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Technology Brief

Non-permitted Synthetic Dye — Food Safety

Highly-Sensitive and Rapid Analysis of Auramine O in Durian with the LCMS-8060NX

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Highlights

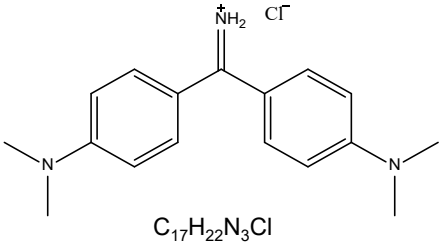
- Simple and effective LC-MS/MS solution for the accurate quantification of Auramine O in durian
- Highly sensitive detection of Auramine O at 5 pg/mL within 5 minutes

Technologies Featured

Shimadzu LCMS-8060NX

SHIMSEN QuEChERS Extraction Salt

Introduction



$C_{17}H_{22}N_3Cl$

Fig. 1. Chemical Structure and molecular formula of Auramine O

Auramine O (AO), also known as Basic Yellow 2, is a synthetic dye with a wide range of applications. However, it is classified as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC) [1], therefore raising significant concerns about its impact on human health.

As a result, regulatory bodies such as the US FDA, the European Commission, and China's GB Standards have restricted its use in food products.

Despite that, presence of AO has been detected in imported durians, causing authorities to implement stricter measures to ensure food safety and public health.

Therefore, there is an urgent need for reliable and sensitive analytical methods to monitor AO levels in food products, especially as consumer awareness of food safety rises.

Advanced analytical techniques like liquid chromatography-tandem mass spectrometry (LC-MS/MS) offer the sensitivity and specificity needed to detect trace levels of AO, ensuring that contaminated products are swiftly identified and removed from the market. This underscores the vital role of analytical techniques in protecting public health and ensuring the integrity of food supply chains.

Comparing to Tech Brief EC-2501, which used LC-PDA to quantitate AO, we present an optimized LC-MS/MS method that is 1000x more sensitive for detecting and quantifying AO in durian samples. This method utilizes the enhanced sensitivity and specificity of the Shimadzu LCMS-8060NX, making it an invaluable tool for regulatory compliance and food safety monitoring. This brief demonstrates how this method effectively addresses the challenges of detecting low levels of AO in complex food matrices.

Table 1. LC parameters for AO analysis

Column	Shim-pack Velox SP-C18, 1.8 µm, 2.1 x 100 mm (P/N: 227-32001-03)
Mobile Phases	A: Ammonium Acetate + Formic Acid in Water B: Acetonitrile
Flow Rate	0.4 mL/min
Elution Mode	Gradient elution, 5 minutes
Oven Temp.	40 ˚C
Injection Vol.	5 µL

Table 2. MRM Transitions for AO

Quantifier ion (m/z)	268.1 > 147.2 (+)
Reference ion (m/z)	268.1 > 252.15 (+)

Table 3. LCMS parameters for AO analysis

Interface	IonFocus™ ESI
Acquisition Mode	MRM, Positive Mode
Heat Block Temp.	300 ˚C
DL Temp.	150 ˚C
Interface Temp.	200 ˚C
Nebulizing Gas Flow	3.0 L/min
Drying Gas Flow	3.0 L/min
Heating Gas Flow	15.0 L/min

Sample Preparation and Analysis Conditions

Reagents and Chemicals

Auramine O was obtained from Sigma-Aldrich. SHIMSEN QuEChERS extraction salt (4 g $MgSO_4$, 1 g NaCl, P/N: 380-00156) and SHIMSEN QuEChERS II (25 mg PSA, 25 mg C18, 150 mg $MgSO_4$, P/N: 380-00990-22) were obtained from Shimadzu. All organic solvents used are of LCMS grade.

Stock and Intermediate Solution Preparation

Auramine O standard was dissolved in a mixture of ethanol and acetonitrile (ACN) to obtain a stock solution of 1000 µg/mL. The prepared stock was stored at 4 ˚C. Amber vials were used for storage. AO stock is prepared fresh to prevent degradation and ensure accurate quantitation.

Intermediate stock solutions were prepared by diluting the stock solution in 100% LCMS grade ACN via serial dilution. These intermediate stocks were used for the preparation of calibration standards and for the recovery study.

Calibration Standards

Three different sets of calibration standards (neat, pre-spiked, and post-spiked) were prepared at concentrations of 5, 10, 50, 100, 500, 2500 and 5000 pg/mL.

Sample Preparation

The durian sample was homogenized, weighed and vortexed in water and ACN. The SHIMSEN QuEChERS extraction salt was added, and the mixture was vortexed and centrifuged for separation. The supernatant was then transferred into the SHIMSEN QuEChERS II dSPE tube, vortexed and centrifuged. Finally, the extract was filtered with a 0.22 µm PTFE filter.

