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AURAMINE O IN FOOD PRODUCTS: IMPROVED QUECHERS EXTRACTION COUPLED WITH UHPLC-MS/MS QUANTITATION

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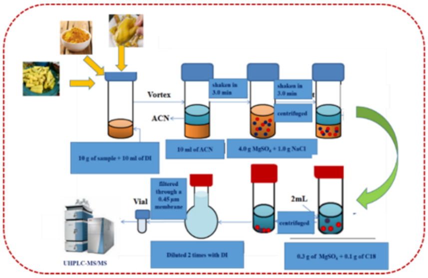
Graphical abstract



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Abstract: Auramine O, also known as basic yellow, is a prohibited substance in food. Its presence in food products may result from fraudulent practices or a lack of understanding of its effects. This research presents a novel method for quantifying Auramine O in food products by utilizing an optimized QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction coupled with UHPLC-MS/MS. This optimization resulted in significantly improved analytical performance, achieving substantially lower limits of detection (LOD) and quantification (LOQ) of 0.012 ppb and 0.036 ppb, respectively. These values represent a considerable improvement over the Vietnamese standard method (TCVN 12267-2018) and other previously reported methods. The method demonstrated excellent linearity ($R^2 > 0.999$) across a relevant concentration range and high recovery rates (95.0-97.0%) in various food matrices (chicken, bamboo shoots, curry powder). Furthermore, both intra-day and inter-day precision were excellent, confirming the method's reliability and reproducibility. Application to real samples revealed detectable levels of AO in a proportion of tested food items. The sensitivity and efficiency of this method provide a powerful tool for monitoring AO in food, thereby strengthening food safety and consumer protection efforts.

Keywords: QuEChERS, Auramine O, Basic yellow 2, Auramin, Dyer contamination

Introduction

Auramine O (AO), also referred to as Basic yellow 2, or Auramin, belongs to the ketoimin subclass, with the chemical nomenclature of diarylmethane and the IUPAC name: 4,4-carbonimidoylbis [N,N-dimethylbenzenamine]. In the medical field, AO can be utilized as a fluorescent agent for the detection of acid-fast bacteria in infected tissues,[1,2] and in combination with rhodamine for the detection of *Mycobacterium tuberculosis.*[3,4] In industrial applications, Auramine O serves as a synthetic dye employed for tinting various products, including fabrics, wood, and paper, and is also used in wall paint formulations. This dye is predominantly utilized in the leather, paper, and paint industries, as well as in hair dyes.[5]

Biologically, AO is an extremely hazardous toxic agent. Upon dermal contact, it can cause allergic reactions or dermatitis, and irritation to the eyes. Inhalation exposure may result in coughing, throat irritation, shortness of breath, and chest pain.[6,7] Continuous accumulation in the body can have detrimental effects on the liver, kidneys, reproductive system, and nervous system, and may also pose a carcinogenic risk.[8,9] The International Agency for Research on Cancer (IARC) has classified AO as Group 2B, indicating that it is possibly carcinogenic to humans.[5] Notably, AO found in tobacco smoke has been shown to induce cellular mutations.[10] Numerous studies have been conducted to assess the harmful effects of AO in animal models, with results indicating that it causes DNA damage in the liver, kidneys, and bone marrow of mice, leading to mortality or the emergence of lymphomas in these animals.[11]

The recent presence of AO in food products is attributable to either fraudulent practices or a lack of knowledge regarding its implications. Due to its striking yellow color, AO is often misused to enhance the appearance of food items. For example, it may be used to dye fresh bamboo shoots, chicken post-slaughter, processed feed, and to achieve an attractive hue in egg yolks. Furthermore, the lack of awareness regarding of consumers the associated risks of this substance has raised concerns about food safety among consumers and regulatory agencies. In Europe, the production of AO must comply with the regulations outlined in Directive 2004/37/EC, which applies to activities involving worker exposure to carcinogens or mutagenic substances.[12] The European Spice Association (ESA) has also included AO in its list of substances prohibited for use in food products.[13] In Vietnam, the Ministry of Agriculture and Rural Development has issued Circular No. 26/2012 and the supplementary Circular No. 42/2015, which prohibit the production, importation, trade, and use of AO and its compounds in livestock and poultry feed.[14]

