Application Note

Food Safety Testing

Determination of Auramine O in Durian Using Agilent LC/MS/MS According to China BJS 202204

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Abstract

This application note details the detection of Auramine O in durian matrices using an Agilent 6495 triple quadrupole LC/MS system (LC/TQ), following the China BJS 202204 method. The performance of the 6495D LC/TQ was demonstrated by its excellent repeatability and high sensitivity, achieving detection at the parts-per-trillion (ppt) level for Auramine O in the samples. The method showed excellent selectivity, sensitivity, and linearity (R^2 > 0.999) over the range of 0.05 to 5.0 µg/L. Accuracy tests at 0.5 and 2.5 µg/kg achieved recoveries of 73 to 104%, with minimal matrix effects. Precision ranged from 1 to 6% RSD, meeting the Association of Official Analytical Chemists (AOAC) Appendix F guidelines.

Introduction

Auramine O (BY2) is a synthetic diarylmethane dye known for its bright yellow color and low manufacturing cost. Despite its industrial applications, BY2 is classified as a Group 2B possible human carcinogen by the International Agency for Research on Cancer (IARC) because of its mutagenic and carcinogenic potential.[1] Consequently, its presence in food is strictly banned. However, recent concerns have emerged about the illegal use of BY2 to improve the appearance of agricultural products, especially durian, a high-value export fruit in Southeast Asia.[2]

In China, the largest importer of durian, national food safety regulations explicitly prohibit the use of BY2 in all food products. This prohibition is reaffirmed in GB 2760-2024 National Food Safety Standard: Standards for the Use of Food Additives, which allows only substances listed in its approved additive catalog. Because BY2 is omitted from this catalog, it is classified as strictly banned.[3] Enforcement efforts have also intensified, with the National Health Commission (NHC) and the General Administration of Customs of China (GACC) conducting routine border inspections and surveillance to ensure full compliance.[4] Therefore, developing rapid, sensitive, and reliable methods to detect BY2 is essential for protecting consumers and upholding trade standards.

Testing for basic yellow 2 (BY2) in durian is difficult because the complex matrix of the fruit. This matrix contains high levels of lipids, sugars, and sulfur-containing volatiles in the pulp, along with lignin, cellulose, phenolic acids, glycosidic phenolics, flavonoids, coumarins, triterpenes, and polysaccharides in the husk. These components can cause significant matrix interference, reduce extraction efficiency, and lead to ion suppression during analysis. Additionally, the dye tends to bind to natural pigments and organic matter, complicating cleanup procedures and potentially affecting quantification accuracy. Therefore, food safety labs need analytical methods that provide high sensitivity, selectivity, robustness, and throughput that is suitable for regulatory monitoring.

Liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) is the preferred method for BY2 analysis because it provides excellent specificity, low detection limits, and efficient matrix effect reduction.[5]

This application note demonstrates the analytical performance of the Agilent 6495D LC/TQ in detecting BY2 in durian husk and pulp based on the China BJ 202204 method. The application illustrates how the Agilent method uses optimized sample preparation, chromatographic separation, and mass spectrometry setup and can deliver quick, sensitive, and reliable detection, helping labs comply with the current Chinese standard for Auramine O.

Experimental and chemicals

Standards and chemicals

Auramine O analytical standard (solid form) was obtained from LGC (product code DRE-C16971353). The standard materials were stored at –18 °C in a freezer, as recommended by the manufacturer.

All chemicals used in this study were of analytical grade or higher. Anhydrous sodium sulfate (Na_2SO_4, Merck, 1066491000) was used for moisture removal. Sample cleanup involved a dSPE mixture containing PSA (50 mg), C18 (50 mg), and $MgSO_4$ (150 mg) in a 2 mL vial (Agilent part number 5982-5122). Formic acid (99%, Merck, 8222541001) and HPLC gradient-grade acetonitrile (Merck, 1.00030.4000) were used for sample extraction. For mobile phases, acetonitrile (Merck, 1000294000), ammonium acetate (Merck, 5330040050), formic acid (Merck, 5330020050), and ultrapure water (Merck, 1.15333.2500) at LC-MS grade were used.

