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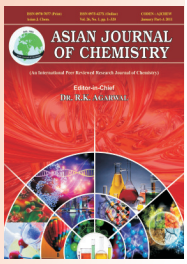

Simultaneous Determination of Organic Acids in Chinese Liquor by GC-MS Method

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A reliable method for determining the organic acids in Chinese liquors by derivatization GC-MS is reported. Acetic acid, butyric acid, isovaleric acid, pentanoic acid, caproic acid, heptylic acid and lactic acid were quantified in this text. The SIM mode was employed in the analysis and presented high accuracy, reproducibility and reduced the matrix effect effectively. The calibration curves were obtained with a satisfactory correlation coefficient of 0.9929 to 0.9994. The precision results showed that the relative standard deviations (RSD) of the repeatability were < 3.01 %. The accuracy of the method was confirmed with an average recovery ranging between 82.43 % and 109.38 %. The proposed method was used for the determination of organic acids in Chinese liquor. Application to different kinds and ages of Chinese liquor confirmed good repeatability and wide variation range for organic acids.

Keywords: Organic acids, Chinese liquor, Derivatization, GC-MS.

INTRODUCTION

Chinese liquor is a traditional distillate fermented from grain, corn, wheat, rice, or highland barley. It processes unique characteristics with excellent colour, aroma, taste and special fragrance lasting a long time and enjoys a good market all over the world.

In the long-term development, Chinese liquor has become a precious heritage of the spirit culture. Along with Brandy, Whisky, Rum, Vodka, Gin and other distillates, they are called the seven distilled spirits in the world[1].

It is well known that distilled spirits typically contain hundreds of fragrant and flavor matters which occupied 2 % of the total weight aside from the main ethanol-water matrix[2]. These micro aroma substances, such as alcohols, esters, acids, acetals, ketones, aldehydes, sulfur-containing compounds, lactones and heterocyclic compounds, are important in wine as they make a great contribution to the fragrance, style and quality of the final product[3,4]. As one of the most important classes of aroma compounds in alcoholic beverages, acids are the precursors of esters whose contents have direct influence on the quality of wine. The liquor smells bad when the acid content is high and tasteless when it's opposite. Proper amount of acids contributes to sweaty, cheesy, strawberry odors[2,3,5,6]. Thus, it is important to precisely determine the acids in liquors for quality control.

There are number of reports on acids determination and quantitative analysis. Previously, titration methods were used to determine total acids in wine[7,8]. In recent years, gas chromatography is one of the most common techniques for the analysis of wine and spirits[9-11]. It has also been used in the quantification of acids[12,13]. Several articles have been published on the application of ion chromatography[14], liquid chromatography[15-17] and supercritical fluid chromatography[18] to determine acids in different matrix. However, titration methods are commonly used to determine total acids; ion chromatography, liquid chromatography and supercritical fluid chromatography suffer from high cost and complex sample preparations, which limit their use in most laboratories; moreover, lactic acid, which is the one of main acids in Chinese liquor, could not be detected by means of injecting directly into a capillary gas chromatographic system. Furthermore, gas chromatographic analysis requires volatilization as well as reduction of adherence to the walls of the column[19]. Owing to the low concentration in liquors and strong polarity, acids have to be derivatized before they are injected into GC system.

The object of this work was to develop a method for determining the acids in alcohol beverage with GC/MS in selective ion monitoring (SIM) mode. GC/MS showed lots of advantages, including less solvent consumption and less toxicity. What's more, applying SIM mode during MS measuring makes the method more reliable, sensitive and reproducible[20]. To

verify this method, a validation was performed to determine the linearity of the standard solution, the limit of detection, the limit of quantification and the precision and recovery of the method. Finally, samples were analyzed with the parameter setting of the method and achieved a satisfactory result.

EXPERIMENTAL

Standard fatty acid (acetic acid, lactic acid, *n*-butyric acid, pentanoic acid, isovaleric acid, hexylic acid, heptylic acid) were purchased from Sigma-Aldrich (Shanghai, China). 2-Ethyl butyric acid was as internal standard and was purchased from Acros organics. Tetrabutyl ammonium hydroxide (10 %) was purchased from Shanghai chemical reagent Co. Ltd (Shanghai, china) and was prepared to 0.1 mol L^{-1}. Analytical grade ethyl bromide and absolute ethyl alcohol were obtained from Beijing Chemical Works (Beijing, China). All chemicals were analytical-reagent grade.