To determine the content of AO in analytical samples, numerous studies have been published. Commonly used methods include

HPLC-PDA,[15–17] HPLC-MS/MS,[15] carbon nanodots,[18,19] voltammetry,[20,21] microchip capillary electrophoresis,[22] and raman spectroscopy.[23] Among these, the HPLC method is the most widely employed, with particular emphasis on sample preparation procedures. In Vietnam, the official method for quantifying AO is outlined in the standard TCVN 12267-2018, which specifies that samples are treated with acetonitrile (ACN), filtered through 0.22 μm filter paper, and analyzed using LC/MS/MS, achieving a limit of detection (LOD) of 3 ppb.[24] A study conducted by Ha et al. (2019) indicated that when AO is directly extracted from food matrix samples using a MeOH:H_2O (9:1) solvent in 0.1% formic acid, with the assistance of ultrasound and analyzed by UHPLC-MS/MS, an LOD of 0.11 mg/kg was obtained [25]. It is evident that direct sample analysis without a purification step does not yield a satisfactory LOD.

In recent years, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction technique has been recognized as a rapid, simple, cost-effective, efficient, and robust method for sample preparation.[26,27] Originally developed for analyzing pesticide residues in high-moisture fruits and vegetables,[28] this technique has gained popularity for analyzing a variety of compounds across diverse matrices.[29,30] QuEChERS offers several advantages over most traditional extraction methods, including rapid implementation and the ability to process a large number of samples, with approximately 10 to 20 samples being processed within 30 to 40 minutes by a technician. Additionally, QuEChERS aligns with green chemistry principles due to its reduced solvent consumption, absence of chlorinated solvents, and generation of minimal waste.[31,32] Some authors have combined this extraction technique with solid-phase extraction (SPE) for cleanup and quantified AO using LC-MS/MS, achieving LOD values of 0.3 ppb[33] or 0.06 ppb.[34] However, the utilization of SPE column increases analytical costs and limits the number of samples processed simultaneously.

In this study, we propose to combine the QuEChERS extraction technique with a simple cleanup stage utilizing magnesium sulfate ($MgSO_4$) and C18 powder to enhance sensitivity and reduce the limit of detection (LOD) in conjunction with the UHPLC-MS/MS method. To our knowledge, this technique has not been previously reported. Therefore, this research aims to simplify the process, increase sample throughput, reduce analytical costs, and enhance sensitivity through improved LOD values. Consequently, this study is anticipated to serve as a valuable reference for scientists, regulatory authorities, and stakeholders involved in the analysis, evaluation, and control of this prohibited substance in food products.

Materials and methods

Chemicals and equipment

The chemicals used were of analytical grade. AO in solid form with a minimum purity of 99.0% was purchased from Sigma - Aldrich. Anhydrous magnesium sulfate ($MgSO_4$) 98.0%, sodium chloride (NaCl) 99.5%, sodium acetate ($NaCH_3COO$) 99.0%, ammonium hydroxide (NH_4OH) 25%, acetic acid 100%, and formic acid (FA) were obtained from Merck. Deionized water, acetonitrile, and methanol suitable for HPLC were sourced from Fisher Scientific. C18 powder 99.5% and primary secondary amine (PSA) 99.5% were acquired from a supplier in Canada.

The equipment used in this study included a high-performance liquid chromatography (HPLC) system (UHPLC Ultimate 3000, Thermo Fisher Scientific, Bremen, Germany) combined with a Thermo Endura QqQ mass spectrometer. The system operated in positive electrospray ionization (ESI) mode coupled with selected reaction monitoring (SRM) for data acquisition.

Preparation standard solution

A stock solution of AO with a concentration of 1000 ppm was prepared by accurately weighing approximately 10 mg of AO (99.0% purity) into a 10 mL volumetric flask, dissolving it in methanol, and bringing it to volume. This solution was stored at -20 °C for a duration of 12 months. Lower concentration standard solutions were prepared by serial dilutions from this stock solution.