Instruments

Chromatographic separation was performed on an Agilent 1290 Infinity III LC system equipped with a built-in degasser, temperature-controlled autosampler, and thermostatted column compartment. An Agilent ZORBAX RRHD Eclipse XDB-C18 column (2.1 × 50 mm, 1.8 µm) with a matching UHPLC guard column was used for separation. The mobile phases consisted of water with 5 mM ammonium acetate and 0.1% formic acid (A) and acetonitrile (B), with a flow rate of 0.3 mL/min and a 2 µL injection volume, under the gradient conditions shown in Table 1.

A 6495D LC/TQ system equipped with an Agilent Jet Stream (AJS) electrospray ion source was operated in positive ionization and dynamic multiple reaction monitoring (MRM) mode. Source conditions and MRM parameters are summarized in Table 1. All data acquisition and processing were performed using Agilent MassHunter software version 12.

Table 1. Agilent 6495D LC/TQ parameters.

Parameter	Value
LC	
HPLC	Agilent 1290 Infinity III LC with built-in degasser; autosampler with temperature control; thermostatted column compartment
Column	ZORBAX RRHD Eclipse XDB-C18; 80Å; 2.1 × 50 mm, 1.8 µm (p/n 981757-902)
Guard Column	ZORBAX Eclipse XDB-C18; 80Å; 2.1 mm; 1.8 µm UHPLC guard (p/n 821725-903)
Column Temperature	35 °C
Mobile Phase	A) H_2O contains 5 mM ammonium acetate and 0.1% formic acid (v/v) B) Acetonitrile
Flow Rate	0.3 mL/min
Injection Volume	2 µL
Gradient Elution Profile	Time (min) %A %B 0.0 80 20 3.0 10 90 4.0 10 90 Post time: 1.5 min
ESI MS/MS Conditions	
MS	6495D TQ with Agilent JetStream ESI (AJS)
Polarity	Positive ionization
Drying Gas Temperature	200 °C
Drying Gas Flow Rate	13 L/min
Nebulizer Gas Pressure	33 psi
Sheath Gas Temperature	350 °C
Sheath Gas Flow Rate	12 L/min
Capillary Voltage	3,000 V
Mode	Dynamic MRM MRM 1: 268 → 252 (CE: 36 V) MRM 2: 268 → 147 (CE 30 V)

Standard solution and matrix-matched calibration preparation

The 1,000 mg/L Auramine O stock solution was prepared from solid Auramine O (product code DRE-C16971353) using acetonitrile as the solvent. Intermediate standard solutions of 10 mg/L and 1 mg/L were subsequently prepared from the stock solution. All standard solutions were stored in a deep freezer (–18 °C) and were used within six months. Working standard solutions in acetonitrile at concentrations of 0.5, 1, 2, 5, 10, and 50 µg/L were prepared from the intermediate standards. The matrix-matched calibration solutions, with concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, and 5.0 µg/L, were made ready by a tenfold dilution of the corresponding working standards in blank durian extract.

Sample preparation procedure

Figure 1 shows the sample preparation process for analyzing Auramine O in durian (including pulp and husk), involving a two-step extraction and cleanup, described in more detail.

Step 1: Extraction

- Weigh 1 gram of homogenized sample (durian pulp or husk) into a 15 mL Falcon tube. Add 5 mL of acetonitrile (contains 0.5% formic acid, HPLC grade), and then 2 grams of anhydrous sodium sulfate.
- Vortex for 1 minute followed by ultrasound for 10 minutes (repeat this step 2 times)
- Centrifuge was used to separate the sample and the ACN phase

Step 2: Cleanup

- Draw 1 mL of supernatant of the sample and add it to a 2 mL dSPE tube containing material cleanup.
- Vortex for 30 seconds and centrifuge for 2 minutes.
- Filter using a 0.22 µm nylon syringe membrane.
- Transfer to a 2 mL vial and analyze by Agilent 6495D LC/TQ.

