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The Effects of Different Solvents on the Determination of Lead in Pork Were Discussed

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Abstract: [Objective]: To optimize the graphite furnace atomic absorption spectrometry, compare the effects of blank pork matrix and 1% nitric acid as solvent on the detection of lead in pork, and establish a method for quantitative detection of lead in pork. [Methods]: The graphite furnace heating program and matrix modifier were optimized, and the method was screened. The results of three-level standard addition test and quality control product test of these two solvents were compared. [Results]: Under the screening conditions, with blank pork matrix as solvent, the correlation coefficient of the standard curve was 0.9999, the recovery rate of the three levels was 91.9% ~ 97.9%, and the coefficient of variation was 4.9% ~ 13.1%. The detection limit was 0.0134 mg / Kg. The test results of quality control products were within the allowable range; the test results with 1% nitric acid as solvent did not meet the standard requirements. [Conclusion]: Under the screening conditions, the accurate detection of lead in pork can be achieved by graphite furnace atomic absorption spectrometry with blank pork matrix as solvent.

Keywords: Graphite Furnace-atomic Absorption Method; Matrix; Matrix Modifier; Detection Limit

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1 Introduction

Pork is one of the meat foods in China, which is loved by most people and is rich in protein, minerals, vitamins and trace elements [1]. In order to improve the production of livestock and poultry, some businessmen have used heavy metals as feed additives [2, 3]. There have been heavy metal pollution incidents in the society [4]. The state has put forward higher and higher requirements for food quality and safety. With the rapid development of economy, the problem of ecological environment pollution has become increasingly apparent, and the problem of heavy metal pollution of animal-derived meat has attracted international attention [5]. In the latest national food safety standard GB 2762-2022, the limit of pollutants in food [6], the limit of lead pollutants in food ranks first. Lead is the most common heavy metal pollutant in the world, especially affecting children's intellectual growth and development [7]. Lead mainly invades hematopoietic organs, nervous system and kidney, and eventually causes anemia [8].

In view of the safety hazards of some heavy metal lead pollution in pork, it is more urgent to establish an accurate detection method for lead in pork. The existing detection methods of heavy metal elements in food are: inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma emission spectrometry (ICP-AES), atomic absorption spectrometry, atomic fluorescence spectrometry, electrochemical analysis, colorimetry, high performance liquid chromatography and so on [9-11]. By comparison, it is found that atomic absorption spectrometry has the advantages of high cost performance, low detection limit, high precision and accuracy, good aging, and many determination elements. It has been widely used in the determination of heavy metals in food [9-11]. In this study, the determination of lead in food of

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GB5009.12-2017 national food safety standard was detected by graphite furnace atomic absorption spectrometry [12]. In order to achieve the purpose of accurate quantitative detection, the heating program and matrix modifier were optimized. Under the screening conditions, the three-level standard addition experiment and quality control experiment results with 1% nitric acid and with blank pork matrix as solvent were compared. The results show that under the selected conditions, the matrix of blank pork can be used as a solvent to achieve the accurate detection of lead in pork by graphite furnace atomic absorption spectrometry, This can provide technical support for the relevant food regulatory authorities.

2 Materials and Methods

2.1 Materials and Reagents

GBW(E) 080129 lead single element standard solution 100 μg/mL (16121 batches, China Institute of Scientific Metrology); GSB-04-1743-2004 palladium single element standard solution 1000 μg/mL (196049, National Center for Analysis and Testing of Nonferrous Metals and Electronic Materials); GBW(E)100351 standard material for composition analysis of rice flour (Institute of Analysis and Testing, General Institute of Iron and Steel Research); nitric acid (electronic grade); argon gas (Beijing helium gas industry, more than 99.99%), laboratory water for ultra-pure water meter is now the conductivity of 18.25 MΩ/cm pure water; the glassware and digestion tubes used were soaked in nitric acid (20%) for more than 24 h, and finally washed with pure water to dry.

2.2 Equipment and Instruments

Atomic absorption spectrophotometer (Shimadzu, AA6880); pyrolysis coated graphite tube (Shimadzu; 206-50588-84); organize the mashing machine; microwave digestion instrument (CEM, MARS6); electronic balance (Mettler, MS204S); ultrapure water meter (MILLI-Q, clear-D24UV).

