An optimized QuEChERS approach for the simultaneous determination of multiple mycotoxins in processed pork

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Abstract. Mycotoxins are fungal metabolites that pose significant food safety concerns. This study developed and optimized a modified QuEChERS extraction method coupled with UHPLC–TCFLD for the simultaneous determination of multiple mycotoxins in processed pork. Extraction parameters, including solvent composition, extraction salts, shaking time, sonication duration, and clean-up procedures, were optimized to improve analytical performance. The method demonstrated acceptable recovery (60.88–96.77%), and good precision (RSD < 10%). Limits of detection ranged from 0.15 to 29.37 μg/kg. The inclusion of n-hexane washing effectively reduced lipid interference and enhanced analytical sensitivity.

1 Introduction

Mycotoxins are fungal-derived toxic metabolites that commonly occur in food and feed products and are recognized as important food safety hazards worldwide [1–3]. Their occurrence remains a major food safety concern because these compounds can persist during food processing and storage and may cause adverse effects in humans and animals [3,4]. Although mycotoxin contamination is commonly associated with cereals and other plant-derived commodities, contaminated feed may also contribute to the occurrence of mycotoxins in animal-derived food products [5]. Recent studies have highlighted the occurrence of mycotoxins in food and feed systems, including animal-derived products, and emphasized the need for improved monitoring and mitigation strategies [6]. Processed pork products are challenging analytical matrices due to their high lipid and protein content, which may interfere with extraction efficiency and chromatographic analysis [7]. Consequently, reliable analytical methods are required for accurate mycotoxin determination in such products. Recent advances in multi-mycotoxin analysis have improved analytical sensitivity and selectivity; however, many reported methods rely on LC–MS/MS instrumentation, which may not be readily accessible for routine monitoring because of its high acquisition and

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operational costs [8,9]. Furthermore, recent reviews have emphasized the ongoing development of analytical methods for identifying various mycotoxins in intricate food matrices [10]. Fluorescence detection provides a practical and cost-effective alternative for naturally fluorescent mycotoxins while maintaining satisfactory analytical performance [8]. To address these challenges, a modified QuEChERS extraction procedure integrated with UHPLC–TCFLD was optimized for the simultaneous detection of eight mycotoxins in processed pork, and its effectiveness for routine food safety surveillance was assessed.

2 Materials and Methods

2.1 Sample Collection and Preparation

Pork samples were purchased from local markets in Taipei City, Taiwan, homogenized, and stored until analysis. Stock solutions of AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, OTA, ZEN, and CIT were prepared in acetonitrile (1 mg/mL) and stored at −20 °C. Working standards and matrix-matched calibration solutions were prepared by serial dilution.

2.2 Preparation of Standard Solution

A modified QuEChERS method was employed for sample preparation. Briefly, 2 g of the homogenized pork sample were extracted using 80% acetonitrile containing 1% acetic acid and partitioned with $MgSO_4$ and sodium acetate. Samples were shaken for 5 min, sonicated for 40 min, and centrifuged. The supernatant was washed with n-hexane to remove lipids, dried under nitrogen, reconstituted in 50% methanol, filtered through a 0.20 μm membrane filter, and transferred to UHPLC vials for analysis.

2.3 UHPLC–TCFLD Analysis and Method Validation

Chromatographic analysis was conducted utilizing an Ultra-High-Performance Liquid Chromatography system integrated with a temperature-regulated fluorescence detector (UHPLC–TCFLD) from Shimadzu Corporation, Kyoto, Japan. Separation was accomplished utilizing a Phenomenex Kinetex PS C18 column (150 × 4.6 mm, 2.6 μm) fitted with a guard cartridge of the identical stationary phase and held at a temperature of 40 °C. The mobile phase consisted of (A) deionized water containing 2% formic acid and (B) acetonitrile/methanol containing 2% formic acid. Gradient elution was performed at a flow rate of 1.0 mL min^{-1} with an injection volume of 10 μL and a total run time of 31 min. Fluorescence detection was performed using wavelength-programming. From 0 to 13 min, excitation and emission wavelengths were set at 360 and 440 nm, respectively, for AFM_1, AFG_2, AFG_1, AFB_2, and AFB_1, while excitation and emission wavelengths of 333 and 470 nm were used from 13 to 20 min for ZEN, OTA, and CIT. Method validation was performed by evaluating linearity, recovery, precision, limits of detection (LOD), and limits of quantification (LOQ). Matrix-matched calibration curves were prepared using multiple concentration levels of a mixed mycotoxin standard and evaluated by linear regression. Recovery experiments were conducted in triplicate using spiked blank matrices. Signal-to-noise ratios of three and ten, respectively, were adopted to calculate LOD and LOQ. Each experiment was carried out in groups of three, and the results were reported as mean ±standard error.

3 Results and Discussion

The developed UHPLC–TCFLD method enabled the simultaneous determination of eight mycotoxins in processed pork matrices. Under the optimized chromatographic conditions, all target analytes were successfully separated within approximately 31 min with well-defined and symmetrical peaks (Fig. 1), illustrating the method's applicability for multi-residue analysis in complex lipid-rich food matrices.