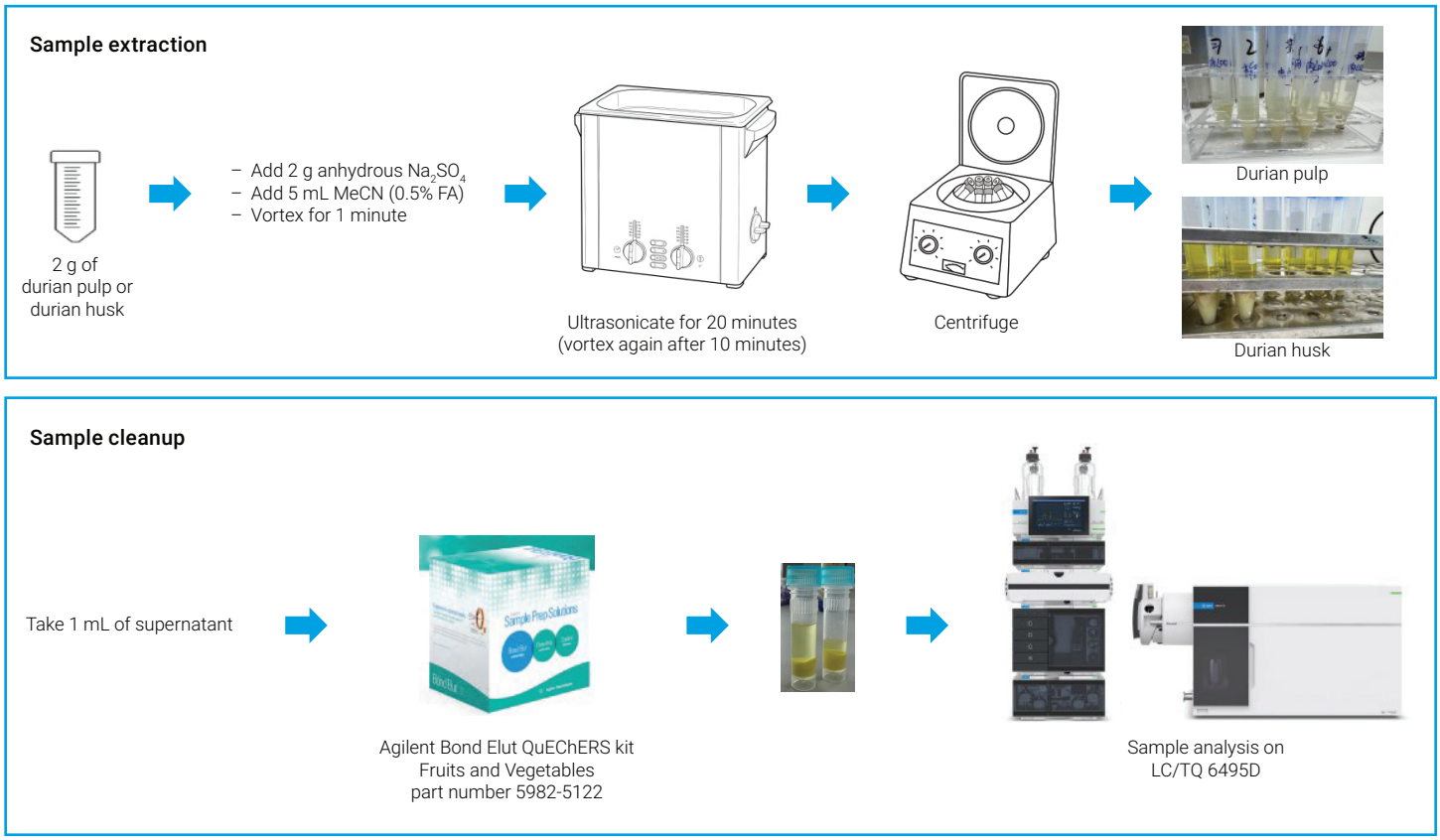


Figure 1. Sample preparation flowchart including extraction, followed by Agilent dSPE cleanup.

