Determination of Captan Residues in Milk Powder by Gas Chromatography-Tandem Mass Spectrometry

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Abstract [**Objectives**] To establish a gas chromatography-tandem triple quadrupole mass spectrometry (GC-MS/MS) method for the determination of captan residues in milk powder. [**Methods**] Milk powder samples were extracted with acidic acetonitrile, and proteins and fats were effectively removed using C_{18} solid-phase extraction cartridges. The residues were reconstituted in n-hexane-acetone (3 +1, *v/v*) prior to injection. Key parameters, including extraction solvent volume, clean-up procedure, and chromatographic-mass spectrometric conditions, were systematically optimized. Quantification was performed using the external standard method. [**Results**] The method exhibited good linearity in the range of 5 -500 ng/mL (R^2 =0.999 7), with a limit of detection of 2 μg/kg and a limit of quantification of 5 μg/kg. At three spiked levels (5, 10, and 50 μg/kg), recoveries ranged from 70.87% to 103.99%, with relative standard deviations (*RSDs*) of 5.53% -7.69%, all meeting the requirements of GB 5009.295-2023 standard. [**Conclusions**] The method is simple, efficient, sensitive, and broadly matrix-compatible, making it suitable for routine monitoring and risk assessment of captan residues in various types of formulated milk powders.

Key words Captan, Milk powder, Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS), Detection

0 Introduction

To meet the ever-expanding demand for food supply, global agriculture has become increasingly dependent on pesticides year by year, leading to a sustained rise in annual pesticide usage. Captan is an organosulfur fungicide belonging to the thiophthalimide class[1-3]. As a contact fungicide, it has been used for decades to protect a wide range of crops against various diseases, and its usage is likely to remain high in the coming decades[4-5]. Captan is one of the most widely used non-systemic fungicides in modern agriculture. The US Environmental Protection Agency (EPA) has classified captan as a probable human carcinogen, and the ecological effects and biological toxicity caused by its environmental residues have attracted widespread concern[6-8]. On July 11, 2024, Slovakia issued a notification through the EU Rapid Alert System for Food and Feed (RASFF) reporting that infant formula exported from the Czech Republic exceeded the maximum residue limit for pesticide residues. Specifically, the captan residue level in the infant formula with notification number 2024.5318 was 0.026 mg/kg, exceeding the maximum residue limit of 0.01 mg/kg.

Milk powder serves as a vital nutritional source for infants and the general consumer population, and its quality and safety are closely linked to public health and well-being. The milk powder matrix is rich in proteins and fats, exhibiting considerable compositional complexity, which makes the analysis of trace pesticide residues highly susceptible to matrix interference. This imposes stringent demands on sample pretreatment strategies as well as on the selectivity and sensitivity of detection methods[9-11]. At present, the established standard methods for captan residue detection are predominantly focused on plant-derived matrices such as fruits, vegetables, and cereals[12-14]. However, dedicated methodological studies targeting milk and dairy products, which are characterized by high protein and high fat content, remain insufficient. Furthermore, existing standards rarely include validation data specifically for milk powder matrices. Given the sustained growth in milk powder consumption and the increasing stringency of food safety monitoring requirements, there is an urgent need to develop an accurate and efficient analytical method suitable for the determination of captan residues in milk powder.

At present, the analytical methods for captan residues mainly include gas chromatography (GC)[15-17], high performance liquid chromatography (HPLC)[18-20], and gas chromatography-tandem mass spectrometry (GC-MS)[21-23]. Although conventional gas chromatography is widely employed, it is susceptible to matrix interference, often leading to false positive results or inaccurate quantification in trace analysis of complex samples. The triple quadrupole gas chromatography-tandem mass spectrometry (GC-MS/MS), operating in multiple reaction monitoring (MRM) mode, offers superior selectivity and anti-interference capability compared with single quadrupole mass spectrometry. It can effectively reduce matrix effects and achieve reliable qualitative and quantitative analysis of trace targets[24-26]. In recent years, GC-MS/MS has demonstrated favorable application performance in the detection of captan residues in complex matrices such as tea and Chinese medicinal herbs. However, pretreatment optimization and systematic methodological studies specifically targeting milk powder matrices remain to be supplemented. This study aims to establish a method for the determination of captan residues in milk powder based on triple quadrupole gas chromatography-tandem mass

Received: July 11, 2026 Accepted: September 20, 2026

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spectrometry. Through systematic optimization of key parameters, including extraction solvent volume, clean-up efficiency of different solid phase extraction cartridges, and various chromatographic conditions, the study strives to achieve satisfactory sensitivity, recovery, and precision in the complex milk powder matrix, thereby providing reliable technical support for routine monitoring and risk assessment of captan residues in milk powder.

