

Simultaneous Analysis of Mycotoxins in Dairy Products by Liquid Chromatography - Quadrupole Orbitrap Mass Spectrometry

Complete method: Jia, W. et al, Multi-Mycotoxin Analysis in Dairy Products by Liquid Chromatography Coupled to Quadrupole Orbitrap Mass Spectrometry, *J. Chrom. A.*, 2014, 1345, pp 107-114, DOI: 10.1016/j.chroma.2014.04.021

Highlights

- Validated method can simultaneously analyze 58 mycotoxins in dairy products at low concentration levels.
- Extraction recoveries ranged between 86.6 and 113.7%, with the coefficient of variation < 6.2%.
- Limits of detection were 0.001–0.92 µg/kg.
- Sample preparation is simple, robust, and inexpensive

Introduction

Mycotoxins are a diverse group of highly toxic secondary metabolic products from various fungal species. Their analysis can be challenging because the physicochemical properties of these compounds vary widely. Liquid chromatography (LC) coupled to tandem quadrupole mass spectrometry (MS) has been widely used for structural characterization, identification, and quantitative analysis of mycotoxins. However, this technique is not suitable for simultaneous screening of large numbers of mycotoxins. Instead, ultrahigh-performance LC coupled with hybrid quadrupole-Orbitrap MS can provide both detailed structural information and effective screening of multiple mycotoxins.

Experimental

Sample Preparation

Dairy samples included milk, milk beverages, and yogurt.

Mycotoxins were extracted using a slightly modified QuEChERS procedure. Extraction with acetonitrile/water containing 1% acetic acid, magnesium sulfate, and sodium acetate was followed by cleanup by dispersive solid-phase extraction using magnesium sulfate, primary-secondary amine, and C₁₈.

LC-MS Conditions

UHPLC analysis was performed using the Thermo Scientific™ Accela™ 1250 LC pump and an open autosampler with a Thermo Scientific™ Accucore™ aQ column (100 × 2.1 mm, 2.6 µm) connected to an Accucore aQ guard column (10 × 2.1 mm). Mobile phases were (A) water and (B) methanol, each containing 4 mM ammonium formate + 0.10% formic acid. The injection volume was 5 µL, and the flow rate was 300 µL/min. A Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer with a heated electrospray ionization probe was used for analysis. All quantitative data were acquired in full MS scan mode. If a targeted compound was present, its precursor ion scan triggered a data-dependent MS² (dd-MS²) scan.

Spray voltage:	3.5 kV (positive ionization), 3.0 kV (negative mode)
Sheath gas (N ₂):	18 arbitrary units
Aux gas (N ₂):	3 arbitrary units
Capillary temperature:	320 °C
Heater temperature:	350 °C
Normalized collision energy:	35%
Resolution:	70,000 (<i>m/z</i> 200, FWHM) 17,500 for dd-MS ²

Data

The multi-mycotoxin method allowed simultaneous analysis of the 58 mycotoxins with extraction recoveries between 86.6% and 113.7%. Matrix-matched calibration curves were used for quantification and were linear for all analytes between 0.001 and 100 µg/kg with R² values greater than 0.99. The detection limits ranged between 0.001 and 92 µg/kg, with coefficients of variation of less than 6.2%. The test and validation results for each analyte are given in Table 2.