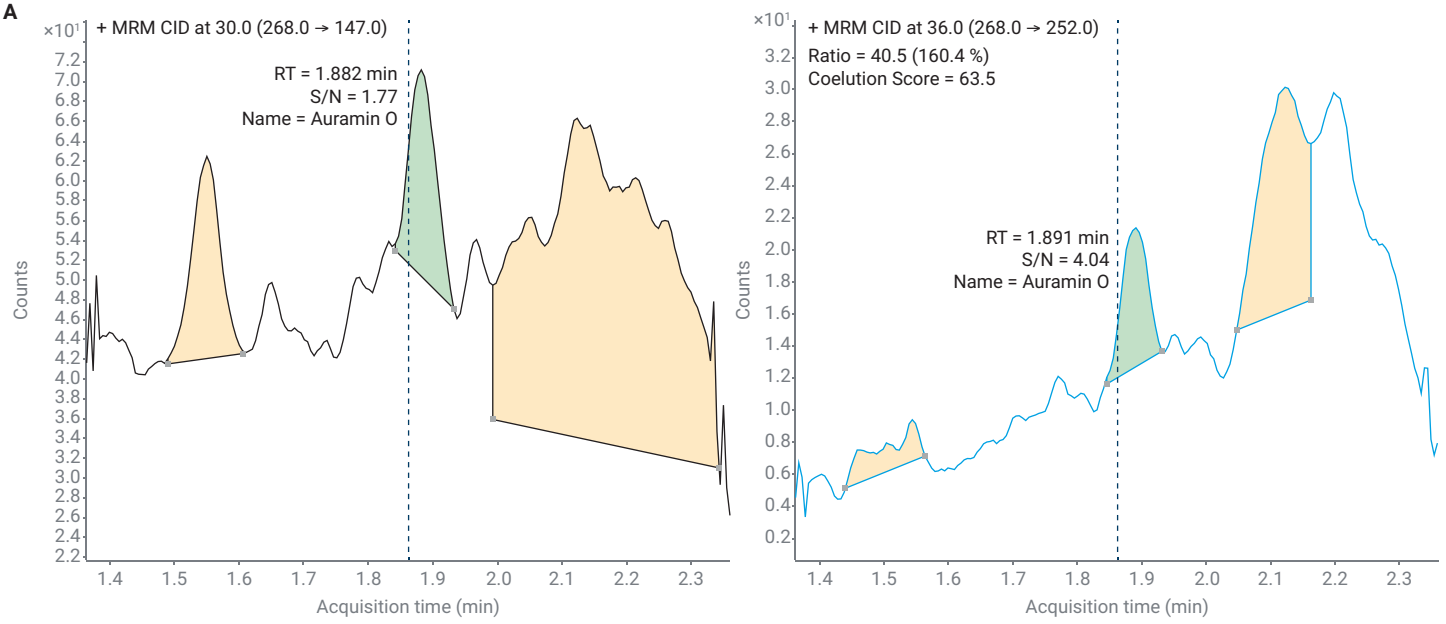
Results and discussion

Selectivity and sensitivity

To evaluate the sensitivity and selectivity of the 6495D LC/TQ system for the analysis of Auramine O, five replicate injections were performed at 2.5 ng/L in the durian husk extract matrix, the results are shown in Figure 2. The feature observed in the blank is likely an interference, as the coelution between the quantifier and qualifier transitions is only 63.5% (Figure 2A), which does not meet typical chromatographic confirmation criteria. Moreover, the ion ratio between the qualifier and quantifier transitions falls outside the accepted tolerance range for Auramine O, further indicating that the signal does not correspond to the authentic analyte. The magnitude of the interference is also negligible, with a response of only ~ 60. This is substantially lower than the response of Auramine O at 2.5 ng/L (~ 330), demonstrating that the interference does not meaningfully contribute to the analyte signal.

For the chromatogram spike of 2.5 ng/L, a well resolved Auramine O peak with a signal-to-noise ratio greater than 3 (ASTM algorithm) demonstrates reliable detection at trace levels even in a highly complex matrix. No matrix-derived interferences were observed at the analyte retention time, confirming the strong selectivity offered by the optimized MRM transitions. In addition, the peak-area responses from the five replicate injections exhibited RSD < 2%, illustrating excellent repeatability and instrumental stability. Such low variability at ppt levels provides compelling evidence of the 6495D LC/TQ robustness and high sensitivity performance.