1 Materials and methods

1.1 Materials and reagents Captan reference standard was purchased from Shanghai ANPEL Experimental Technology Co., Ltd. Various types of formula milk powder were obtained from commercial sources. Acetonitrile, n-hexane, and acetone were supplied by Shanghai ANPEL Experimental Technology Co., Ltd. Sodium chloride was purchased from Tianjin Guangfu Technology Development Co., Ltd. Anhydrous sodium sulfate and phosphoric acid were obtained from Tianjin Kermel Chemical Reagent Co., Ltd.

1.2 Instruments and equipment The following instruments were used in this study: a triple quadrupole gas chromatography-tandem mass spectrometry system (Thermo Fisher Scientific, USA); an XPR 205DU electronic balance (precision: 0.000 01 g, Mettler Toledo, Switzerland); a 3-20R refrigerated centrifuge (Hunan Kecheng); an MTN-2800W nitrogen evaporator (Tianjin Autoscience); an LC-SPE-24 + solid phase extraction apparatus (Hunan Lichen Instrument Technology Co., Ltd.); an NY-4sx vortex mixer (Changzhou Enyi); a K550 platform shaker (IKA, Germany); and a DTC-27G ultrasonic cleaner (Hubei Dingtai Hengsheng).

1.3 Extraction of milk powder samples A 3.0 g (accurate to 0.000 1 g) portion of milk powder sample was weighed into a 50 mL stoppered centrifuge tube. Then, 5 mL of deionized water was added, and the mixture was vortexed until homogeneous. Subsequently, 5 mL of 10 % phosphoric acid solution was added to the centrifuge tube, and the mixture was allowed to stand for 5 min. Next, 20 mL of acetonitrile and 4 g of sodium chloride were added, followed by vortex oscillation for 10 min. The mixture was then centrifuged at 5 000 r/min for 10 min at 4 ℃. A 10 mL aliquot of the upper organic layer was transferred to a 15 mL centrifuge tube and evaporated to approximately 1 mL under a nitrogen stream at 40 ℃, prior to purification.

1.4 Purification of milk powder samples The C_{18} solid phase extraction cartridge was preconditioned by adding 0.2 g of anhydrous sodium sulfate and then activated with 3 mL of acetonitrile. The entire extract obtained from the previous step was loaded into the cartridge and eluted with 8 mL of acetonitrile. The eluate was collected completely into a glass nitrogen blowing tube and evaporated to near dryness under a gentle nitrogen stream at 40 ℃. The residue was reconstituted in 1 mL of n-hexane-acetone (3 + 1, *v*/*v*), vortexed to mix thoroughly, and filtered through a 0.22 μm organic membrane prior to GC-MS/MS analysis.

1.5 Analysis and detection

1.5.1 Standard working solutions. An appropriate volume of captan standard solution (1 μg/mL) was accurately transferred and diluted to volume with acetone to prepare a series of standard solutions at concentrations of 5, 10, 20, 50, 100, 200, and 500 ng/mL. Blank samples were subjected to the same pretreatment procedure as the actual samples. After the purified extract was evaporated to dryness under nitrogen, the residue was reconstituted in 1 mL of each of the above serial standard solutions, vortexed to homogeneity, and filtered through a 0.22 μm membrane. The supernatant was then analyzed by gas chromatography-tandem triple quadrupole mass spectrometry. A standard curve was constructed by plotting the peak area of the quantitative ion of captan on the ordinate against the concentration of the captan standard solution on the abscissa.

