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The Feasible Discussion about determination of diisobutyl phthalate in potato liquor by HPLC

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Abstract

In this study, the feasibility of determination of diisobutyl phthalate (DIBP) as a plasticizer in potato liquor by HPLC was studied. The standard curve, accuracy, precision, systematic error and sensitivity of diisobutyl phthalate were determined by HPLC method. The results showed that the detection range of DIBP by HPLC method was 0.30 ~ 400.00μg /L, the determination coefficient R^2=0.9995, the relative error of each data was less than 10%, and the average recovery rate of potato liquor was 83.27%. The coefficients of variation of diisobutyl phthalate in potato liquor samples were 5.07%, 6.18% and 2.45%, respectively. The detection sensitivity reached the minimum detection limit of 0.30μg /L, and the actual concentration of DIBP in the detected potato liquor was 154.44μg /L，the uncertainty of class A was 7.56%. Less than the national standard GB 5009.271-2016 stipulated in 500μg /L, in line with the national requirements.

Keywords

HPLC Diisobutyl phthalate (DIBP) Potato liquor plasticizer.

1. Preface

The current standard for the detection of diformate esters in white wine in China is GB 5009.271-2016 "Determination of phthalate esters in food". This standard uses gas chromatography to determine phthalic esters in food and stipulates that their content should be less than 500 μg/L [1]. However, the cost of meteorological chromatography is relatively high and the detection speed is not fast enough. In this experiment, based on the method of GB 5009.271-2016 and related literature, the DIBP content of potato white wine was analyzed and determined by gas high performance liquid chromatography (HPLC) [2]. It is beneficial to broaden the detection method of phthalate esters and improve the level of the analysis method of plasticizer content in potato liquor in China.

2. Materials and Methods

2.1 Experimental reagents and materials

n-hexane reagent, extracted with acetonitrile, Analytical pure reagents; Mobile phase acetonitrile, Chromatographic purity; Trifluoroacetic acid, Analytical pure reagents; DIBP standard, China Bureau of Standards and Metrology; 52° potato white wine, Sichuan Potato Engineering and Technology Center [3].

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2.2 Instruments and equipment

HPLC, reversed-phase C18 column, Agilent 1100; GC-MS 3100 benchtop gas chromatography (quadrupole) mass spectrometer, (Beijing East-West Analytical Instruments Co., Ltd.) electronic balance, Shanghai Lichen Instruments Co., Ltd.; micro adjustable pipette gun (100-1000μL), Shanghai Dalong Xingchuang Co., Ltd.; drying oven, high-speed centrifuge, Chengdu Shengjie Technology Co; Electric oscillator, ultra-pure water machine, Millipore, USA; refrigerator, Guangdong Kolon Instrument Co.

2.3 Test Methodology

2.3.1 Determination of DIBP concentration in potato white wine by GC-MS

To determine the validity of the HPLC results, the concentration size of DIBP in potato white wine was determined by GC-MS using the detection method in GB 5009.271-2016 "Determination of phthalate esters in food". Nine samples of 1.00 mL potato white wine were taken and accurately injected for detection under the gas chromatographic conditions specified in the national standard. The concentration of DIBP in potato white wine was processed and calculated under the national standard detection method.

2.3.2 HPLC plotting the standard curve of DIBP

To determine the detection range of the assay, a standard curve for the determination of DIBP by HPLC is required. The DIBP standard solution was diluted in a downward gradient at the concentrations of 400.00 μg /L, 200.00 μg /L, 100.00 μg /L, 50.00 μg /L, 25.00 μg /L, and 0.00 μg /L, and the standard sample peak area was obtained by sequentially testing the standard sample according to the operation steps of HPLC, and the peak area was used as the horizontal coordinate X and the concentration of the standard solution as the the vertical coordinate Y to plot the standard curve [4].

2.3.3 Accuracy of DIBP determination by HPLC

The accuracy of this HPLC method was measured based on the relative error of the measurement results. To study the accuracy of DIBP determination by HPLC, 50 μL of DIBP standard solutions of 0.00 μg /L, 25.00 μg /L, 50.00 μg /L, 100.00 μg /L and 200.00 μg /L were accurately aspirated and measured three times for each concentration gradient, and the relative error was calculated, which should be less than 10%.

$$\text{Relative Error} = \frac{\text{Measured value - true value}}{\text{true value}} \times 100\% \quad (1)$$

