three times. Linearity was evaluated by plotting detector response (peak area, A) against analyte concentration (C, mg/L) to obtain the calibration curve and correlation coefficient (R^2). Standard curves of 13 organic acids were linear in the investigated range and the R^2 values of 13 organic acids were found to be ≥ 0.9991, suggesting an excellent linearity of analytes. Results obtained were summarized in Table 1.

The detection sensitivity can be assessed by LOD which is the lowest concentration that can be detected. LOQ is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. LOD was the minimal concentration of the analyte giving a peak height that was three times the noise base line and 10 times for LOQ. LOD and LOQ were separately determined by diluting the standard solution. LOD and LOQ were separately determined by diluting the standard solution. Results obtained listed in Table 1.

To validate the accuracy of the method, a known amount (low, medium and high) of the thirteen standard references were spiked into wine samples. The percent recoveries of the organic acids in wine samples were determined. The average recovery rate of each organic acid standard was calculated in Table 2. Good recoveries which ranged from 85.44 to 106.68% were obtained for each added concentration, confirming that the method was accurate.

Intra-day precision (repeatability, six successive injections on the same day) and the inter-day precision (intermediate precision, six injections on three different days), were both expressed as a relative standard deviation (RSD %). RSD of citric acid showed an unstable state and one of its RSD values was over 5%, which may due to its high content. RSD of acetic acid for both intra-day precision (n = 6) and inter-day precision (n = 3) was over 5% because of its volatility

Table 2: Results of accuracy of the method

Organic acid	Initial amount (mg/L)	Added (mg/L)	Found Mean value ±SD (mg/L)	RSD (%)	Recovery (%)
Oxalic acid	2.20	1.76	3.84±0.09	2.23	96.98
		2.21	4.38±0.05	1.15	99.15
		5.63	7.99±0.02	0.24	102.15
Gluconic acid	60.00	48.66	109.72±1.02	0.93	100.98
		54.10	112.08±1.28	1.14	98.23
		56.29	116.28±0.98	0.84	99.99
Tartaric acid	0.00	31.90	31.66±0.37	1.16	99.24
		42.50	41.66±0.61	1.46	98.03
		69.19	70.25±0.72	1.02	101.53
Formic acid	0.00	10.65	10.10±0.20	1.97	94.85
		14.20	13.52±0.28	2.07	95.24
		15.98	16.49±0.22	1.35	103.23
Pyruvic acid	1.48	1.18	2.42±0.06	2.29	90.92
		1.49	2.80±0.02	0.58	94.02
		1.65	3.28±0.06	1.95	104.91
Malic acid	15.50	12.33	27.37±0.40	1.46	98.33
		12.55	28.06±0.25	0.89	100.02
		29.77	45.25±0.75	1.66	99.97
Isocitric acid	96.07	21.05	116.38±2.18	1.87	99.37
		64.42	159.52±2.28	1.43	99.40
		106.18	202.99±2.98	1.47	100.37
Shikimic acid	1.17	0.94	2.14±0.03	1.51	101.17
		1.26	2.43±0.01	0.28	100.02
		2.30	3.47±0.02	0.61	99.99
Lactic acid	10.13	8.10	17.34±0.33	1.90	95.11
		10.72	22.24±0.37	1.65	106.68
		14.59	23.95±0.39	1.63	96.86
Acetic acid	35.65	5.00	35.42±0.80	2.26	87.14
		39.44	64.16±0.61	0.96	85.44
		43.49	71.09±3.40	4.79	89.83
Citric acid	190.10	44.08	245.16±1.92	0.78	104.69
		102.86	294.36±1.59	0.54	100.48
		240.85	433.19±3.70	0.85	100.52
Succinic acid	55.40	24.86	80.31±0.75	0.94	100.07
		28.70	83.76±0.42	0.50	99.59
		50.93	106.28±1.78	1.67	99.95
Propionic acid	30.18	64.91	94.43±0.34	0.36	99.31
		72.60	101.94±0.55	0.54	99.18
		74.00	103.13±0.77	0.74	98.99

Table 3: Contents of organic acids (mg/L) in different species of blueberry juices (n = 3)