Sample extraction

The sample processing procedure is adapted from previous work with some minor modification,[35] as follows: approximately 10.0 g of the sample is accurately weighed, and 0.1 mL of a 2 ppm AO standard solution is added, followed by the addition of 10 mL of water, which is then vortexed for 3 minutes. Next, 10 mL of acetonitrile (ACN) is added, and the mixture is vigorously shaken on a vortex mixer for 3 minutes. Subsequently, a salt mixture composed of 4.0 g $MgSO_4$ and 1.0 g NaCl is added, and the mixture is shaken vigorously for an additional 3 minutes before being centrifuged at 4000 rpm for 3 minutes. Afterward, 1.0 mL of the supernatant is transferred into centrifuge tubes containing 0.3 g $MgSO_4$ and 0.1 g C18 for cleanup. This mixture is then vortexed for 3 minutes and centrifuged at 4000 rpm for another 3 minutes. The resulting supernatant is diluted with distilled water and filtered through a 0.22 µm membrane prior to analysis by LC-MS/MS. Each experiment is conducted in triplicate to ensure the accuracy and reliability of the results.

To investigate the impact of various sample processing factor on the recovery, the following factors are systematically evaluated: extraction solvent systems A1 (100% of ACN) and A2 (99% of ACN and 1.0% pure acetic acid); extraction salts B1 (4.0 g NaCl), B2 (4.0 g $MgSO_4$ + 1.0 g NaCl), B3 (6.0 g $MgSO_4$ + 1.5 g $NaCH_3COO$), and B4 (4.0 g Na_2SO_4 + 1.0 g NaCl + 0.5 g $MgSO_4$); and cleanup salts C1 (0.3 g $MgSO_4$ + 0.1 g C18), C2 (0.6 g $MgSO_4$ + 0.1 g C18), C3 (0.3 g $MgSO_4$ + 0.1 g PSA), and C4 (0.6 g $MgSO_4$ + 0.1 g C18 + 0.1 g PSA). Each experimental condition is repeated three times to calculate the mean value and standard deviation.

Method validation

Linear concentration range

The linear range of the method is established by analyzing standard solutions of AO at concentrations ranging from 0.2 to 20 ppb (0.2, 0.5, 1.0, 2.0, 5.0, 10, and 20 ppb). This concentration range is designed to encompass the expected levels of AO contamination in food, ranging from trace amounts to elevated levels. The peak area of AO is measured and plotted against concentration to construct a calibration curve. Linearity is assessed based on the correlation coefficient (R^2).

Recovery efficiency evaluation (R%)

To evaluate the recovery efficiency of the method, standard AO is spiked into matrices of chicken meat, bamboo shoots, and curry powder, which do not contain the analyte, at three concentration levels: 2.0, 5.0, and 10.0 ppb. Each experiment is repeated seven times at every concentration level to ensure the accuracy and reliability of the obtained data.

Assessment of Repeatability and Reproducibility

To assess inter-day repeatability as well as reproducibility across intra-days (specifically on the second, third, and fourth days), seven replicates are performed on matrices of chicken meat, bamboo shoots, and curry powder, all spiked with the analyte at a concentration of 10 ppb. This evaluation helps determine the consistency and reliability of the method under varying conditions.

Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ is crucial for assessing prohibited substances in food. These values not only establish the measurement limits but also assist regulators in setting concentration thresholds for effective management. Several methods for determining LOD have been developed and published.[29,36] The procedure is as follows: 0.1 mL of a 20 ppb AO standard solution is added to 10.0 g of sample and processed according to the optimized sample preparation protocol. The experiments are conducted eleven times to calculate the standard deviation (SD), from which LOD and LOQ are derived using the formulas: LOD = 3 × SD and LOQ = 10 × LOD.