A



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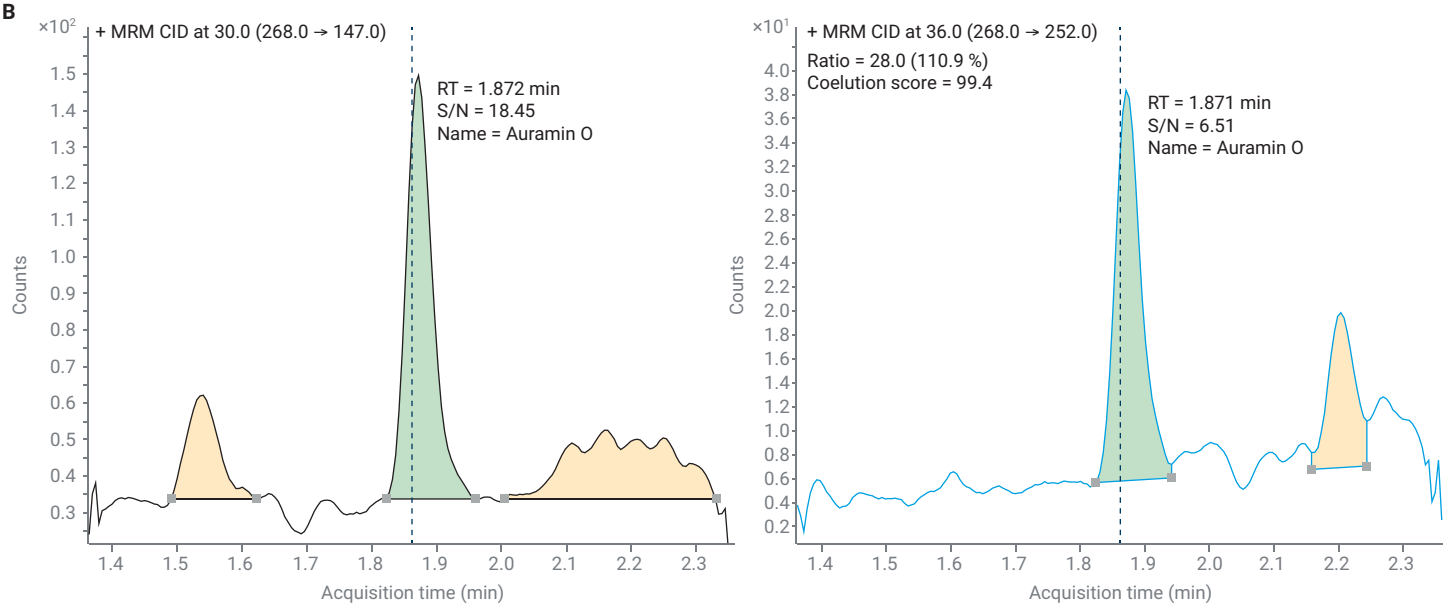


C

Sample			Auramin...	Auramin O Results		Qualifier (268.0 -> 252.0) Results
Data File	Type	Level	Exp. Conc.	RT	Resp.	Ratio
blank matrix-r004.d	Sample			1.882	60	40.5
STD 0.0025ppb-r001.d	Sample			1.871	324	28.4
STD 0.0025ppb-r002.d	Sample			1.866	337	27.0
STD 0.0025ppb-r003.d	Sample			1.872	329	28.0
STD 0.0025ppb-r004.d	Sample			1.872	332	27.8
STD 0.0025ppb-r005.d	Sample			1.866	335	26.2

Figure 2. Chromatogram of (A) blank durian husk matrix solution, (B) post-spike Auramine O at 2.5 ng/L in durian husk matrix solution, and (C) response of five replicates of Auramine O.

Linearity and matrix effect

Calibration curves were built for both matrices (durian pulp and husk) using matrix-matched standards ranging from 0.05 to 5 µg/L with six calibration points. Linearity was evaluated using linear regression with a 1/x weighting factor and without forcing the origin. The obtained calibration functions showed excellent linearity for Auramine O, with $R^2 > 0.999$ in both matrices (as shown in Figure 3).

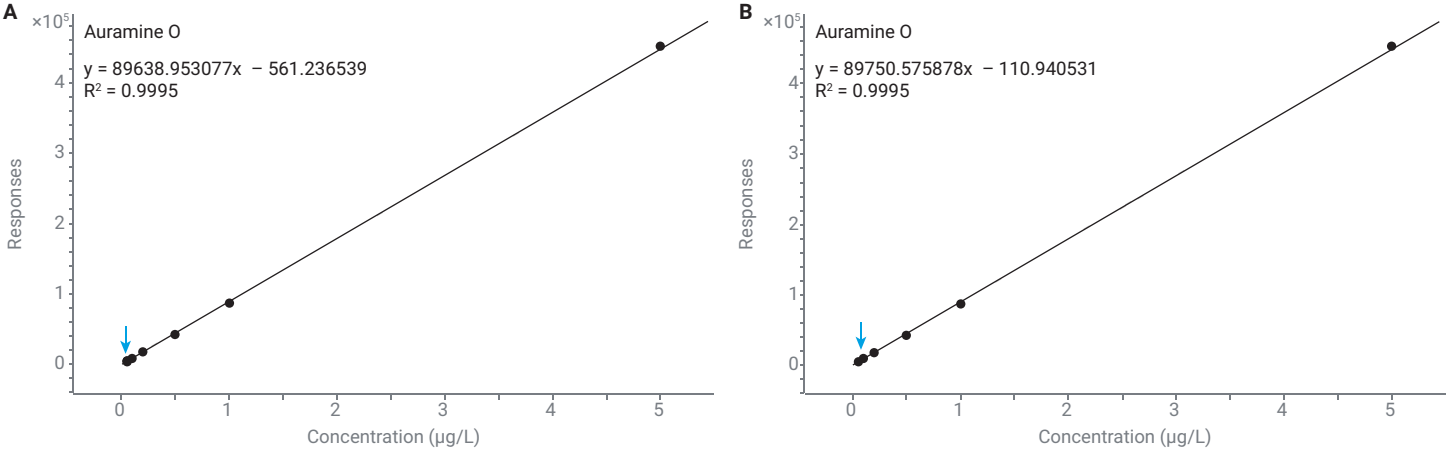
In addition, potential differences in matrix effect between pulp and husk were evaluated using analysis of covariance (ANCOVA), matrix effect (ME) calculations, and quality control (QC) samples. The comparison of calibration slopes using ANCOVA, based on the regression model $y = \beta_0 + \beta_1 x + \beta_2 M + \beta_3 (x \times M)$, showed that the slope interaction term β_3 was not significant ($p > 0.05$, 95% confidence level), indicating no matrix-dependent change in response.