	Species of blueberry					
Organic acid	Sharpblue	Misty	Britewell	Premier	Anna	Bluecrop
Oxalic acid	104.60±1.60	110.08±2.25	101.41 ±3.78	96.76 ±4.55	127.95 ±4.88	94.34 ±2.17
Gluconic acid	2076.98±36.64	2079.08 ±38.11	2789.28 ±24.82	1950.04 ±11.84	2882.79 ±143.90	2288.38 ±30.11
Tartaric acid	ND[1]	ND	ND	ND	ND	ND
Formic acid	ND	ND	ND	ND	ND	ND
Pyruvic acid	ND	ND	ND	ND	ND	ND
Malic acid	1142.07±69.19	478.90 ±21.06	3319.66 ±133.45	2603.77±101.35	955.28 ±39.78	496.09 ±30.23
Isocitric acid	ND	ND	ND	ND	ND	ND
Shikimic acid	46.49±1.65	83.03 ±4.00	85.66 ±2.33	58.98 ±2.59	71.44 ±1.52	15.98 ±0.74
Lactic acid	ND	ND	ND	ND	ND	ND
Acetic acid	ND	ND	ND	ND	ND	ND
Citric acid	14962.5±470.39	11813.07 ±573.33	2464.50 ±110.30	1327.43 ±64.63	12214.94 ±216.89	10316.82 ±435.23
Succinic acid	ND	ND	ND	ND	ND	ND
Propionic acid	ND	ND	ND	ND	ND	ND

[1]ND: Not Detected

characteristic possible. RSD of most organic acids for both intra-day precision (n = 6) and inter-day precision (n = 3) were below 5%, demonstrating that the method were precise.

Analysis of organic acids of blueberry: According to the method described above, organic acids in six species of blueberry juices and fermented wines were analyzed. Data of juices were summarized in Table 3. Oxalic acid, gluconic acid, malic acid, shikimic acid and citric acid were detected in six species of blueberry juices (Fig. 1). However, different species owe different content of these organic acids. The major acid in four kinds of tested species (Sharpblue, Misty, Anna and

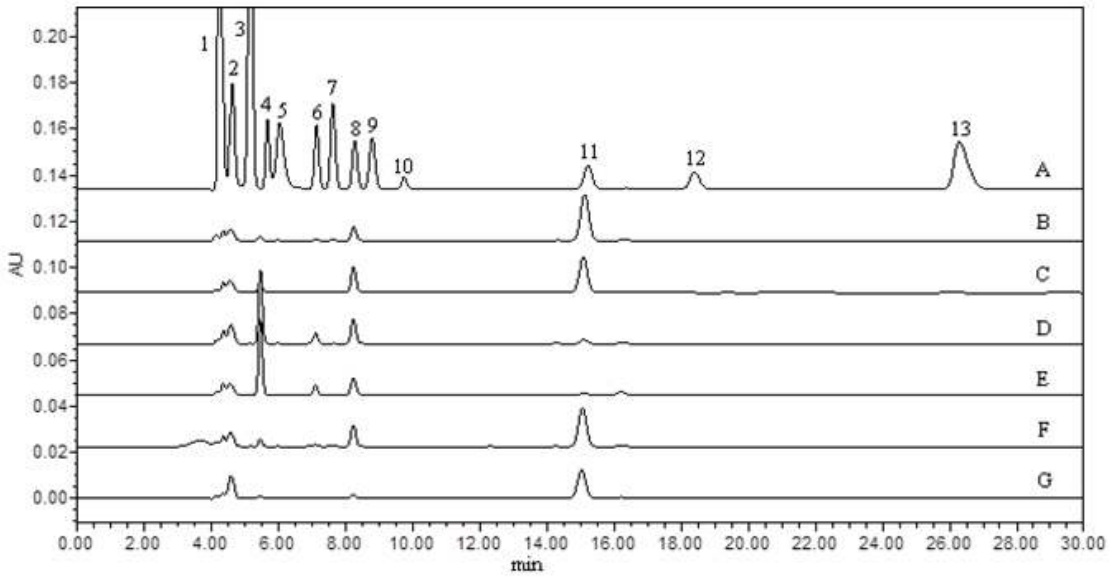

Fig. 1: Chromatograms of blueberry juice samples and organic acid standards; Peak 1: Oxalic acid; 2: Gluconic acid; 3: Tartaric acid; 4: Formic acid; 5: Pyruvic acid; 6: Malic acid; 7: Isocitric acid; 8-Shikimic acid; 9: Lactic acid; 10: Acetic acid; 11: Citric acid; 12: Succinic acid; 13: Propionic acid. A: organic acid standards; B: Sharpblue; C: Misty; D: Britewell; E: Premier; F: Anna; G: Bluecrop