Results and Discussion

Liquid Chromatography Conditions

The application of modern analytical techniques such as LC-MS/MS enhances the reliability and selectivity of the method, as the analyte AO is quantified based on the fragmentation of parent ions into characteristic daughter ions. Selecting an appropriate mobile phase that complements the ionization properties of the analytes is of importance. Previous studies have indicated that the most suitable ionization mode for AO is positive ionization (+).[33,34] Therefore, in this study, we selected the positive ionization mode and a mobile phase containing acid to ensure maximum sensitivity. The ion source parameters were configured as follows: ionization source: H-ESI (+); spray voltage: 3500 V; vaporization temperature: 300 °C; ion transfer tube temperature: 300 °C; sheath gas: 40 arb; auxiliary gas: 5 arb; and CID gas: 2 mTorr. Data collection and processing were conducted using TraceFinder 3.3 software (see more Compound Optimization Report on Figure S1).

Chromatographic conditions were established using an Accucore C18 chromatography column (100 x 2.1 mm, particle size 2.6 µm), with the column oven temperature set at 40 °C and an injection volume of 10 µL. The optimized gradient program for the mobile phase is detailed in Table 1.

Table 1. Mobile phase program for the method.

Time	MeOH 5mM $HCOONH_4$ 0.1% FA	H_2O 5mM $HCOONH_4$ 0.1% FA	Flow rate (mL/min)
0	20	80	0.3
1.0	20	80	0.3
2.0	100	0	0.3
4.0	100	0	0.3
4.1	20	80	0.3
6.0	20	80	0.3

To identify the parent ion of AO, a 10 µL aliquot of a 10 ppb AO standard solution was injected directly into the mass spectrometer (without a column). AO has a molecular weight of 268 g/mol and a pKa of 9.6; upon dissolution in solution, it dissociates into Cl^- and a positively charged ion. Consequently, a positive ionization scan mode was employed with a scanning range of m/z 100 to 300. The resulting mass spectrum revealed fragment ions with the highest relative abundances of m/z = 268.44 (100%), and m/z = 269.44 (24.71%) (see more Figure S2). After identifying the parent ion at m/z = 268.44 with the highest intensity, this parent ion was subjected to fragmentation to obtain the corresponding daughter ions in the auto-tune mode of the MS/MS instrument. Figure S1 illustrates the plotted signal of m/z values and their corresponding intensities. The identified daughter ions were m/z 122.60, 147.50, and 252.50. Among these, the ion fragment at m/z 147.50 exhibited the highest intensity and was selected as the quantification ion, while the two daughter ions at m/z 122.60 and m/z 252.50, which had lower intensities, were chosen as confirmation ions. The obtained data were also consistent with previous publications.[25,34]

Figure 1 shows that, under the chromatography conditions presented in Table 1, the retention time of AO in the standard sample is 3.80 minutes, while the retention time of the actual sample is 3.79 minutes. This indicates that the influence of the matrix on the retention time is insignificant.

Influence of sample preparation on the recovery

Effect of extraction solvent

The extraction solvent is one of the key factors influencing the extraction efficiency of the analyte from the sample matrix. In the QuEChERS extraction technique, acetonitrile (ACN) is commonly used due to its excellent extraction capability for organic compounds, particularly those with low to medium polarity. Since Auramine O exhibits moderate polarity, the use of ACN is appropriate.

The results of the investigation indicate that the use 100% of ACN and 99% of ACN with 1.0% pure acetic acid yielded nearly equivalent extraction efficiencies, both exceeding 98%. Therefore, to simplify the experimental procedure, we selected the ACN extraction solvent for subsequent analyses.