1.5.2 Chromatographic Conditions. A 5% phenyl-methylpolysiloxane fused-silica capillary column (TG-5SILMS) with dimensions of 30 m × 0.25 mm i.d. × 0.25 μm film thickness, or an equivalent column, was used. The inlet temperature was set at 250 ℃. The column temperature was programmed as follows: the initial temperature was 50 ℃, held for 1.0 min; then ramped at 20 ℃/min to 200 ℃ and held for 1.5 min; finally ramped at 10 ℃/min to 300 ℃ and held for 2.0 min. Helium with purity ≥ 99.999% was used as the carrier gas in constant flow mode at a flow rate of 1.0 mL/min. Splitless injection mode was adopted with an injection volume of 1 μL.

1.5.3 Mass spectrometric conditions. The ion source was operated in electron impact (EI) mode with an electron energy of 70 eV. The ion source temperature was set at 230 ℃, and the transfer line temperature was maintained at 250 ℃. Quantitative analysis was performed in selected reaction monitoring (SRM) mode. The retention time of captan (CAS No. 133-06-2) was 13.43 min. The quantitative ion pair was 149.1 > 70.0 *m/z*, and the two qualitative ion pairs were 149.1 > 105.0 *m/z* and 116.9 > 81.9 *m/z*, respectively. The corresponding collision voltages for these three ion pairs were 15, 5, and 25 V, respectively.

1.6 Calculation of captan content The captan content in the sample was calculated according to Equation (1):

$$X = \frac{\rho \times V \times 1\,000}{m \times 1\,000} \quad (1)$$

where X is the captan content in the sample, μg/kg; ρ is the concentration of captan obtained from the standard calibration curve, μg/mL; V is the final volume of the sample solution, mL; m is the mass of the sample, g; 1 000 is the unit conversion factor. The result was rounded to two decimal places.

2 Results and analysis

2.1 Comparison of chromatographic conditions (temperature programming) Under the column temperature program recommended in the industry standard SN/T 0654-2019, the target analyte captan eluted at 18.9 min with a total run time of 24.5 min. As a single-compound detection method, the retention time was relatively late; therefore, optimization experiments were conducted to shorten the elution time of the target compound. Given

that captan belongs to the organosulfur fungicide class, and in consideration of maintaining consistency with the laboratory's multi-residue methods, temperature program 1 (the chromatographic temperature program used for multi-residue screening) was initially adopted. Under this condition, the target compound eluted at 16.56 min, with a total run time of 35.6 min. The improvement in retention time was not significant, and the pretreatment results indicated that the multi-residue screening method was not suitable for captan detection. Therefore, temperature program 2 was designed for further optimization, under which the target compound eluted at 13.57 min with a total run time of 22 min (Table 1).

Taking all factors into consideration, the optimal column temperature programming conditions selected for this method was as follows: 50 ℃ held for 1 min, ramped at 20 ℃/min to 200 ℃ and held for 1.5 min, then ramped at 10 ℃/min to 300 ℃ and held for 2 min. Under these conditions, the target compound exhibited the earliest retention time and the shortest total run time.

Table 1 Comparison of different chromatographic temperature programming conditions

Conditions	Initial temperature	Temperature rise rate
1	Held 1.5 min at 40 ℃	The column temperature was programmed as follows: ramped at 25 ℃/min to 90 ℃ and held for 1.5 min; then ramped at 25 ℃/min to 180 ℃; then ramped at 5 ℃/min to 280 ℃; and finally ramped at 10 ℃/min to 300 ℃ and held for 5 min.
2	Held 1 min at 50 ℃	The column temperature was programmed as follows: ramped at 20 ℃/min to 200 ℃ and held for 1.5 min; then ramped at 10 ℃/min to 300 ℃ and held for 2 min.

2.2 Comparison of extraction solvent volumes in pretreatment The sample matrix in this experiment was milk powder, which is characterized by high protein and fat content, leading to significant matrix interference. Therefore, sample pretreatment was required prior to detection to extract the target analytes using appropriate extraction solvents and suitable volumes. In this experiment, different volumes of acetonitrile (10, 15, 20, and 25 mL) were compared as the extraction solvent. Blank milk powder samples were spiked with captan standard solution at 10 μg/kg and extracted with the respective solvent volumes, while all other operations were kept consistent. Each condition was tested in triplicate, and the results are shown in Fig. 1. One-way analysis of variance (ANOVA) was performed on the recovery data obtained with different extraction solvent volumes, yielding a P-value of 0 (<0.05), indicating a statistically significant difference. The average recoveries of captan from milk powder samples under the four solvent volumes were 75.1%, 92.1%, 99.2%, and 88.7%, respectively, with the highest average recovery achieved at 20 mL. Therefore, 20 mL of acetonitrile was selected as the optimal extraction solvent volume for this method.