Liquor samples were purchased in the market and different ages of Fenjiu were provided by Shanxi Xinghuacun Fenjiu Group.

Preparation of the standard solution: The acetic acid, butyric acid, pentanoic acid, caproic acid, heptylic acid, isovaleric acid stock standard were prepared by dissolving them in 60 % ethanol and then yielded the concentration of 2 mg mL^{-1} for acetic acid, 0.5 mg mL^{-1} for butyric acid, pentanoic acid, caproic acid, heptylic acid and isovaleric acid. Exception was lactic acid which dissolved in purified water and yielded the concentration of 2 mg mL^{-1}.

2-Ethylbutyric acid was employed as the internal standard with the concentration of 10.0 mg mL^{-1} prepared by dissolving in 60 % ethanol.

Sample preparation: 10 mL of Chinese liquor sample was transferred quantitatively into a 50 mL beaker and then 0.05 mL internal standard was added. Adjusted the solution to approximately pH = 9.5 by use of 0.1 mol L^{-1} tetrabutyl ammonium hydroxide (pH was monitored with a PHS-4CT pH meter; Shanghai, China) and recorded the volume of it. Then, dried this solution on a water bath, dissolved the residues completely into a 10 mL volumetric flask in N, N-dimethyl lacetamide and added bromoethane whose dosage was calculated as Formula (1). After that, set the volume to the mark with N,N-dimethyl lacetamide. At last, the solution was injected directly in the GC/MS after left quiescent at room temperature for 1 h.

$$v(\mu L) = \frac{108.97 \times c \times V}{1.4612} \times 2 \quad (1)$$

where c is the concentration of tetrabutylammonium hydroxide (mol/L); V is the volume of tetrabutylammonium hydroxide (L); 108.97 is the molar mass of bromoethane; 1.4612 is the density of bromoethane; 2 is the excessive multiples of bromoethane which is to make sure complete derivatization.

Gas chromatographic-mass spectrometry analysis: GC-MS analysis was carried out with a 7890 gas chromatograph interfaced to a 5975C mass selective detector (Agilent Technologies, USA). The chromatograph separation was operated on a HP-FFAP ms column(30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA) using helium as the carrier gas at a constant flow rate of 1 mL min^{-1}. Injector temperature was maintained at 250 °C in split mode with a ratio of 45:1 and injection volume was 1 μL. The oven temperature was held at 45 °C for 4 min, then raised to 230 °C at the rate of 3.5 °C min^{-1}. The transfer line temperature was set at 280 °C. The mass detector was operated in electron impact mode with the energy of 70eV and scanning from *m/z* 15 to 400. The quadrupole and ion source were 150 °C and 230 °C, respectively. Quantitative analyses were carried out in the SIM mode.

RESULTS AND DISCUSSION

Optimization of different base: 0.1 mol L^{-1} tetrabutyl ammonium hydroxide, 0.1 mol L^{-1} sodium hydroxide and 0.1 mol L^{-1} ammonia were tested following the procedure reported above. The results showed that the inorganic salt generated by the reaction of sodium hydroxide with acids could not be dissolved completely resulted to affect the quantitative results. The weak alkaline of ammonia caused acids can not be esterified. The results using tetrabutyl ammonium hydroxide were acceptable. Thus, the optimized base was tetrabutyl ammonium hydroxide.

Optimazation of pH: The solution's pH after neutralization was further optimized. pH at 8.0, 9.5, 10.5 were respectively analyzed. The results are shown in Table-1. All data are the mean recoveries of triplicate analyzing.

TABLE-1
INFLUENCE OF THE pH (8.0, 9.5, 10.5) ON THE RECOVERIES (%). CONDITION:1 mL STANDARD SOLUTION OF A MIXTURE OF ACIDS FOLLOWING THE PROCEDURE REPORTED IN TEST

Acids	pH = 8.0	pH = 9.5	pH = 10.5
Acetic acid	63.5 ± 5.7	103.2 ± 3.5	105.3 ± 6.6
Butyric acid	71.3 ± 3.5	94.2 ± 0.6	97.4 ± 1.8
Isovaleric acid	75.2 ± 4.2	101.4 ± 2.3	103.6 ± 11.0
Pentanoic acid	65.7 ± 3.3	99.7 ± 7.6	105.2 ± 3.6
Caproic acid	52.1 ± 1.2	90.1 ± 7.6	88.6 ± 2.9
Heptylic acid	63.8 ± 6.8	99.2 ± 5.8	99.3 ± 8.8
Lactic acid	48.7 ± 2.7	91.2 ± 1.6	97.9 ± 4.1

When the pH at 8, the recoveries of acids were low especially caproic acid and lactic acid. But the recoveries were acceptable at higher pH at 9.5 and 10.5. Higher than 9.5 did not increase the recovery significantly. Finally, the optimized pH was 9.5.