2.3.4 Precision of DIBP determination by HPLC

The precision of the HPLC method was measured according to the coefficient of variation of the measurement results. To study the precision of HPLC determination of DIBP, 9 samples of 5.00 mL potato white wine were taken, and the content of DIBP was measured after treatment, and its coefficient of variation was calculated by the standard deviation formula, which is inversely proportional to the precision. When the value of the coefficient of variation of the test results was less than 10%, it indicated that the precision of this test was good.

$$SD = \sqrt{\frac{\sum \left(X_i - \overline{X}\right)^2}{n-1}} \qquad CV = \frac{SD}{\overline{X}} \quad (2)$$

In the equation:

SD----Standard deviation

X_i ----The amount of DIBP measured in each group

$\overline{X}$ ----The average content of DIBP in this sample

n ----Number of sample groups

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CV ----Coefficient of variation

2.3.5 Systematic errors in the determination of DIBP by HPLC

The systematic errors of the HPLC method were examined by calculating the spiked recoveries of the samples. To accurately determine the systematic error of DIBP, samples with different contamination levels were first prepared, and DIBP standard solutions with concentrations of 12.50 μg /L , 25.00 μg /L , 50.00 μg /L , 100.00 μg /L , and 200.00 μg /L were added to 5.00 mL of potato white wine samples, and then the spiked samples were extracted, purified, and derivatized, and then the concentration of DIBP in the spiked samples was detected by HPLC [5]. The recoveries were not less than 70%.

$$P = \frac{C_2 - C_1}{C_3} \times 100\% \quad (3)$$

In the equation:

P ----Spiked recovery rate.

C_1 ----Specimen concentration.

C_2 ----Spiked specimen concentration.

C_3 ----Spiked amount.

2.3.6 Sensitivity of DIBP determination by HPLC

The signal-to-noise ratio was used to measure the sensitivity of the method [6]. To study the sensitivity of HPLC for the determination of DIBP, a blank solvent was used and the noise peak height N was measured. 0.10 μg /L, 0.20 μg /L, 0.30 μg /L, 0.40 μg /L of DIBP standard solution was then injected, and when the signal-to-noise ratio S/N = 3, i.e., there exists a measured concentration, at which the measured peak height is three times the noise height. This concentration can be considered as the minimum detection sensitivity of DIBP.

2.4 The method for the determination of DIBP content by HPLC

Take 5mL of potato white wine sample which was fully shaken into a centrifuge tube, add 20.00mL of 80% acetonitrile solution, shake the sample fully for 30min using an electric shaker and leave it for 15min, and take the supernatant as the sample to be tested.

Pipette about 6 ml of supernatant into the sample bottle of the multifunctional clean-up column, insert the packing tube of the multifunctional clean-up column into the sample glass bottle and slowly push the packing tube to allow the clean-up solution to enter the collection cell of the functional clean-up column.

Avoiding light operation, feed 2 μL of the clean-up solution of the liquid chromatography column into a formula vial and dry it with a drying oven at 80°C. 200.00μL of trifluoroacetic acid and 400.00μL of hexane solution were added and shaken well. Then derivatize in a drying oven at 40°C for 15 min, dissolve with 200.00 μL of 80% acetonitrile solution, shake and mix well and leave for 15 min, and take the supernatant to the sample bottle of the HPLC as the sample to be measured [7].

Precisely pipette 1.00 mL of 4.00 μg/mL of DIBP standard into a 10 mL volumetric flask to configure a standard solution with a concentration of 400.00 μg/L; then take 5.00 mL of 400.00 μg/L standard solution into a 10 mL volumetric flask to prepare a standard solution of 200.00 μg/L; repeat the above operation steps to configure the standard solution in order of concentration gradient 400.00μg /L, 200.00μg /L, 100.00μg /L, 50.00μg /L, 25.00μg /L of DIBP standard solution [8]; then aspirate 200.00μL of each standard solution in a sample bottle, and blow dry in a dryer at 80°C. The derivatization method is the same as 1.4.3.

Chromatographic column: 4.6X150mm, 5μm, C18; column temperature: 25℃; mobile phase: acetonitrile (chromatographic purity) 20%, water 80%; flow rate: 0.50ml/min; injection volume: 20Ml.

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After the peak of the chromatograph, the peak area of the standard (X) and the concentration of the sample (Y) are used to draw the standard curve, and the peak time of DIBP is determined by comparison with the retention time of the standard chromatogram and the peak is determined, and the DIBP content in the sample is calculated by the DIBP standard curve and the peak area in the sample based on the regression equation obtained, and the concentration of DIBP in the sample is also calculated according to the following formula.

$$C = \frac{A \times V}{m \times f} \tag{4}$$

In the equation:

C----The concentration of DIBP in the sample in μ g /L.