Bluecrop) was citric acid, which accounted for a percentage >75% of the whole content of organic acids. In the other two species (Britewell and Premier), malic acid, gluconic acid and citric acid owned a mean percentage of 40, 32 and 25%, respectively. Oxalic acid is naturally present in blueberry and its content between six species had no significant difference. In grape, oxalic acid originates from the ascorbic acid metabolic pathway together with tartaric acid (Debolt *et al*., 2004; Oliveira *et al*., 2010). Gluconic acid, a permitted food additive, was detected in large amounts in blueberry juices. It did not frequently exist in other fruit juice (Eyéghé-Bickong *et al*., 2012; Tezcan *et al*., 2009; Zhang *et al*., 2008). In grape, gluconic acid presents in small concentrations naturally, but it was found in higher amounts when the grape was infected with Botrytis cinerea (Noble Rot) (Albanese *et al*., 2014). It could also be added in foodstuffs together or in substitution of citric acid to exploit flavor and antioxidant or preservative properties (Larcher *et al*., 2009). Malic acid, accompanies with citric acid, is certainly the substrates that wine lactic acid bacteria degrade most frequently in their natural environment (Saguir and Manca de Nadra, 2002). Its content between six species varies a lot. Britewell and Premier owed a large amount of malic acid, suggests that a malo-lactic fermentation is desired to obtain a good taste. Content of shikimic acid was low in six speices which only range from 15.98 to 85.66 mg/L. Citric acid was conductive to improve the anthocyanin stability (Gauche *et al*., 2010). However, citric acid is known to be metabolized by lactic acid bacteria to produce lactic acid, diacetyl, acetoin and acetic acid. Diacetyl, which is a common flavor substance in wine, could give rise to an acid spoiled taste when it is excessive.

Data of wines were summarized in Table 4. After yeast fermentation and aging, several acids from blueberries were found decreased. Content of oxalic acid and gluconic acid had a sharp fall, while the fall of malic acid and citric acid was milder. Content of shikimic acid had a smooth change. Besides, six new acids appeared in wine (Fig. 2). The general result was similar with grape wine that acetic acid and succinic acid were both detected in blueberry wine and grape wine (Xu *et al*., 2003). Pyruvic acid, an important intermediate product and is produced during the Embden-Meyerhof-Parnas (EMP) pathway, was detected in wine samples. It was affected significantly by sulfide dioxide and indicates the course of fermentation. Isocitric acid, produced in the Tricarboxylic Acid Cycle (TCA) cycle, was detected in Sharpblue, Misty, Anna and Bluecrop. Lactic acid tastes smooth and can improve the sensory characteristics of wine. Content of lactic acid detected in Britewell was higher than other species, showing that Britewell might have a better sensory characteristic than others. Content of acetic acid in six species was <1 g/L, suggesting that the juice was not been infected with acetic acid bacteria during fermentation and was able to show a good quality. Succinic acid, a normal product of yeast fermentation, could help to form rich esters during the maturing of wine. Content of succinic acid in six species had no significant differences except in Premier. Propionic acid, which showed a high content in wine samples, is a precursor for ethyl propionate which is a significant flavor substance to the

Table 4: Contents of organic acids (mg/L) in different species of blueberry wines (n = 3)

	Species of blueberry					
Organic acid	Sharpblue	Misty	Britewell	Premier	Anna	Bluecrop
Oxalic acid	25.32 ±0.50	26.17 ±0.28	31.11 ±0.97	36.07 ±0.92	68.55 ±0.88	25.47 ±0.24
Gluconic acid	766.34 ±25.06	923.17 ±52.31	979.9 ±9.97	1180.06 ±64.05	3854.52 ±175.00	1393.38 ±34.62
Tartaric acid	ND[1]	ND	ND	ND	ND	ND
Formic acid	ND	ND	ND	ND	ND	ND
Pyruvic acid	18.43 ±0.26	17.2 ±0.49	41.27 ±0.35	31.3 ±0.48	45.73 ±0.84	27.1 ±0.59
Malic acid	326.17 ±7.04	271.59 ±11.78	608.03 ±1.16	808.67 ±10.63	397.4 ±10.13	299.51 ±4.78
Isocitric acid	2862.97 ±52.34	2906.29 ±100.86	ND	ND	202.13 ±26.62	1055.56 ±58.36
Shikimic acid	41.19 ±0.13	65.99 ±0.99	89.93 ±1.26	59.69 ±0.058	64.83 ±0.59	22.33 ±0.23
Lactic acid	315.59 ±3.07	286.92 ±4.30	740.93 ±4.45	286.76 ±4.78	264.25 ±6.34	332.49 ±4.28
Acetic acid	618.287 ±11.96	890.163 ±18.78	881.165 ±16.14	645.58 ±19.63	739.98 ±15.03	914.06 ±30.15
Citric acid	10092.5 ±125.02	8449.04 ±357.01	2064.36 ±14.82	757.71 ±22.12	9831.05 ±179.43	7736.19 ±81.82
Succinic acid	2001.42 ±53.74	1845.32 ±24.87	2614.28 ±73.19	3533.72 ±70.44	2346.46 ±70.44	1850.66 ±44.15
Propionic acid	1253.84 ±29.54	1083.54 ±26.27	1547.42 ±50.56	1417.19 ±32.29	1536.61 ±27.66	1047.78 ±22.54