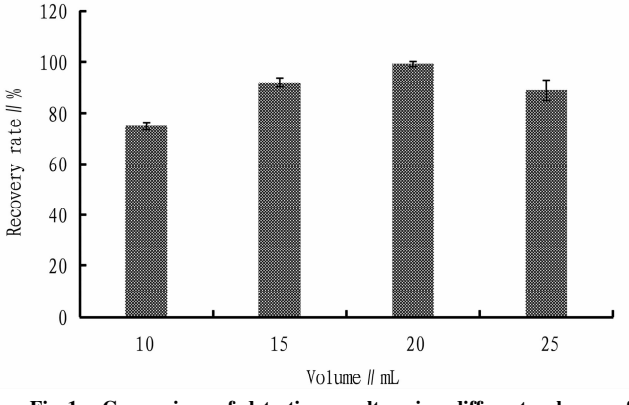


Fig. 1 Comparison of detection results using different volumes of extraction reagents ($n=3$)

2.3 Optimization of purification methods in pretreatment In the analysis of trace contaminants, the purpose of purifying the extract is to further isolate and enrich the target analytes. Solid phase extraction (SPE) cartridge purification has been widely applied in the field of food contaminant detection. In this study, different types of SPE cartridges were compared for the purification of milk powder matrix. Blank milk powder samples were spiked with captan standard solutions at concentrations of 10, 20, and 100 μg/kg. After extraction, the samples were purified using either C_{18} SPE cartridges or HLB SPE cartridges. The cartridges were then eluted with 8 mL of acetonitrile, and the eluates were collected into glass nitrogen blowing tubes. The eluates were concentrated to near dryness under a gentle nitrogen stream at 40 ℃, reconstituted in 1 mL of n-hexane-acetone (3 + 1, v/v), vortexed, and filtered through 0.22 μm membranes prior to GC-MS/MS analysis. The recovery results are presented in Fig. 2. As shown in Fig. 2, at all three spiking levels, the mean recoveries obtained with C_{18} cartridges (107.8%, 93.6%, and 83.8%, respectively) were higher than those obtained with HLB cartridges (107.4%, 79.4%, and 59.3%, respectively). Therefore, the C_{18} SPE cartridge was selected as the purification sorbent for the milk powder matrix in this method.

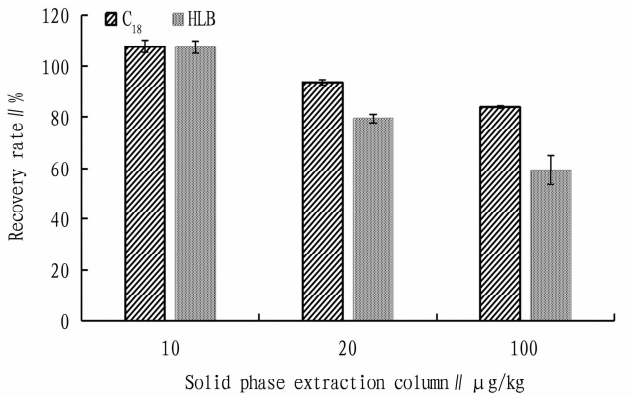


Fig. 2 Comparison of purification by different types of solid phase extraction columns ($n=3$)