A----The measured concentration of the sample through in the standard curve (external standard method) in μ g/L.

V----The volume of the extract of the sample in ml.

m----The amount of sample taken in the measurement, in g.

f----Concentration times of the sample solution before and after derivatization.

3. Results and Analysis

3.1 Experimental results for the determination of DIBP concentration in potato white wine by GC-MS

Nine 1.00 mL potato white wine samples were taken and accurately injected for testing under the gas chromatographic conditions specified in the national standard. Three potato white wine samples were used as the samples to be tested, and after treatment, each sample was repeated three times to determine the content of DIBP and calculate its DIBP concentration, as shown in Table 1 below.

Table 1 The concentration of DIBP was determined by GC-MS

Sample to be tested	Peak area1	Peak area2	Peak area3	Average peak area value	Calculated concentration (μg/L)
Sample Wine #1	58.24	58.79	59.93	58.99	158.87
Sample Wine #2	57.68	60.12	58.35	58.72	161.56
Sample Wine #3	56.57	56.83	58.66	57.35	161.42
Average concentration					160.62

From Table 1, it can be seen that the average concentration of DIBP in potato white wine samples determined by GC-MS method in the national standard is 160.62 μg /L, which is less than the 500 μg /L specified in the national standard GB 5009.271-2016, indicating that the content of DIBP in potato white wine meets the national requirements.

3.2 Standard curve test results of DIBP

The DIBP standard solution was purified by derivatization at the concentrations of 0.00μg/L, 25.00μg/L, 50.00μg/L, 100.00μg/L, 200.00μg/L and 400.00μg/L, and 20μl of DIBP was injected sequentially at the column temperature of 28℃, and the peak areas of DIBP were determined as shown in Figure 1 below.

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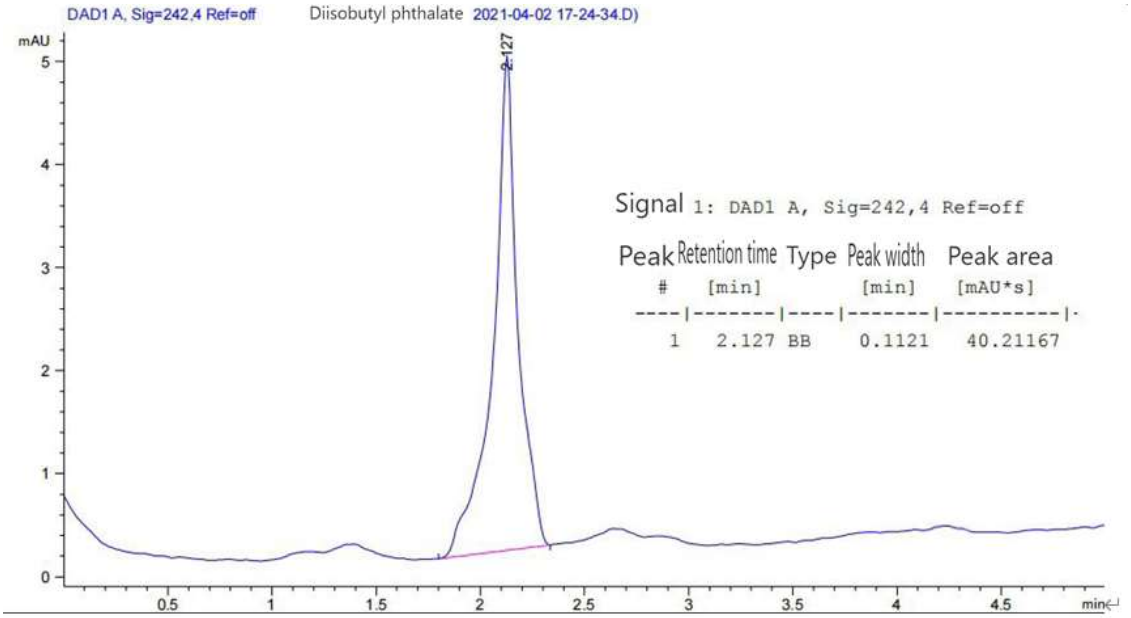

Figure1 The chromatogram of the DIBP

The standard curve of DIBP was plotted with the peak area of DIBP as the horizontal coordinate X and the concentration of the standard sample as the vertical coordinate Y. The regression equation Y=3.7226X-4.9260, R2=0.9995 was calculated and the standard curve of DIBP content is shown in Figure 2. As can be seen from Figure 2, the peak area is positively correlated with the concentration of DIBP, and if the peak area is larger, it means that the concentration of DIBP is larger, and its coefficient of determination R^2=0.9995 is close to 1, which indicates that the linear correlation of the standard curve of DIBP drawn by HPLC method is good in the range of 0.00~400.00μg /L.