2 Table 2. Analyte and method validation parameters.

Compound Name	RT (min)	Elemental Composition	Molecular Ion	Extraction Recoveries (%)	Dynamic Range ($\mu\text{g}/\text{kg}$)	CC α ($\mu\text{g}/\text{kg}$)	CC β ($\mu\text{g}/\text{kg}$)	RSD% Level 1	RSD% Level 2	RSD% Level 3
Moniliformin	0.75	C ₄ HNaO ₃	[M - Na] ⁻	89.3	0.02–5	0.01	0.02	4.9	4.8	5.3
Patulin	0.85	C ₇ H ₆ O ₄	[M + NH ₄] ⁺	90.2	1–100	0.49	0.82	5.9	5.1	5.5
Kojic acid	1.62	C ₆ H ₆ O ₄	[M + H] ⁺	95.8	0.1–10	0.04	0.08	3.4	2.8	5.6
T-2 tetraol	3.81	C ₁₅ H ₂₂ O ₆	[M + NH ₄] ⁺	93.2	0.005–1	0.003	0.005	3.0	5.6	3.1
Nivalenol	3.84	C ₁₅ H ₂₀ O ₇	[M + FAc] ⁻	91.5	1–100	0.07	0.12	1.6	3.4	3.9
Deoxynivalenol	4.32	C ₁₅ H ₂₀ O ₆	[M + H] ⁺	93.6	1–100	0.09	0.15	4.2	5.7	5.6
Deoxynivalenol 3-glucoside	4.37	C ₂₁ H ₃₀ O ₁₁	[M + FAc] ⁻	97.5	1–100	0.28	0.47	2.9	2.1	2.3
Fusarenol X	4.84	C ₁₇ H ₂₂ O ₆	[M + NH ₄] ⁺	97.9	1–100	0.06	0.11	4.6	5.9	4.8
Deepoxy-deoxynivalenol	4.89	C ₁₅ H ₂₀ O ₅	[M + H] ⁺	96.0	0.1–10	0.01	0.03	5.2	4.0	5.6
Penicillic acid	4.89	C ₈ H ₁₀ O ₄	[M + H] ⁺	98.2	0.02–5	0.006	0.01	4.9	3.2	4.4
Neosolaniol	5.00	C ₁₉ H ₂₆ O ₈	[M + NH ₄] ⁺	95.4	0.1–10	0.03	0.06	3.5	4.9	2.3
3-Acetyldeoxynivalenol	5.35	C ₁₇ H ₂₂ O ₇	[M + H] ⁺	97.0	1–100	0.07	0.12	5.9	4.1	5.7
15-Acetyldeoxynivalenol	5.35	C ₁₇ H ₂₂ O ₇	[M + NH ₄] ⁺	87.4	1–100	0.07	0.13	5.8	2.6	4.7
Agroclavine	5.47	C ₁₆ H ₁₈ N ₂	[M + H] ⁺	90.3	1–100	0.55	0.92	6.0	6.1	3.5
Ochratoxin- α	5.75	C ₁₁ H ₉ O ₅ Cl	[M - H] ⁻	95.2	0.002–0.5	0.0012	0.002	3.7	4.4	4.7
Aflatoxin M1	5.75	C ₁₇ H ₁₂ O ₇	[M + H] ⁺	92.3	0.001–0.5	0.0006	0.001	2.3	2.1	2.0
Aflatoxin G2	5.76	C ₁₇ H ₁₄ O ₇	[M + H] ⁺	90.3	0.01–5	0.004	0.007	2.9	4.9	3.9
15-Acetoxyiscripenol	5.78	C ₁₇ H ₂₄ O ₆	[M + NH ₄] ⁺	95.8	1–100	0.11	0.19	4.7	4.6	4.2
Aflatoxin G1	5.91	C ₁₇ H ₁₂ O ₇	[M + H] ⁺	87.3	0.01–5	0.003	0.005	6.1	4.4	6.4
Aflatoxin B2	6.08	C ₁₇ H ₁₄ O ₆	[M + H] ⁺	90.4	0.01–5	0.005	0.009	5.1	3.9	5.9
Gliotoxin	6.20	C ₁₃ H ₁₄ O ₄ N ₂ S ₂	[M + H] ⁺	95.0	0.1–10	0.03	0.06	2.2	4.4	3.9
Diacetoxyiscripenol	6.20	C ₁₉ H ₂₆ O ₇	[M + NH ₄] ⁺	106.6	0.1–10	0.01	0.03	5.7	4.6	4.1
Aflatoxin B1	6.25	C ₁₇ H ₁₂ O ₆	[M + H] ⁺	91.2	0.01–5	0.005	0.009	4.3	4.1	3.4
Ergosine	6.31	C ₃₀ H ₃₇ N ₅ O ₅	[M + H] ⁺	99.9	0.1–10	0.02	0.04	2.1	4.2	2.2
Wortmannin	6.31	C ₂₃ H ₂₄ O ₈	[M + NH ₄] ⁺	89.4	0.1–10	0.05	0.09	4.3	2.6	4.5
T-2 triol	6.33	C ₂₀ H ₃₀ O ₇	[M + NH ₄] ⁺	89.3	0.01–5	0.003	0.006	5.8	4.4	5.3
Citrinin	6.34	C ₁₃ H ₁₄ O ₅	[M - H] ⁻	103.2	0.1–10	0.04	0.08	5.4	2.3	6.1
Meleagrins	6.41	C ₂₃ H ₂₃ N ₅ O ₄	[M - H] ⁻	95.6	1–100	0.35	0.59	3.1	5.6	2.3
Ergocornine	6.65	C ₃₁ H ₃₉ N ₅ O ₅	[M - H] ⁻	92.3	0.1–10	0.01	0.03	2.2	1.3	5.3
HT-2 toxin	6.69	C ₂₂ H ₃₂ O ₆	[M + NH ₄] ⁺	104.2	0.1–10	0.05	0.09	4.9	4.4	6.0
Dihydroergocristine	6.75	C ₃₅ H ₄₁ N ₅ O ₅	[M + H] ⁺	87.5	1–100	0.28	0.47	4.6	3.4	5.8
Ergocryptinine	6.82	C ₃₂ H ₄₁ N ₅ O ₅	[M + H] ⁺	113.7	0.1–10	0.03	0.05	2.0	4.5	5.5
α -Zearalanol	6.85	C ₁₈ H ₂₆ O ₅	[M + H] ⁺	98.2	1–100	0.07	0.12	2.3	3.4	6.0

Table 2. Analyte and method validation parameters (continued).