Optimazation of esterifying agent: In order to esterified acids completely, esterifying agent of benzyl bromide α-bromotoluene and bromoethane were tested. Experiments were executed and the results showed that recoveries of the two agent were both acceptable. But benzyl bromide α-bromotoluene is a strong lachrymator and irritating to skin, we chose bromoethane as the esterifying agent in this experiment.

Working with standard solutions, seven acids were analyzed by the method above. Fig. 1 illustrates the TIC of these acids standard solution and Table-2 lists the selected ions used for quantification.

Calibration curves: Calibration curves were obtained with increments in six concentrations of the stock standard mixtures consisting of seven organic acids. Calibration sample was prepared by the method mentioned above. Each calibration sample was injected in triplicate. Calibration curves for each acid were obtained by plotting concentration to peak area ratio (peak area of acid derivative to peak area of internal standard).

TABLE-2
LINEAR RANGE OF ACIDS' DERIVATIVES, IONS SELECTED FOR ANALYSIS AND CHARACTERISTICS OF THE CALIBRATION CURVES

Acid	Linear range (μg mL^{-1})	Quantitative ion (*m/z*)	Qualitative ion (*m/z*)	k	c	R
Acetic acid	10-2000	43	29, 61	1.1483	0.4009	0.9983
Butyric acid	0.5-1000	71	43, 29	0.9206	0.05258	0.9994
Isovaleric acid	0.5-1000	88	29, 57	1.0155	0.03701	0.9994
Pentanoic acid	0.5-1000	88	29, 85	1.0403	0.04285	0.9993
Caproic acid	0.5-1000	88	43, 99	0.4270	0.04240	0.9929
Heptylic acid	0.5-1000	88	29, 43	1.0259	0.04232	0.9993
Lactic acid	1-1500	45	27, 29	2.3689	0.2240	0.9963

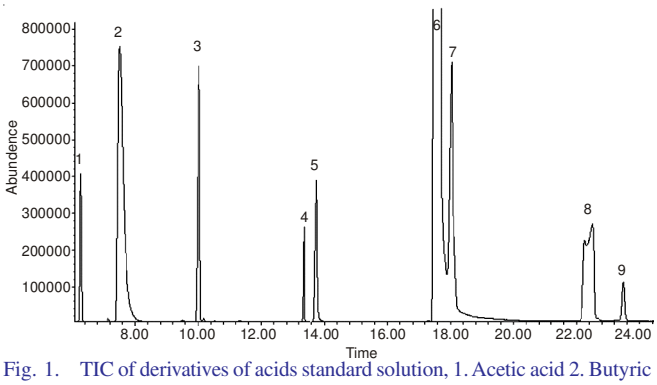

Fig. 1. TIC of derivatives of acids standard solution, 1. Acetic acid 2. Butyric acid 3. Isovaleric acid 4. 2-Ethylbutyric acid 5. Pentanoic acid, 6. Tributylamine 7. Caproic acid 8. Heptylic acid 9. Lactic acid

The linear regression equations for acids were expressed as Y = kx + c, where x is the peak area ratio of the standards solutions to internal standard, Y is the concentration ratio of acids to internal standard, k and c are constants. The results are listed in Table-2. Splendid correlations between Y and x of the acids were observed with a coefficient, R > 0.9929 for each calibration curve.

Limit of detection and limit of quantification: The determination of LOD and LOQ was calculated by 3.3(SD/S) and 10(SD/S), respectively, where SD is the standard deviation of the response and S is the slope of the calibration curves. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines. Table-3 displayed LOD and LOQ of acids.

Precision and recovery: The precision, expressed as the relative standard deviation (RSD), was established by injecting the spiked sample with two concentrations for six times. The recovery procedure was performed on a mixture of acids added to Chinese liquors. Table-3 shows the recoveries and precision of these acids.