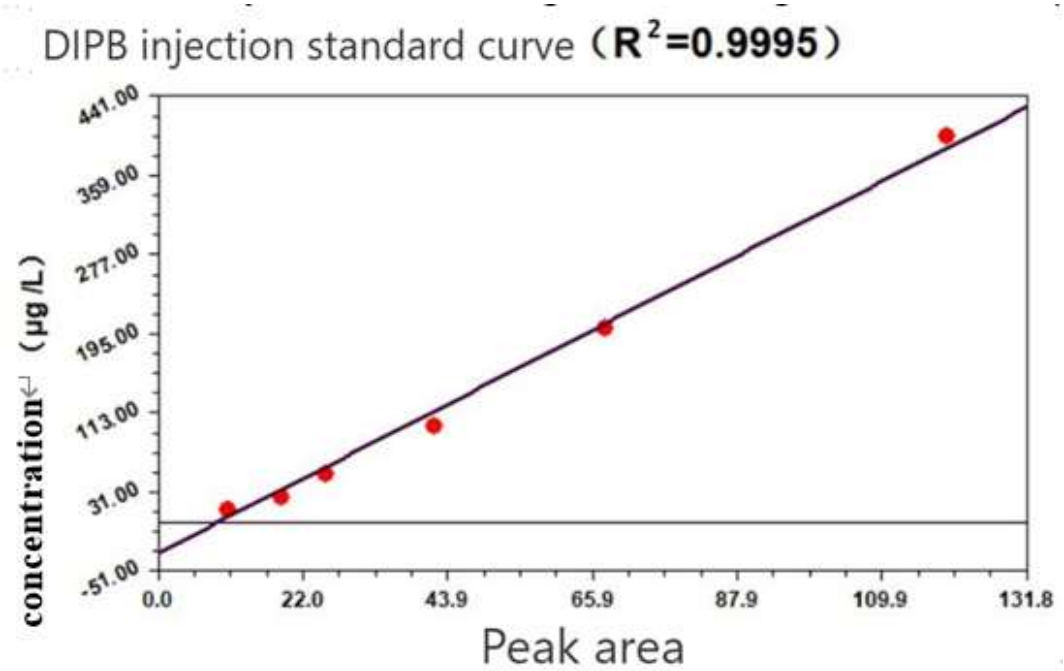

Figure2 HPLC standard curve method for the determination of DIBP

3.3 Accuracy calculation results of DIBP

The relative error can be calculated by precisely aspirating DIBP standard solution 0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L, 400.00μg /L. DIBP standard solution 50μL was repeatedly injected three times as shown in Table 2 below.

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Table 2 Accuracy of HPLC for determination of DIBP

Serial number	DIBP concentration （μg/L）	Peak area 1	Peak area 2	Peak area 3	Average peak area value	Calculated concentration （μg/L）	Relative Error %
1	0.00	0.83	0.65	0.72	0.73		
2	25.00	18.86	21.54	21.07	20.49	27.01	8.04
3	50.00	25.78	27.67	28.23	27.22	52.07	4.14
4	100.00	37.83	41.67	36.87	38.79	95.14	4.86
5	200.00	61.94	61.85	66.21	63.33	186.49	6.75
6	400.00	112.86	122.43	116.57	117.28	387.33	3.17

From Table 2, the relative errors of the HPLC method for the determination of DIBP standard solutions were 8.04%, 4.14%, 4.86%, 6.75% and 3.17%, respectively. It is within 10% of GB 5009.271-2016, which indicates that the accuracy of HPLC method is good.

3.4 Calculation results of the precision of DIBP

To study the precision of DIBP determination by HPLC, three potato white wine samples were used as the samples to be tested, and the content of DIBP was determined three times for each sample after treatment and the coefficient of variation was calculated as shown in Table 3 below.

Table 3 The precision of HPLC method for the determination of DIBP

Sample to be tested	Peak area 1	Peak area 2	Peak area 3	Average peak area value	Calculated concentration （μg/L）	Coefficient of variation （%）
Sample Wine #1	54.43	58.40	54.92	55.92	158.90	5.07
Sample Wine #2	56.48	51.36	52.68	53.51	149.94	6.18
Sample Wine #3	55.69	54.84	53.66	54.73	154.48	2.45

As can be seen from Table 3, the coefficients of variation of DIBP in potato white wine were 5.07%, 6.18% and 2.45% for the three groups determined by HPLC, and the average calculated concentration was 154.44 μg /L, with an error of 4.01% compared to the concentration of 160.62 μg /L detected by GC-MS method in the national standard GB 5009.271-2016, indicating that the detection results were valid . Meanwhile, the national standard stipulates that the coefficient of variation of the assay should be less than 10%, and the above data are less than the value stipulated in the national standard, which indicates that the precision of the HPLC method is better.