The ME was further calculated using the slope-ratio formula, and the slope coefficients for the pulp and husk matrices are shown in Figure 4, with corresponding values of 89638.95 and 89750.57, respectively.

Equasion 1.

$$ME\ (\%) = \left(\frac{\text{Slope (pulp)}}{\text{Slope (husk)}} - 1\right) \times 100$$

The ME value is −0.12%, which is well within the ±20% acceptance limits.[6,7] QC samples in both matrices also demonstrated acceptable accuracy (> 95%) and precision (Figure 3C), confirming that the husk-based calibration curve provides an analytical response equivalent to that of the pulp matrix and can be reliably used for quantification.



C

Sample				Auramin O Method	Auramin O Results				Qualifier (268.0 -> 252.0) Results
Data File	Type	Level	Sample Group	Exp. Conc.	RT	Resp.	Accuracy	Calc. Conc.	Ratio
PI std1 0.05.d	Cal	0.05	Pulp	0.0500	1.861	4733	107.9	0.0540	25.5
PI std2 0.1.d	Cal	0.1	Pulp	0.1000	1.861	8774	99.0	0.0990	25.4
PI std3 0.2.d	Cal	0.2	Pulp	0.2000	1.861	17749	99.5	0.1990	25.6
PI std4 0.5.d	Cal	0.5	Pulp	0.5000	1.861	42708	95.4	0.4771	25.6
PI std5 1.d	Cal	1	Pulp	1.0000	1.861	87092	97.2	0.9716	25.5
PI std6 5.d	Cal	5	Pulp	5.0000	1.861	453070	101.0	5.0493	25.3
ROU Std1 0.05-r003.d	QC	0.05	Husk	0.0500	1.856	4236	96.9	0.0484	25.9
ROU Std2 0.1.d	QC	0.1	Husk	0.1000	1.861	8441	95.3	0.0953	24.6
ROU Std3 0.2.d	QC	0.2	Husk	0.2000	1.861	17137	96.1	0.1922	25.3
ROU Std4 0.5.d	QC	0.5	Husk	0.5000	1.856	42551	95.1	0.4753	25.4
ROU Std5 1.d	QC	1	Husk	1.0000	1.861	87172	97.3	0.9725	25.4
ROU Std6 5.d	QC	5	Husk	5.0000	1.861	451638	100.7	5.0334	25.3

Figure 3. Matrix-matched calibration curve of Auramine O: (A) durian pulp, (B) durian husk, and (C) QC.