In this work, the RSDs of precision are < 3 % and the recovery percentage varies between 82.43 % and 109.38 %. Therefore, based on this information, we could confirm that this method can be used for quantitative analyses of organic acids in Chinese liquors.

Method Application: The acids content of different kinds of Chinese liquor samples are shown in Table-4. Four brands of Chinese liquor were tested. Fenjiu and Fenyangwang is light

TABLE-3
PERFORMANCE CHARACTERISTICS

Acid	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Spiked amount: Acetic acid, lactic acid 100 μg mL^{-1}, respectively, other acids 20 μg mL^{-1}, respectively		Spiked amount: Acetic acid, lactic acid 200 μg, respectively other acids 40 100 μg, respectively	
			Recovery (%)	Precision RSD (%)	Recovery (%)	Precision RSD (%)
Acetic acid	1.6378	4.9630	109.38	0.91	109.32	1.61
Butyric acid	0.1426	0.4322	90.82	0.56	90.78	0.92
Isovaleric acid	0.1018	0.3084	97.28	0.29	99.51	0.74
Pentanoic acid	0.1223	0.3705	98.89	0.33	99.51	1.04
Caproic acid	0.3950	1.1969	83.18	1.99	82.43	3.01
Heptylic acid	0.1075	0.3259	99.11	1.41	97.10	1.23
Lactic acid	0.3764	1.1405	85.54	1.64	100.53	2.90

TABLE-4
CONTENTS OF ACIDS IN CHINESE LIQUOR

Sample	Acetic acid (μg mL^{-1})	Butyric acid (μg mL^{-1})	Isovaleric acid (μg mL^{-1})	Pentanoic cid (μg mL^{-1})	Caproic acid (μg mL^{-1})	Heptylic cid (μg mL^{-1})	Lactic acid (μg mL^{-1})
New brewed Fenjiu	742.99 ± 26.3	11.09 ± 1.09	1.28 ± 0.07	0.73 ± 0.01	3.64 ± 0.22	ND	432.69 ± 18.1
Five-year Fenjiu	1186.38 ± 52.7	14.54 ± 0.77	2.47 ± 0.13	1.83 ± 0.06	4.03 ± 0.09	ND	752.19 ± 21.6
Ten-year Fenjiu	1010.52 ± 28.2	13.97 ± 1.09	3.05 ± 0.10	1.68 ± 0.02	4.80 ± 1.17	ND	810.67 ± 11.3
Twenty-year Fenjiu	1261.39 ± 9.41	16.25 ± 1.47	5.86 ± 0.24	2.47 ± 0.08	5.53 ± 1.09	0.52 ± 0.01	1117.73 ± 33.5
Thirty-year Fenjiu	1472.81 ± 37.3	21.63 ± 2.22	6.66 ± 0.86	2.91 ± 0.08	7.38 ± 1.62	0.94 ± 0.03	1290.58 ± 9.10
Fenyangwang	593.27 ± 8.15	13.35 ± 1.23	0.98 ± 1.09	0.67 ± 0.01	2.20 ± 0.41	1.17 ± 0.03	631.83 ± 27.4
Wuliangye	519.21 ± 3.67	125.43 ± 8.99	40.01 ± 7.09	27.20 ± 1.27	318.75 ± 12.7	ND	576.59 ± 16.5
Maotai	1087.99 ± 12.6	205.17 ± 10.3	11.37 ± 1.63	38.69 ± 3.71	217.84 ± 20.4	6.38 ± 0.59	1006.57 ± 25.3

ND: Not Detected

aroma style, Wuliangye is strong aroma style and Maotai is soy sauce aroma style. As Table-4 displayed, acetic acid and lactic acid are the main acids in light aroma style liquor and four main acids including acetic acid, lactic acid, butyric acid and caproic acid are characteristic of strong aroma style and soy sauce aroma style liquor. While the concentrations of other acids were very low. It can also be found that contents of acids are increasing with the liquor age of Fenjiu.

Conclusion

In conclusion, a reliable and efficient derivatization GC-MS method in SIM mode for the simultaneous determination and quantification of organic acids has been developed and found to be accurate and precise. This method was validated and found to be satisfactorily linear and selective. Our derivatization GC/MS analysis method supplies an effective approach and provides a basis for research on determination of acids. Good results were achieved in testing acids in different varieties of Brandies and ages of Chinese liquor.

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