[1]ND: Not Detected

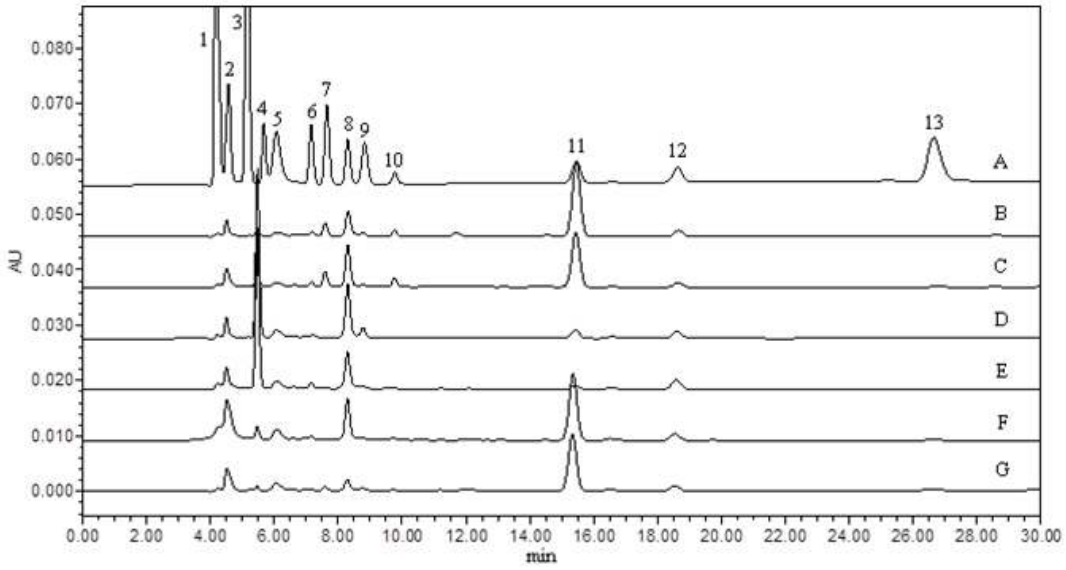

Fig. 2: Chromatograms of blueberry wine samples and organic acid standards; Peak 1: Oxalic acid; 2: Gluconic acid; 3: Tartaric acid; 4: Formic acid; 5: Pyruvic acid; 6: Malic acid; 7: Isocitric acid; 8: Shikimic acid; 9: Lactic acid; 10: Acetic acid; 11: Citric acid; 12: Succinic acid; 13: Propionic acid; A: organic acid standards; B: Sharpblue; C: Misty; D: Britewell; E: Premier; F: Anna; G: Bluecrop

beverage. However, tartaric acid and formic acid were not detected in blueberry wine, which was different from grape wine (Xu *et al*., 2003).

CONCLUSION

In conclusion, a simple, sensitive, good selectivity and high accuracy HPLC analytical method for thirteen organic acids had been developed in blueberry. The chromatographic separation was performed at a final column temperature of 35°C and a flow rate of 0.6 mL/min by using an ammonium hydrogen phosphate buffer (pH 2.8) as mobile phase. Besides, the optimum dilution ratio of samples was 10. The R^2 values of 13 organic acids were found to be ≥ 0.9991, suggesting an excellent linearity of analytes. Good recoveries which ranged from 85.44 to 106.68% were obtained for each added concentration, confirming that the method was

accurate. RSD of most organic acids for both intra-day precision (n = 6) and inter-day precision (n = 3) were below 5%, demonstrating that the method were precise.

Distribution and contents of organic acids of six species of blueberry juice and fermented wine were analysed by this method. Species significantly affected distribution of organic acids in samples, but not the kinds of organic acids. Oxalic acid, gluconic acid, malic acid, shikimic acid and citric acid were detected in blueberry juice. Citric acid, which accounted for a percentage > 75% of the whole content of organic acids, was the major acid in four kinds of tested species (Sharpblue, Misty, Anna and Bluecrop). In the other two species (Britewell and Premier), malic acid, gluconic acid and citric acid owned a mean percentage of 40, 32 and 25%, respectively. After yeast fermentation and aging, several organic acids from blueberries were detected decreased. However, several new organic acids (pyruvic acid, isocitric acid, lactic acid, acetic acid, succinic acid and propionic acid) appeared in wine samples.

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