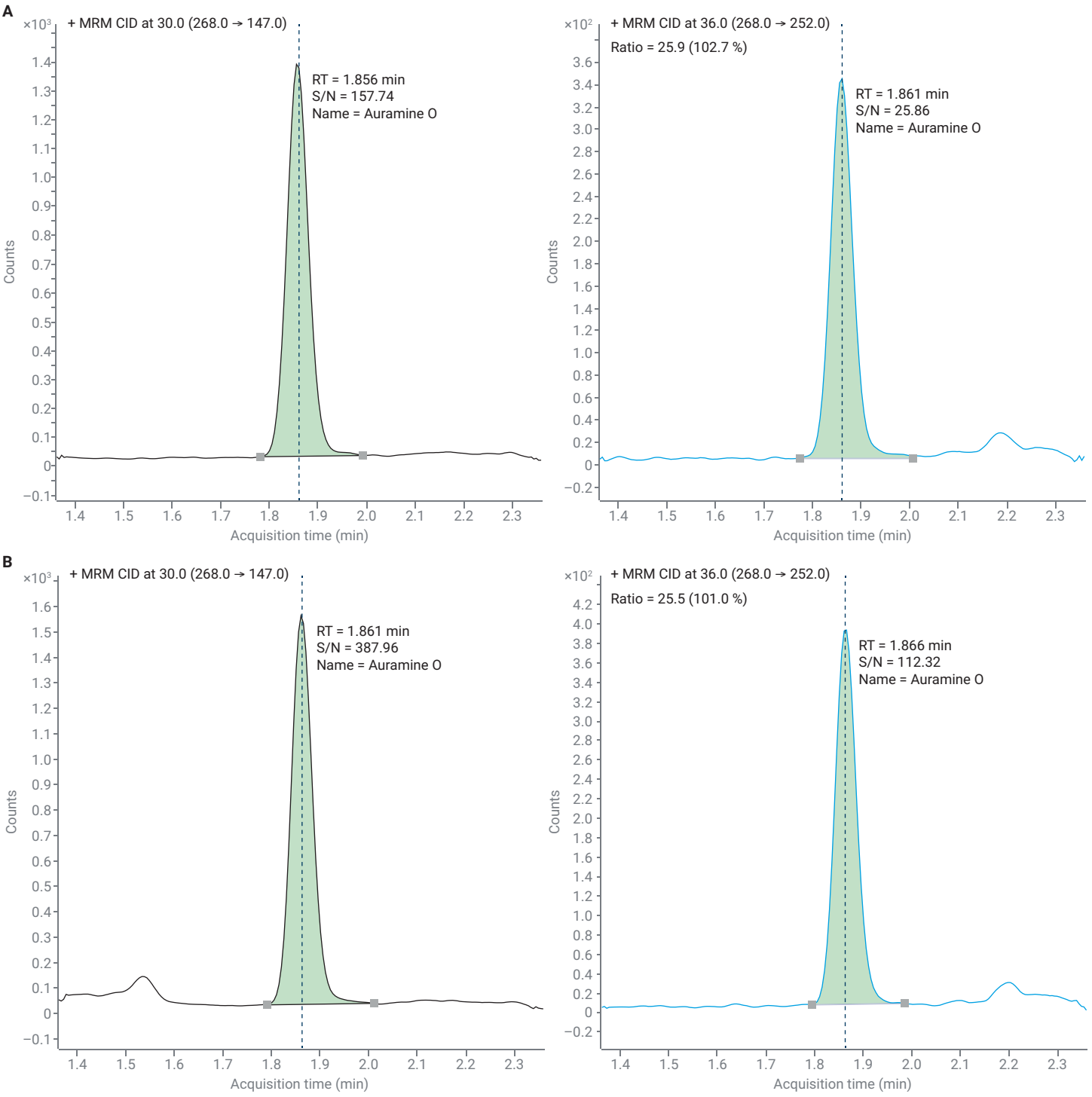


Figure 4. Chromatogram of Auramine O at 0.05 µg/L (lowest calibration point) in (A) durian pulp and (B) durian husk.