2.4 Correlation coefficient, linear range, limit of detection, and limit of quantification of the calibration curve The exter-

nal standard quantification method was employed in this study. A captan reference standard was diluted with acetone to prepare a stock standard solution at a concentration of 100 μg/mL, which was then further diluted 100-fold with acetone to obtain an intermediate standard solution at 1 μg/mL. Aliquots of the intermediate standard solution were accurately transferred and diluted to prepare a series of working standard solutions at concentrations of 5, 10, 20, 50, 100, 200, and 500 ng/mL. Blank samples were pretreated according to the procedure described in Section **1.3** to obtain blank matrix solutions, which were then evaporated to dryness under nitrogen. The residues were reconstituted in 1 mL of each of the above working standard solutions, vortexed, filtered through 0.22 μm membranes, and the supernatants were analyzed by GC-MS/MS. The calibration curve was constructed by plotting the peak area of the quantitative ion of captan on the ordinate against the mass concentration of the standard solutions on the abscissa. The experimental results demonstrated that the calibration curve exhibited good linearity over the concentration range of 10 – 500 ng/mL, with a regression equation of $Y = 1\,136.0X - 3\,632.0$ and a correlation coefficient (R^2) of 0.999 7, meeting the analytical requirements. The limit of detection (LOD) for captan residues in milk powder was determined to be 2 μg/kg, with a signal-to-noise ratio (S/N) greater than 3. The limit of quantification (LOQ) was 5 μg/kg, with an S/N greater than 10.

2.5 Recovery rate and precision of the method In this study, blank formula milk powder samples were used for spiked recovery experiments at three fortification levels: the limit of quantification (5 μg/kg), twice the limit of quantification (10 μg/kg), and ten times the limit of quantification (50 μg/kg). Seven replicate determinations were performed at each spiking level. The recovery and precision results are presented in Table 2. As shown in Table 2, at the LOQ fortification level (5 μg/kg), the recovery rates ranged from 82.72% to 103.17%, with a relative standard deviation (*RSD*) of 7.69%, which met the requirements of GB 5009.295-2023 (recovery between 60% and 120%, and $RSD \leqslant 20\%$ for spiking levels greater than 1 μg/kg and not exceeding 10 μg/kg). At the two-fold LOQ (10 μg/kg) and ten-fold LOQ (50 μg/kg) levels, the recovery rates ranged from 70.87% to 90.68% and from 75.24% to 90.61%, with *RSD* values of 5.53% and 6.83%, respectively. All results complied with the acceptance criteria specified in GB 5009.295-2023.

Table 2 Results of standard recovery rates and precision tests ($n = 7$)

Spiking level μg/kg	Measured value μg/kg	Recovery rate %	Precision *RSD* %	Spiking level μg/kg	Measured value μg/kg	Recovery rate %	Precision *RSD* %	Spiking level μg/kg	Measured value μg/kg	Recovery rate %	Precision *RSD* %
5.04	5.24	103.99	7.69	9.87	7.51	76.09	5.53	49.12	42.13	85.77	6.83
5.05	4.79	94.77		9.96	7.91	79.44		49.91	42.19	84.52	
5.03	4.16	82.72		9.87	7.82	79.24		50.04	45.34	90.61	
5.04	5.20	103.17		10.05	7.12	70.87		49.96	37.58	75.24	
4.95	4.82	97.31		9.95	7.34	73.72		50.33	38.94	77.37	
5.02	5.01	99.85		10.14	8.37	82.52		49.70	41.10	82.69	
4.99	5.16	103.49		9.93	9.00	90.68		49.84	41.61	83.49	

2.6 Analysis of actual samples In this study, different types of commercially available formula milk powders were analyzed, including Beichang goat milk powder, Jinlingguan Zhenhu infant formula, high-iron and high-calcium milk powder for women, pure milk powder, and full-fat sweet milk powder. The five milk powder samples were tested for captan residues following the procedure described in this method. The results showed that captan was not detected in any of the samples.

3 Conclusions

Through systematic optimization and analysis of key parameters, including extraction solvent, purification method, and chromatographic and mass spectrometric conditions, this study established a gas chromatography-tandem triple quadrupole mass spectrometry method for the determination of captan residues in milk powder. The method is applicable to various types of formula milk powders. The calibration curve exhibited good linearity over the concentration range of 5 – 500 ng/mL. The limit of detection (LOD) was 2 μg/kg, and the limit of quantification (LOQ) was 5 μg/kg. This method is simple, efficient, sensitive, and accurate, providing reliable technical support for routine monitoring and risk assessment of captan residues in milk powder.

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