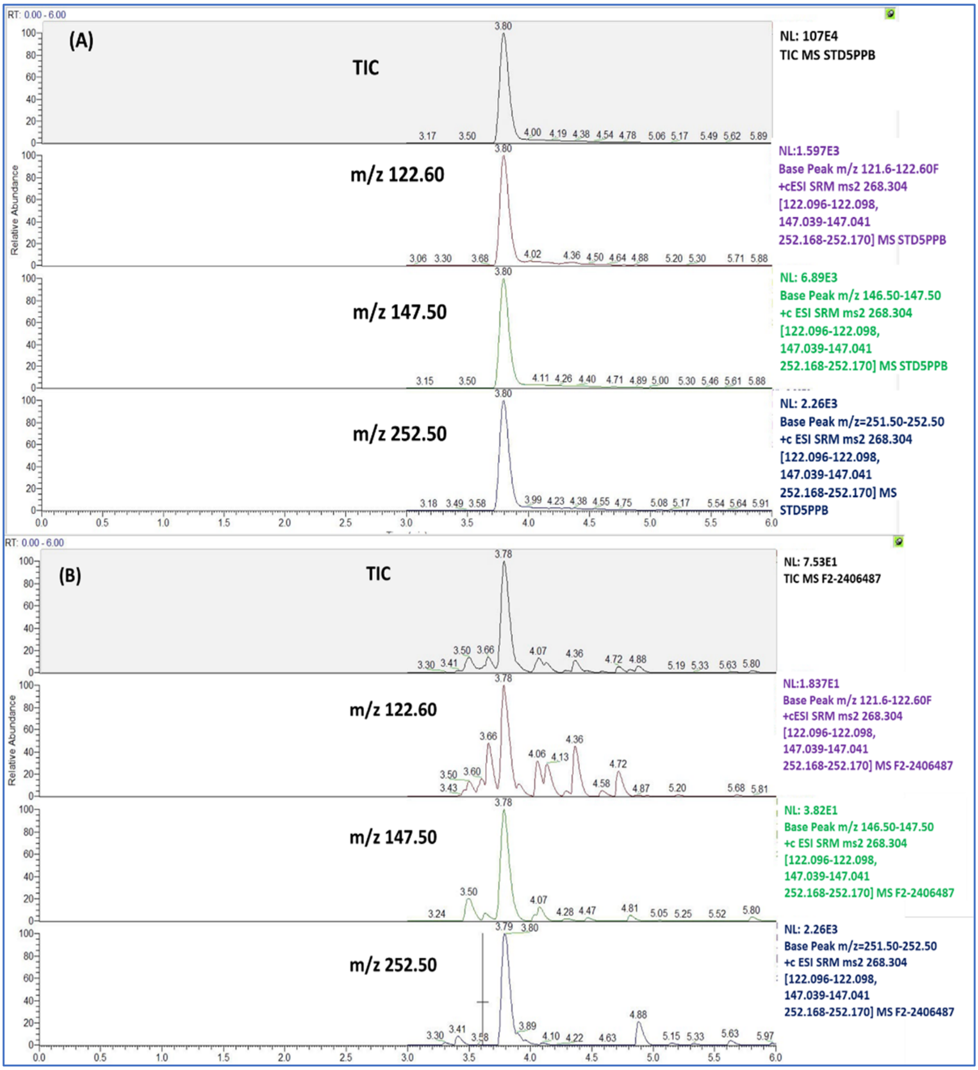

Figure 1. Representative chromatograms of Auramine O (AO): (A) 5.0 ppb standard solution; (B) contaminated Chicken meat sample 3. From top to bottom: the total ion chromatogram (TIC) and extracted ion chromatograms (EICs) for daughter ions m/z 122.60, m/z 147.50, and m/z 252.50.

Extraction salts

The purpose of using extraction salts in the QuEChERS extraction technique is to facilitate the complete separation of the acetonitrile (ACN) phase from the aqueous phase. Currently, many different extraction salts have been developed, each serving to create a specific pH environment for the extraction solvent. However, magnesium sulfate ($MgSO_4$) is the most commonly used extraction salt due to its hygroscopic properties. When $MgSO_4$ is added to the extraction solution, it generates significant heat, thereby improving the extraction process and aiding in the separation between the aqueous and ACN phases.[37]