Instrumentation and Analytical conditions

The analysis was performed using the Shimadzu Nexera™ XS system coupled to a LCMS-8060NX The LCMS analytical conditions are described in Tables 1, 2 and 3 respectively.

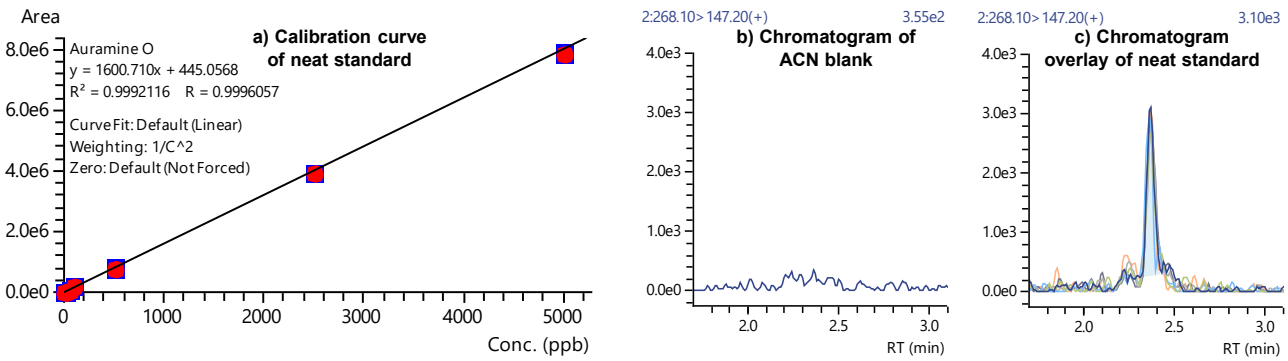


Fig. 2. (a) Calibration curve, (b) blank chromatogram and (c) chromatogram overlay of neat standard at 5 pg/mL

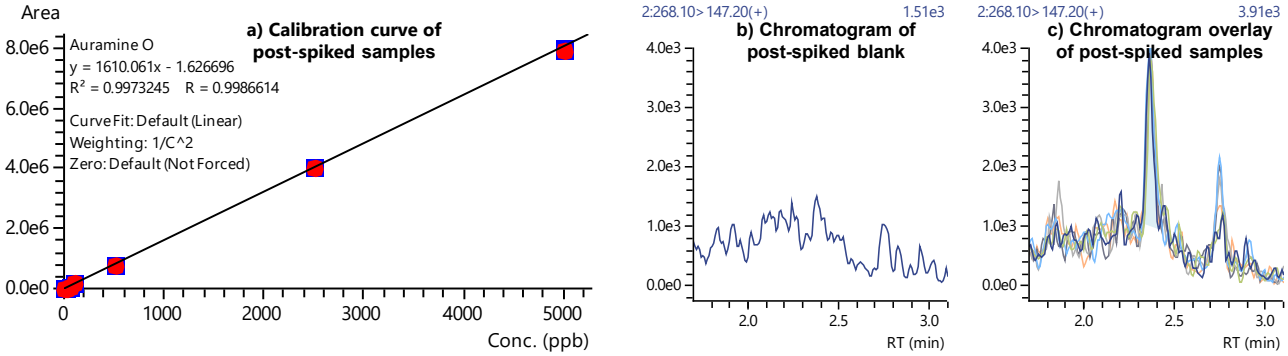


Fig. 3. (a) Calibration curve, (b) post-spike blank chromatogram and (c) chromatogram overlay of post-spiked samples at 5 pg/mL