2.3 Test Method

2.3.1 Optimized Working Conditions of the Instrument

The determination was carried out by graphite furnace atomic absorption spectrometry. The optical parameters were as follows: lamp current 10 mA, characteristic wavelength 283.3 nm, slit width 0.7 nm, deuterium lamp background; the injection method was (20+5)μL of (sample+matrix modifier). The graphite tube heating program: the first stage was heating to 60 ℃ in 3S; the second stage, heating to 120 ℃ in 20S; the third stage heating to 250 ℃ in 10S; the fourth stage heating to 600 ℃ in 10S and hold for 13s; these four stages of the graphite tube heating program were all heating at uniform speed; the fifth stage: rapid heating up to 1700 ℃ and hold for 3s; the sixth stage was rapid heating up to 2300 ℃ and remains for 2S.

2.3.2 Sample Inorganic Pretreatment

The pork samples and blank pork samples were cut into small pieces, crushed by the tissue crusher, loaded in a plastic box, and weighed in the digestion tube. 5 mL of nitric acid was added, pre-digestion at room temperature for more than 0.5 h, microwave digestion, 150 ℃ open cover acid removal, acid removal to about 1 mL, cold cut to room temperature plus 2 mL of pure water, 140 ℃ acid removal to a small drop, constant volume to 25 mL, and reagent blank. This solution can be directly used for graphite furnace atomic absorption spectrometry to detect lead.

2.3.3 Registration of Standard Solution

Using 1% nitric acid as the solvent, the standard solution of each lead element reserve was gradually diluted and configured into a working standard solution of 8ng/mL. The blank pork sample was pretreated to obtain the solution, that is, the blank pork matrix was used as the solvent, and each reserve standard solution was gradually diluted and configured into a working standard solution of 8ng/mL. The blank pork sample was pre-treated to obtain a solution as a solvent to automatically prepare the working standard solution. Palladium standard solution was diluted with 1% nitric acid to obtain palladium working solution 10μg/mL, 20μg/mL, 50μg/mL, 100μg/mL, 200μg/mL, 400μg/mL, 1000μg/mL palladium nitrate and 20mg / mL ammonium dihydrogen phosphate, 200μg/mL palladium nitrate ammonium dihydrogen phosphate-palladium nitrate solution.

2.3.4 Three-level Spiked Recovery Test

Three levels of lead were added to the samples at low, medium and high concentrations. The three levels of lead were 60 μg / kg, 80 μg / kg and 90 μg / kg, respectively. The samples were treated according to 1.3.2, and each level was repeated six times.

2.3.5 Standard Samples

Lead was detected by GBW(E)100351 rice quality control as a standard sample, and the quality control sample was tested after pretreatment and repeated three times.

2.3.6 Data Processing Method

According to GB5009.12-2017, the formula for the determination of lead in food in the national food safety standard was calculated [12], and the detection limit was calculated according to GB/T 27404-2008 laboratory quality control specification food physical and chemical detection formula [13]. Excel 97-2003 was used to analyze the data.

3 Results and Analysis

3.1 Optimization of Heating Process of Graphite Furnace

It is mainly to optimize the fourth stage to the sixth stage of the heating program of the graphite furnace. The results are shown in Table 1. Based on the principle of high sensitivity and small background interference, the optimum temperature of the fourth stage is 600 ℃. The optimum temperature of the fifth stage is 1700 ℃; the sixth stage is the purification temperature. When it is set to 2000 ℃, it is found that there is a memory effect with the increase of the number of injection times. In order toreduce the memory better effect, it is found through experiments that it is more appropriate to set the purification temperature at 2300 ℃.