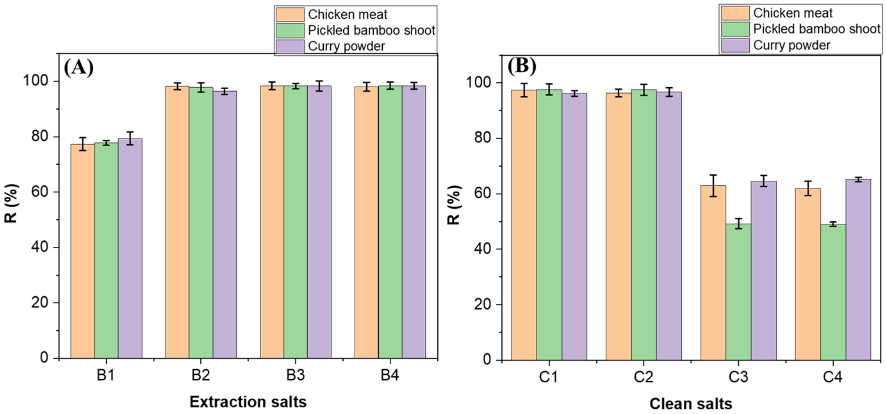

Figure 2. (A): The factors effect on recovery efficiency; (A): extraction salts (B): cleanup salts.

The results shown in Figure 2A indicate that among the four extraction salts investigated, sodium chloride (NaCl) provided the lowest extraction efficiency. The low efficiency of NaCl could be attributed to the fact that 4.0 g of NaCl may not be sufficient to completely separate the ACN phase from the aqueous phase. In contrast, the other three extraction salts demonstrated comparable and relatively good extraction efficiencies. To minimize the amount of waste salts, the optimal extraction salt system selected for subsequent studies is the mixture of 4.0 g $MgSO_4$ and 1.0 g NaCl (B2).

Cleanup salts

Acetonitrile is a widely used solvent in chromatographic analysis due to its strong ability to extract a variety of compounds from food matrices and agricultural products. However, the selectivity of this solvent is relatively low. Consequently, the extract often contains numerous contaminants, including color compounds and organic acids. These impurities can adversely affect the chromatographic column, decrease the sensitivity of the detector, and impact the selectivity of the instrument. Therefore, utilizing cleanup salts to remove some of the impurities from the extract is essential to reduce interference and enhance the accuracy of the analysis.

The cleanup salts typically employed in this extraction technique include C18 powder to remove color from the extraction solution and PSA to eliminate organic acids present in the sample matrix. Additionally, $MgSO_4$ is also included in the cleanup salt mixture to desiccate the extraction solution [30,38]. Figure 2B illustrates the impact of cleanup salts on recovery efficiency. The results indicate that the two treatments, C1 (0.3 g $MgSO_4$ + 0.1 g C18) and C2 (0.6 g $MgSO_4$ + 0.1 g C18), achieved very high and comparable extraction efficiencies. Conversely, in treatments C3 and C4, the presence of PSA significantly affected the extraction process, leading to a considerable decrease in efficiency. This can be attributed to PSA being a primary amine ($R-NH_2$), where PSA molecules interacted with the polar groups on the AO compound through electrostatic interactions, resulting in the entrapment of AO. Therefore, the cleanup salt in treatment C1, with the smallest mass, was selected for subsequent experiments.

Method validation

Linear range

After identifying the optimal conditions from the sample processing procedure, the linear range of an analytical method is defined as the concentration range over which there is a linear dependence between the measured quantity and the analyte concentration. A standard series of AO was prepared, ranging from 0.2 ppb to 20.0 ppb, and analyzed using UHPLC-MS/MS. The results, illustrated in Figure 3, demonstrate that the calibration curves for AO follows a linear relationship within the concentration range of 0.2 to 20.0 ppb, with the correlation coefficient (R^2) over 0.9998. This meets the requirements for establishing the calibration curve, confirming the accuracy and reliability of the quantification method within the studied concentration range. Determining this linear range is essential for verifying the method's applicability to food samples with varying contamination levels, ensuring the accuracy of the quantitative results for AO according to AOAC guidelines.[39]