Optimization experiments were performed to evaluate the effects of extraction solvent composition, extraction salts, shaking time, sonication duration, reconstitution solvent, and n-hexane clean-up on mycotoxin recovery. Higher recoveries were obtained using 80% acetonitrile containing 1% acetic acid compared with the other solvent systems evaluated, while the combination of $MgSO_4$ and sodium acetate provided efficient phase separation and extraction performance. A shaking time of 5 min and sonication duration of 40 min improved analyte extraction without increasing matrix interference. Reconstitution with 50% methanol provided satisfactory analyte solubility, whereas the inclusion of an n-hexane washing step effectively reduced co-extracted lipids and improved chromatographic response. These optimized conditions were subsequently selected for method validation and sample analysis.

Method validation results are presented in Table 1. The recovery values varied from 60.88% to 96.77%, with relative standard deviations staying below 10%, demonstrating acceptable accuracy and precision. The limits of detection and quantification varied from 0.15–29.37 µg/kg and 0.45–89.00 µg/kg, respectively, demonstrating adequate sensitivity for trace-level mycotoxin determination. Matrix-matched calibration curves exhibited excellent linearity (R^2 = 0.9901–0.9997).

The analytical performance obtained in this study was comparable to previously reported QuEChERS-based multi-mycotoxin methods, with recovery, precision, and sensitivity values falling within commonly accepted ranges for food analysis. [9–11]. Although LC–MS/MS remains the preferred platform for comprehensive multi-mycotoxin screening [9,12], the developed UHPLC–TCFLD method offers a practical and cost-effective alternative for routine monitoring of naturally fluorescent mycotoxins in processed pork products.

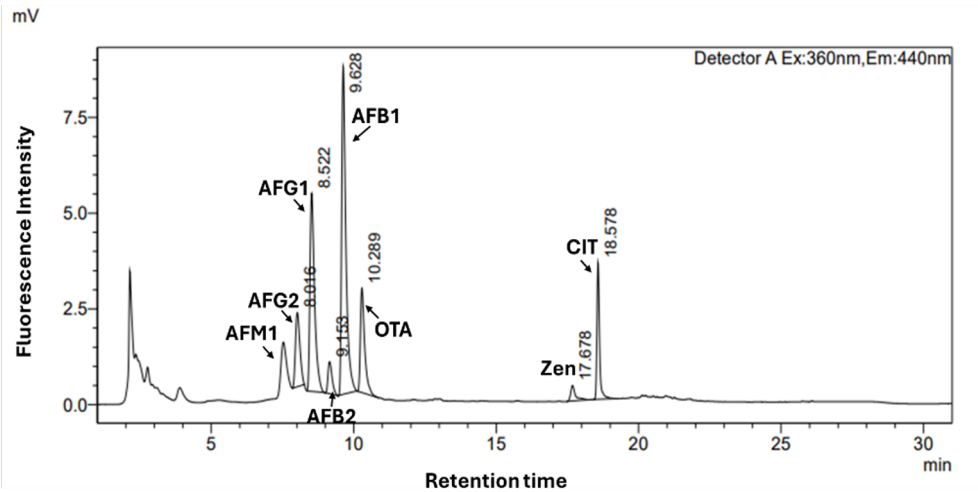

Fig. 1. Chromatogram of eight target mycotoxins obtained using UHPLC–TCFLD.

Table 1. Method validation results for eight mycotoxins

Mycotoxin	Recovery (%)	RSD (%)	LOD (µg/kg)	LOQ (µg/kg)
AFM_1	83.82	2.06	1.19	3.60
AFG_2	60.88	2.05	0.17	0.51
AFG_1	96.77	4.00	6.83	20.70
AFB_2	61.64	9.64	0.15	0.45
AFB_1	70.87	8.47	3.10	6.41
OTA	61.62	6.00	0.34	1.04
ZEN	69.87	3.52	29.37	89.00
CIT	88.64	1.46	8.65	26.21

Notes: Validation experiments were performed in triplicate (n = 3)

4 Conclusion

A QuEChERS–UHPLC–TCFLD method was successfully developed and optimized for the simultaneous determination of eight mycotoxins in processed pork matrices. The optimized extraction procedure, consisting of acidified acetonitrile extraction, $MgSO_4$/sodium acetate partitioning, n-hexane clean-up, and methanol reconstitution, provided satisfactory analytical performance. The method demonstrated good linearity ($R^2 > 0.99$), acceptable recovery (60.88–96.77%), satisfactory precision (RSD < 10%), and adequate sensitivity for trace-level mycotoxin determination. Compared with more sophisticated LC–MS/MS-based approaches, the proposed method offers a simpler and more cost-effective alternative while maintaining acceptable analytical performance for naturally fluorescent mycotoxins. These characteristics make the method suitable for routine food safety monitoring and quality control applications, especially in labs that have restricted access to sophisticated mass spectrometry equipment.

A limitation of the present study is that only eight target mycotoxins were evaluated. Future studies should investigate the applicability of the method to a wider range of animal-derived food products and explore the inclusion of additional mycotoxins and emerging metabolites. Nevertheless, the developed method provides a practical analytical tool for multi-mycotoxin monitoring and contributes to ongoing efforts to strengthen food safety surveillance in animal-derived products.

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