3.5 Systematic error calculation results of DIBP

The systematic error of the method was measured by the sample spiked recovery test. The average calculated concentration of 154.44 μg /L of the three groups of sample wines in 2.4 was taken as the value of DIBP content in the samples, to which 50 μL of DIBP standard solution with concentrations of 0.00 μg /L, 25.00 μg /L, 50.00 μg /L, 100.00 μg /L, 200.00 μg /L, 400.00 μg /L were added to 5.00

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mL of potato white wine samples to prepare samples with different contamination levels. After extraction, purification and derivatization of the contaminated samples, the content of DIBP in the spiked samples was determined according to the HPLC method as shown in Table 4 below.

Table 4 Determination of Potato liquor of DIBP in the rate of HPLC method

Serial number	DIBP content in samples（μg/L）	Adding DIBP concentration（μg/L）	Detection concentration（μg/L）	Recovery rate（%）
1	154.44	0.00	128.68	83.32
2	154.44	25.00	155.67	86.76
3	154.44	50.00	167.01	81.69
4	154.44	100.00	215.18	84.57
5	154.44	200.00	281.92	79.54
6	154.44	400.00	461.69	83.73
Average recovery rate				83.27

From Table 4, the spiked recoveries of DIBP in potato white wine ranged from 79.54% to 86.76% in the range of 0.00-400.00 μg/L. The average recovery was 83.27%, and the minimum required recovery should be greater than 70% as required in GB/T 26792-2019. It indicates that the systematic error of DIBP determination in potato white wine using HPLC method is small and within the specified range, and the method can be used for the determination of DIBP in potato white wine.

3.6 Sensitivity calculation results of DIBP

The noise signal N near the outgoing peak was measured at 0.0020 mAU for the 0 μg /LDIBP standard, and then the DIBP standards of 0.10 μg /L, 0.20 μg /L, 0.30 μg /L, and 0.40 μg /L were injected, and the signal S was measured and S/N was calculated as shown in Table 5 below.

Table 5 Sensitivity of HPLC for determination of DIB

Concentration / Peak High	0.10μg /L	0.20μg /L	0.30μg /L	0.40μg /L
Peak High S	0.0034mAU	0.0052mAU	0.0060mAU	0.0068mAU
S/N	1.7	2.6	3	3.4

From Table 5, S/N (chromatographic peak/noise peak) = 3 when and only when the concentration of DIBP sample is 0.30 μg /L. Therefore, the minimum detection concentration of DIBP is 0.30 μg /L.

4. Discussion and Conclusion

This experiment not only explored the test parameters for the analysis of DIBP by HPLC, but also made a detailed analysis of the performance and reliability of DIBP detection in potato white wine using HPLC method. The results showed that: the plotted standard curve of HPLC method with the

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coefficient of determination R2=0.9995, which is in line with the detection requirements; and within the detection range of 0.30~400.00μg /L, its detection results are in 4.01% error with the national standard GB 5009.271-2016 GC-MS method, which indicates its good detection performance; the spiked recovery rate of DIBP in potato white wine ranged from 79.54% to 86.76%, which was in line with the national standard; the average recovery rate was 83.27%, which was higher than the minimum recovery rate of 70% required by the national standard. The coefficients of variation of DIBP in potato white wine determined by HPLC were 5.07%, 6.18% and 2.45%, which were in accordance with the requirements of less than 10% as stipulated in the national standard GB 5009.271-2016. The minimum detection concentration of DIBP by HPLC was 0.30 μg /L, and its class A uncertainty was 7.56%, which was less than 10% as stipulated in the national standard GB/T 26792-2019.

In summary, the HPLC method can be used for the detection of DIBP in potato white wine, and it can be used to some extent instead of gas chromatography (GC--MS) to help us analyze and detect plasticizers in potato white wine. Of course, the results of this test need to be further broadened, such as whether the feasibility of extending the HPLC method for the detection of plasticizers to all alcohols is still high; whether the precision of the detection of different types of phthalates will be affected; and in the process of reagents and detection, the impact of the B uncertainty on the accuracy of the test results introduced by the instrument operation, standard preparation, environmental factors, still need more experiments to prove perfect.

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