Method accuracy and precision

According to the Chinese method BJS 202204, the required limit of quantitation (LOQ) for the determination of Auramine O is 2 µg/kg. In this application note, the high sensitivity of the Agilent 6495D LC/TQ allowed us to assess method accuracy at substantially lower concentrations, specifically at 0.5 µg/kg and 2.5 µg/kg, in order to demonstrate robust analytical performance well below the regulatory LOQ threshold. Accordingly, the accuracy was evaluated by prespiking Auramine O at these two levels into both durian husk and pulp matrices, using 12 replicates in total (six per analyst). The distribution of recovery performance is presented in Figure 5 and Table A1. Overall, Auramine O recoveries were consistently above 70% across both matrices. In the durian husk matrix, recoveries ranged from 80.0 to 86.7% at 0.5 µg/kg and from 73.0 to 79.9% at 2.5 µg/kg, indicating a narrow distribution. In contrast, the durian pulp matrix exhibited a wider, yet still acceptable, recovery range (87.8 to 104.3% at 0.5 µg/kg and 79.6 to 93.6% at 2.5 µg/kg). The slightly lower recovery observed at 2.5 µg/kg compared with 0.5 µg/kg may be attributed to the high levels of lipids, cellulose, lignin, proteins, and polyphenols present in both durian pulp and husk. At low spiking levels, the small amount of Auramine O can be extracted efficiently. However, at higher concentrations, the dye interacts more strongly with matrix components, particularly lignin and cellulose, through π–π interactions, electrostatic attraction, and hydrogen bonding. As a cationic compound, Auramine O tends to be increasingly retained by hydroxyl-and phenolic-rich structures, leading to reduced extraction efficiency at elevated spiking levels. Although a slight decrease in recovery is observed at the higher concentration, the method still meets the accuracy requirements outlined in AOAC Appendix F, confirming its overall reliability for quantitative analysis.[6]

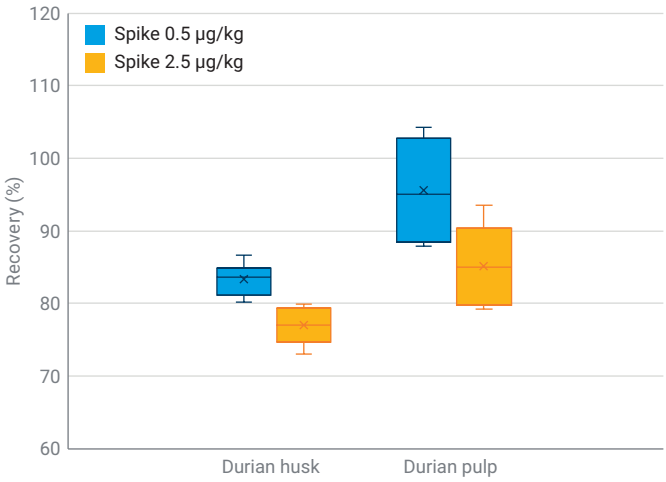


Figure 5. Recovery of Auramine O with prespike at 0.5 and 2.5 µg/kg in durian husk and pulp.

Repeatability, expressed as within-analyst relative standard deviation (RSD_r) across six replicates per analyst, remained low under all conditions, ranging from 0.90 to 1.72% in pulp and 0.94 to 1.33% in husk, well below the AOAC Appendix F performance limits.[6] Reproducibility (RSD_R), calculated across all 12 measurements from both analysts, also showed excellent interanalyst consistency, with values of 2.01 to 2.17% in husk and 4.53 to 6.14% in pulp.

Collectively, robust recoveries, low repeatability errors, and strong reproducibility confirm the reliability of this method for quantifying Auramine O at trace levels in complex durian matrices.

Conclusion

This study demonstrates the analytical performance of the Agilent 6495D triple quadrupole LC/MS system coupled with an Agilent 1290 Infinity III LC for the detection of Auramine O in durian matrices. The 6495D LC/TQ instrument showed excellent sensitivity, detecting at the ppt level, along with good repeatability, supported by 4th generation Agilent iFunnel technology, which optimizes compound-specific transitions for maximum sensitivity. Method performance was further validated through recovery efficiency, repeatability, and reproducibility, all of which met the AOAC Appendix F criteria. In addition, the Agilent 6400 LC/TQ series system provides fast, reliable analysis, making it ideal for high-throughput monitoring of Auramine O in durian matrices.

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Appendix

Table A1. Recovery of Auramine O.

Lab Technical	Recovery (%)			
	Durian Husk		Durian Pulp	
	Spike 0.5 µg/kg	Spike 2.5 µg/kg	Spike 0.5 µg/kg	Spike 2.5 µg/kg
1-1	80.98	74.29	102.73	90.37
1-2	84.01	74.00	102.68	93.60
1-3	82.79	73.09	101.02	87.73
1-4	81.09	76.04	103.84	90.18
1-5	81.40	75.79	99.93	86.27
1-6	80.23	76.68	104.29	91.32
2-1	83.96	77.43	90.12	79.64
2-2	84.87	79.45	88.07	79.18
2-3	86.66	78.98	88.20	80.05
2-4	85.36	79.57	89.29	79.21
2-5	84.74	78.75	87.84	81.18
2-6	83.39	79.91	89.14	83.75

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