3.2 Optimization of the Concentration of Matrix Modifier

The standard solution of palladium nitrate was prepared with 1% nitric acid in the concentration range of (10~1000)μg/mL. The optimum concentration of ammonium dihydrogen phosphate was 20mg/mL, and the optimum concentration of palladium nitrate was 200μg/mL. The blank pork digestion solution was used as the solvent to configure the standard solution. In the concentration range of 10μg/mL ~ 1000μg/mL, the optimal concentration was 200μg/mL palladium nitrate solution. The selection principle is that the standard curve has good correlation and high sensitivity. The specific results are shown in table 2. When the optimal concentration of palladium nitrate was selected, the correlation coefficient reached more than 0.999, and the sensitivity was also relatively good.

3.3 Detection Limit and Recovery Test of Two Solvents

The optimized instrument parameters were used for the detection. The former used 1% nitric acid as the solvent to configure the standard solution, and the latter used the blank pork digestion solution as the solvent. The detection limit of the former was 11.1 μg/kg, which of the latter was 14.3 μg/kg; The recoveries of the former ranged from 42.5% to 114.1%, and which of the latter 91.9% to 97.9%; The coefficient of variation of the former ranged from 4.0% to 29.6%, and the latter 4.9% to 13.1%. The specific results are shown in table 3. It can be seen that although the former meets the requirements of the detection limit of lead in GB5009.12-2017 national food safety standard-determination of lead in food by atomic absorption spectrometry (the first method) [12], the recovery rate meets the requirements when the medium level is added, and the coefficient of variation meets the requirements when the high level is added. In general, the recovery rate and coefficient of variation of the former three levels can not fully meet the requirements of GB/T 27404-2008 laboratory quality control standard for food physical and chemical testing [13]. The detection limit of the latter, the recovery rate and coefficient of variation of the three-level addition recovery are in line with the relevant requirements of the national testing standards.

3.4 Quality Control Results of Two Solvents

The optimized instrument parameters were used for the detection. The former used 1% nitric acid as the solvent to configure the standard solution, and the latter used the blank pork digestion solution as the solvent. GBW (E) 100351 rice powder was used as quality control. The quality control results were shown in table 4. The detection result of the former was 131 μg/kg; the average value of the latter was 112 μg/kg, and the standard value ranged from (100 to 120)μg/kg. The former was not in the range, exceeding its maximum value of 120 μg / kg; the latter is in this range.

Table 1 Optimization of lead heating procedure

Optimization stage	**Fourth stage (ashing)**	**The fifth stage (atomization)**	**The sixth stage (purification)**	**Lead concentration (ng/mL)**	**Absorbance value**	**Background value**
Fourth stage (ashing)	700	1700	2000	1	0.0073	0.0194
	650	1700	2000	1	0.0058	0.0036
	600	1700	2000	1	0.0100	0.0039
	550	1700	2000	1	0.0107	0.0043
	500	1700	2000	1	0.0106	0.0061
	450	1700	2000	1	0.0105	0.0080
The fifth stage (atomization)	600	1700	2000	2	0.0209	0.0226
	600	1800	2000	2	0.0124	0.0260
	600	1650	2000	2	0.0118	0.0269

Table 2 Selecting the levels of $Pd(NO_3)_3$

NO	**Matrix modifiers**	**1% nitric acid as solvent**		**Blank pork digestion solution as solvent**	
	μg/mL	**The equation of standard curve**	**Correlation coefficient /r**	**The equation of standard curve**	**Correlation coefficient/ r**
1	1000 $Pd(NO_3)_2$	Y=0.0056600X- 0.0056400	0.9724	Y=0.015249X+0.0091625	0.9766
2	400 $Pd(NO_3)_2$	Y=0.0070925X+0.0041025	0.9916	Y=0.013314X-0.0010108	0.966
3	20000$NH_4H_2PO_4$ 200$Pd(NO_3)_2$	$Y=0.020324X-6.3924e^{-0.05}$	0.9998	Y=0.011906X-0.0064712	0.9982
4	200 $Pd(NO_3)_2$	Y=0.026322X-0.0053748	0.9867	Y=0.016764X-0.00028671	0.9999
5	100 $Pd(NO_3)_2$	$Y=0.022748X-9.3513e^{-0.05}$	0.9945	Y=0.011739X+0.0095357	0.9776
6	50 $Pd(NO_3)_2$	Y=0.022078X+0.0030644	0.9985	Y=0.012746X-0.0025077	0.995
7	20 $Pd(NO_3)_2$	Y=0.018636X+0.0053524	0.9959	Y=0.010838X-0.0022680	0.9984
8	10 $Pd(NO_3)_2$	Y=0.017295X+0.0063508	0.9950	Y=0.0093352X+0.0034004	0.9984