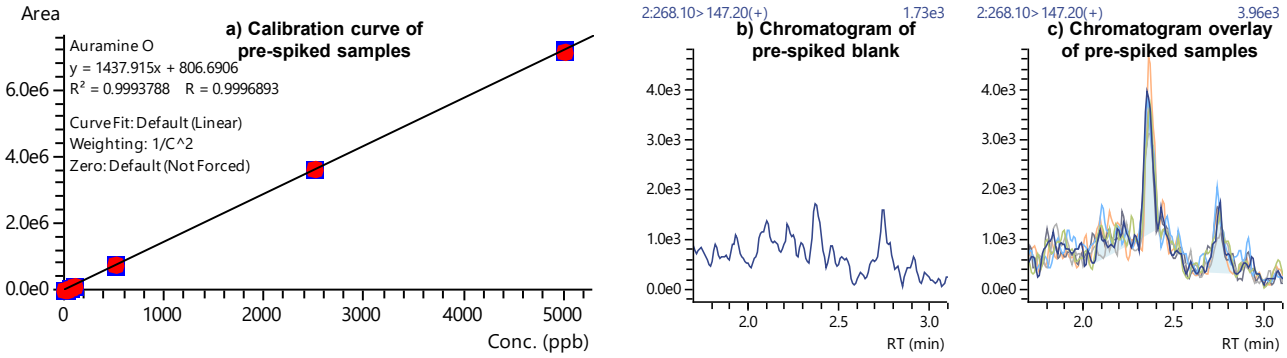


Fig. 4. (a) Calibration curve, (b) pre-spike blank chromatogram and (c) chromatogram overlay of pre-spiked samples at 5 pg/mL

Results & Discussion

Calibration and Linearity

Calibration curves were established for neat standards as well as for matrix-matched samples, including pre-spiked and post-spiked configurations, across seven calibration levels ranging from 5 to 5000 pg/mL, as illustrated in Fig. 2a, 3a and 4a. All three calibration curves exhibited excellent linearity, achieving R^2 values greater than 0.997.

Sensitivity and Specificity

The method's sensitivity was evaluated by calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ) from the signal-to-noise (S/N) ratios of 5 pg/mL injections from each calibration set. Based on pre-spiked samples, the calculated LOD was 2.13 pg/mL and the LOQ was 6.45 pg/mL as shown in Table 4, demonstrating the method's capability to detect trace concentrations of AO.

Reference ion ratio is set at 30.92% (peak area) and it is confirmed to be within ±30% relative across all calibration curves.

Table 4. S/N, LOD and LOQ of each calibration set at 5 pg/mL

Sensitivity	Neat	Post-spiked	Pre-spiked
S/N	37.79	9.58	8.04
LOD *	0.44	1.83	2.13
LOQ *	1.35	5.54	6.45

* Values are estimates calculated from 5 pg/mL

Table 5. Peak area %RSD comparison

% RSD (n=6)	Neat	Post-spiked	Pre-spiked
Retention Time	0.157	0.172	0.225
Peak Area	4.96	5.78	5.13

Table 6. %Accuracy comparison

Accuracy (%)	Neat	Post-spiked	Pre-spiked
5	101.50	108.99	102.23
10	96.16	90.65	95.68
50	102.98	101.15	99.33
100	102.57	104.47	99.50
500	100.07	100.08	101.98
2500	98.22	100.44	101.30
5000	98.50	98.87	99.98

The analysis confirmed that AO was not detected in all the ACN and sample blanks, as indicated in Fig. 2b, 3b and 4b.

Accuracy and Precision

Precision testing was conducted for all three calibration sets with six repeated injections at a concentration of 5 pg/mL; these chromatograms are shown in Fig. 2c, 3c and 4c.

The relative standard deviation (RSD) for retention time and peak area at 5 pg/mL were less than 0.25% and 6% respectively, as seen in Table 5, indicating robust precision in the measurement even at such a low concentration.

As depicted by Table 6, the accuracy results for all the calibration sets all fall within ±10% , adhering to the guidelines of ICH Q2(R2).

Recovery and Process Efficiency

Recovery studies were performed to evaluate the analytical performance of the method by comparing the results of the three calibration sets. The calculated values for matrix effect, sample preparation recovery and process efficiency are presented in Table 7.

The process efficiency results, all within ±15%, indicate that the method is both effective and robust, allowing any of the three sample configurations to be used for constructing the calibration curve.

Table 7. %Recovery at each concentration levels

Concentration (pg/mL)	Matrix Effect (%)	Sample Preparation Recovery (%)	Process Efficiency (%)
5	98	97	95
10	92	100	92
50	98	89	87
100	102	86	87
500	101	91	92
2500	103	90	93
5000	101	90	91

Conclusion

This technology brief presents a simple and effective solution for analysing AO using the Shimadzu LCMS-8060NX. The method demonstrates exceptional sensitivity, detecting trace concentrations as low as 5 pg/mL, along with excellent linearity, process efficiency, accuracy, and precision. This capability allows for the easy detection and removal of contaminated products from the market, ensuring regulatory compliance and safeguarding public food safety.

References

1) IARC. Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risks Hum. 2010;99:1–678

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SHIMSEN QuEChERS Extraction Salt

QuEChERS, which stands for "Quick, Easy, Cheap, Effective, Rugged, and Safe," is a sample preparation technique used in analytical chemistry. It has revolutionized the way samples are prepared for analysis, especially in the fields of food safety, environmental monitoring, and pesticide residue testing. QuEChERS is designed to simplify and streamline the extraction and purification of complex sample matrices for subsequent analysis.

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