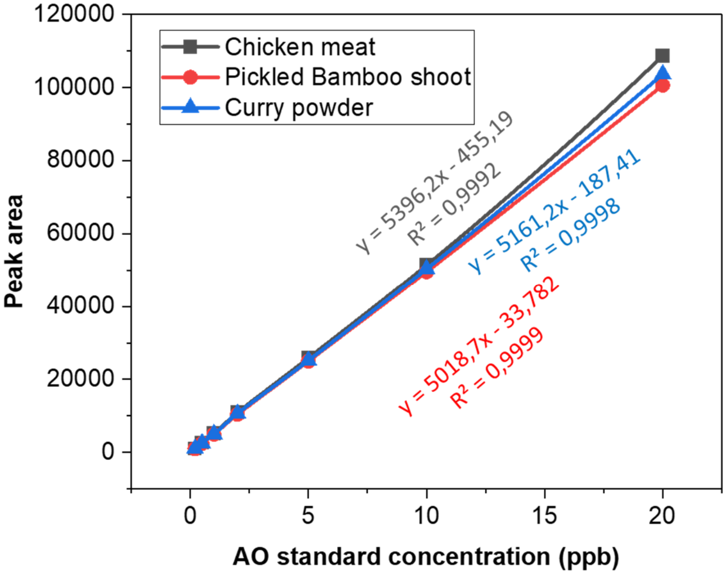

Figure 3. Calibration curve for the determination of AO in various sample matrices.

Repeatability and reproducibility

The repeatability of the method was evaluated to assess the stability of the analytical process and the consistency of performance among technicians. This parameter is crucial for evaluating the effectiveness of the analytical method. The requirements for repeatability must adhere to the stringent criteria established by the AOAC at specific concentration levels. The repeatability analysis was performed seven times on chicken meat, bamboo shoots, and curry powder samples containing AO to determine the method's repeatability. The analytical results are presented in Table 2. The findings indicate that the method's within-day repeatability and inter-day reproducibility meet AOAC standards[39].

Table 2. Validation of parameter of the UHPLC-MS/MS method

Samples	**The correlation coefficient, R^2**	**Recovery, R(%)**	**LOD, ppb**	**LOQ, ppb**	**Repeatability, RSD (%)**	
					Intraday	**Interday**
Chicken meat	0.9992	95.1÷96.3	0.012	0.036	1.5	4.5
Pickled Bamboo Shoots	0.9991	96.0÷97.3	0.012	0.036	1.9	3.7
Curry powder	0.9993	95.5÷96.8	0.012	0.036	1.2	3.3

Recovery efficiency

Evaluating the recovery efficiency of the method is essential for assessing the accuracy of the analytical process. The recovery efficiencies of the method, as shown in Table 2, indicate that the obtained values from various sample matrices meet the criteria established by AOAC.

LOD and LOQ

The study results demonstrate that the LOD for AO is 0.012 ppb, while the LOQ is 0.036 ppb for all three sample matrices: chicken meat, bamboo shoots, and curry powder. These results confirm that the LOD and LOQ values of the method are significantly lower than those reported in previous studies, while also meeting the objectives outlined in this work. According to TCVN 12267:2018,[24] the LOD for AO in meat samples is 3.0 ppb and 5.0 ppb in bamboo shoot samples. It is evident that the conventional method per TCVN 12267:2018 involves direct extraction with ACN, leading to a high dilution factor. Notably, the absence of a cleanup step prior to analysis significantly impacts the method's sensitivity, resulting in an LOD of 3.0 ppb for meat samples and 5.0 ppb for bamboo shoot samples. For prohibited substances, such as AO, it is crucial to determine the lowest possible LOD, as this provides a basis for regulatory agencies to impose strict penalties on facilities deliberately using prohibited substances, even at trace levels.

Other studies, such as those conducted by Thanh-Thien et al., (2020),[33] investigated the quantification of Auramine O in food and spices using UPLC-MS/MS. Their analysis involved extracting 1.0 g of the sample using an extraction solvent (50 mM ammonium acetate: methanol: formic acid, 79:20:1, v/v/v). The sample was then cleaned and concentrated on a solid-phase extraction (SPE) column using a 2.0% NH_4OH in methanol elution solvent. Finally, the extract was evaporated to dryness under N_2, reconstituted in 1 mL of ammonium formate solvent containing 0.1% formic acid: methanol (1:1, v/v), and analyzed using LC-MS/MS. The results showed that the method achieved an LOD of 0.1 ppb with recovery efficiencies ranging from 80.1% to 99.4%. Similarly, recent research by