Table 3 Three-level mark-adding test

Standard addition concentration (μg/kg)	**Average recovery (%)**	**Coefficient of variation (%)**	**GB/T27404-2008**		**Linear equation**	**Correlation coefficient (r)**	**Detection limit (μg/kg)**	**Solvent**
			recovery (%)	**Coefficient of variation (%)**				
60	42.5	29.6	60~120	15~21	$Y=0.020324X-6.3924e^{-0.05}$	0.9998	11.1	1%nitric acid
80	90.7	16.5	60~120	11~15				
90	114.1	4.0	80~110	11~15				
60	97.9	11.0	60~120	15~21	Y=0.016764X-0.00028671	0.9999	13.4	Blank pork digestion solution
80	91.9	4.9	60~120	11~15				
90	97.8	13.1	80~110	11~15				

Table 4 quality control results of Certified Reference Materials (N = 3)

Standard substance	**Standard value (μg/kg)**	**The average value of detection (μg/kg)**	**Coefficient of variation (%)**	**Solvent of standard solution**
GBW (E) 100351 rice flour composition analysis reference material	100-120	131	10.2	1% nitric acid
		112	13.3	Blank pork digestion solution

4 Discussions

4.1 Microwave Digestion Atomic Absorption Method

Due to the time-consuming and poor parallelism of wet digestion, and the cumbersome operation of pressure tank digestion, this study selected microwave digestion with high efficiency, high systematization and good parallelism [14-16]. Atomic absorption method has the advantages of high detection sensitivity, good accuracy, many measurable elements and relatively affordable instrument price [9-11]. It has been widely used in the detection of heavy metals. The content detected by graphite furnace atomic absorption method is generally ng/mL. The content detected by flame atomic absorption method is generally at the level of μg/mL, and the lead content in food belongs to the trace range. At the same time, graphite furnace atomic absorption method is preferred for safety and practicability.

4.2 Condition Optimization

For simple matrix, there is no need to add matrix modifier, and for complex matrix, appropriate matrix modifier is added as needed [17]. In the detection of lead in pork, it was found that the background interference was large and the matrix was complex. Through experiments, it was found that the recovery rate and precision could not meet the requirements if the matrix modifier was not added. Therefore, the commonly used palladium nitrate matrix modifier was added in the experiment and the concentration was optimized. Matrix modifiers are used to increase the ashing temperature of the element lead to be tested or to form volatile substances in the matrix, thereby eliminating matrix interference [18, 19]. This study optimizes the fourth stage to the sixth stage of the graphite furnace heating program, covering the key stages of ashing and atomization of the graphite furnace heating program. In the optimization, the main reference absorption value and background peak are optimized according to the principle of large absorption value and relatively small background peak.

4.3 Selection of Solvents

The solvents were selected through experiments. The standard working solution was prepared with 1% nitric acid and blank pork elimination matrix as solvents to carry out three-level standard addition recovery experiment and standard substance detection experiment. The results showed that the recovery rate and coefficient of variation of the former and the standard substance as quality control products could not meet the requirements of relevant national standards, while the latter could meet the requirements. This may be because the blank pork digestion solution is used as the solvent to configure the solution, which can offset the interference caused by some pork matrices and improve the recovery rate and precision of the detection. The matrix of different samples is different. It is necessary to confirm whether nitric acid solution is suitable as solvent or sample blank digestion solution is suitable as solvent through experiments. If all of them can meet the requirements, nitric acid is generally preferred as solvent. The latter is considered when the former cannot meet the conditions, and not all samples are suitable for this method.

5 Conclusion

Using blank pork digestion solution as solvent, the optimized graphite furnace atomic absorption method can realize the accurate detection of lead content in pork; Using 1% nitric acid as solvent, the optimized graphite furnace atomic absorption method could not achieved the accurate detection of lead content in pork.

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