Trinh.T and colleagues (2022),[34] reported that their samples were extracted in 1.0% acetic acid in acetonitrile, followed by cleanup and enrichment on an SPE column using a 5.0% NH_3 in methanol elution solvent. The extracts were then evaporated to dryness under N_2, reconstituted in 2.0 mL ACN: H_2O (1:9, v/v), and analyzed using LC-MS/MS. This method achieved an LOD of 0.06 ppb with recovery efficiencies ranging from 77.0% to 99.0%. Both of these studies significantly lowered the LOD values compared to TCVN 12267:2018.[24] However, both methods employed solid-phase extraction (SPE) for sample cleanup and enrichment, which complicated the procedures, extended the analysis time, and increased analytical costs.

Thus, by applying an improved QuEChERS sample extraction technique, we effectively addressed issues related to rapid analysis time, simplified methodologies, reduced analytical costs, and, importantly, improved the method's LOD sensitivity compared to TCVN 12267:2018 and previously published studies.

Determination of AO in real samples

We applied the proposed method to analyze several food samples collected from markets in Ho Chi Minh City, with the results presented in Table 3. Among the six chicken skin samples collected from traditional markets, four showed the presence of the contaminant AO, with concentrations ranging from 0.14 µg/kg to 3.13 µg/kg. For the fresh bamboo shoot samples, three out of six were also found to be contaminated with AO. Notably, all three curry samples were contaminated, indicating a significant health risk associated with AO in food.

In contrast, using the TCVN 12267 method, only five out of fifteen samples were identified as contaminated. This discrepancy indicates that the improved method has successfully reduced the limit of detection (LOD) to a very low level, enabling the detection of the analyte at trace levels. Consequently, this provides a legal basis for regulatory agencies to effectively monitor the presence of AO in food products.

Table 3. Results of AO analysis in various real samples (ND: Not detected).

No	Samples	Proposed method LOD, 0.012 ppb.	TCVN 12267 – 2018[24] LOD, 3.00 ppb
1	Chicken meat 1	0.14 ± 0.02	ND
2	Chicken meat 2	ND	ND
3	Chicken meat 3	2.42 ± 0.25	ND
4	Chicken meat 4	3.13 ± 0.12	3.52 ± 0.09
5	Chicken meat 5	0.40 ± 0.08	ND
6	Chicken meat 6	ND	ND
7	Pickled Bamboo Shoots 1	ND	ND
8	Pickled Bamboo Shoots 2	4.75 ± 0.25	4.76 ± 0.24
9	Pickled Bamboo Shoots 3	2.42 ± 0.17	ND
10	Pickled Bamboo Shoots 4	4.45 ± 0.08	3.92 ± 0.12
11	Pickled Bamboo Shoots 5	ND	ND
12	Pickled Bamboo Shoots 6	2.14 ± 0.13	ND
13	Curry powder 1	16.00 ± 0.19	16.60 ± 1.43
14	Curry powder 2	2.35 ± 0.17	ND
15	Curry powder 3	14.40 ± 0.20	15.20 ± 1.22

Furthermore, these findings highlight the urgent need to establish and publish updated procedures for quantifying AO in food samples, to enhance monitoring efforts and safeguard public health.

Conclusion

In this study, we developed a method for determining AO in chicken meat, bamboo shoots, and curry powder using UHPLC-MS/MS, demonstrating superior sensitivity and lower detection limits compared to the TCVN 12267 method. The method exhibits high reliability and accuracy, as demonstrated by various evaluation metrics, including the linear range, repeatability, recovery efficiency, limit of detection, and limit of quantitation. The application of the QuEChERS sample extraction technique has facilitated shorter and simpler analysis times while significantly reducing the LOD of the method to an impressive 0.012 ppb. Given that AO is categorized as a prohibited substance in food, the research focused on lowering the detection limit provides a vital basis for regulatory agencies to effectively monitor food safety. The data generated from this study have been utilized by the Saigon High-Tech Analysis Center in Vietnam for analytical services. Future research should explore validation across additional food types and assess scalability for routine screening purposes, supporting broader implementation in food safety practices.

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