Compound Name	RT (min)	Elemental Composition	Molecular Ion	Extraction Recoveries (%)	Dynamic Range (µg/kg)	CC _α (µg/kg)	CC _β (µg/kg)	RSD% Level 1	RSD% Level 2	RSD% Level 3
Tentoxin	6.85	C ₂₂ H ₃₀ O ₄ N ₄	[M - H] ⁻	95.7	1–100	0.19	0.32	4.1	5.1	4.4
Ochratoxin B	6.89	C ₂₀ H ₁₉ NO ₆	[M + H] ⁺	98.5	0.01–5	0.06	0.01	4.6	4.9	2.2
Ergocristine	6.89	C ₃₆ H ₃₉ N ₅ O ₅	[M + H] ⁺	94.3	0.1–10	0.02	0.04	2.7	3.5	5.7
Roquefortine C	6.90	C ₂₂ H ₂₃ N ₅ O ₂	[M + H] ⁺	90.2	1–100	0.33	0.56	5.9	4.2	6.1
Mycophenolic acid	6.92	C ₁₇ H ₂₀ O ₆	[M - H] ⁻	99.2	0.1–10	0.03	0.06	4.7	1.9	5.3
Ergocristinine	6.93	C ₃₅ H ₃₉ N ₅ O ₅	[M + H] ⁺	107.7	0.1–10	0.03	0.06	5.9	6.1	4.9
Fumonisin B1	6.96	C ₃₄ H ₅₉ NO ₁₅	[M + H] ⁺	86.6	0.1–10	0.04	0.08	3.4	5.4	1.5
α-Zearalenol	7.01	C ₁₈ H ₂₄ O ₅	[M + H] ⁺	102.4	1–100	0.25	0.42	2.3	5.0	1.6
Alternariol	7.01	C ₁₄ H ₁₀ O ₅	[M - H] ⁻	95.2	0.1–10	0.01	0.03	2.8	4.4	3.4
Trichothecenes	7.01	C ₂₄ H ₃₄ O ₉	[M + NH ₄] ⁺	89.0	1–100	0.52	0.88	2.3	4.3	2.2
α-Zearalanol	7.18	C ₁₈ H ₂₆ O ₅	[M + H] ⁺	101.5	1–100	0.18	0.31	1.4	5.7	1.8
Fumonisin B3	7.21	C ₃₄ H ₅₉ NO ₁₄	[M + H] ⁺	90.3	1–100	0.32	0.54	3.6	2.0	3.6
α-Zearalenol	7.27	C ₁₈ H ₂₄ O ₅	[M + H] ⁺	99.7	1–100	0.19	0.32	5.0	3.8	5.0
Ochratoxin A	7.28	C ₂₀ H ₁₈ NO ₆ Cl	[M + H] ⁺	90.6	0.01–5	0.03	0.009	3.9	3.4	3.5
Citreoviridin	7.32	C ₂₃ H ₃₀ O ₆	[M + H] ⁺	95.2	1–100	0.09	0.16	4.2	6.2	3.4
Zearalanone	7.34	C ₁₈ H ₂₄ O ₅	[M + H] ⁺	95.1	1–100	0.13	0.22	2.9	3.7	4.8
Zearalenone	7.37	C ₁₈ H ₂₂ O ₅	[M + H] ⁺	91.0	0.1–10	0.04	0.08	4.8	4.1	2.9
Fumonisin B2	7.44	C ₃₄ H ₅₉ NO ₁₄	[M + H] ⁺	99.8	0.1–10	0.05	0.09	2.6	5.8	1.0
Streigmatocystin	7.58	C ₁₈ H ₁₂ O ₆	[M + H] ⁺	95.2	0.02–5	0.01	0.02	3.5	5.4	6.2
Verruculogen	7.60	C ₂₇ H ₃₃ O ₇ N ₃	[M + H] ⁺	91.3	0.01–5	0.004	0.008	4.9	3.7	5.9
Stachybotrylactam	7.71	C ₂₃ H ₃₁ NO ₄	[M + H] ⁺	90.2	1–100	0.09	0.15	4.6	6.1	4.7
Altermariolmethylether	7.75	C ₁₅ H ₁₂ O ₅	[M - H] ⁻	87.1	1–100	0.38	0.64	5.1	4.4	5.6
Fumagillin	7.76	C ₂₆ H ₃₄ O ₇	[M - H] ⁻	89.7	1–100	0.05	0.09	5.6	5.0	3.9
Paxilline	8.17	C ₂₇ H ₃₃ O ₄ N	[M + H] ⁺	94.5	1–100	0.25	0.43	3.5	4.5	4.4

Sample Analysis

A total of 30 commercial dairy products, including milk, milk beverages, and yogurt, were analyzed for the 58 mycotoxins. Four mycotoxins (aflatoxin M1, ochratoxin A, ochratoxin-α, and fumonisin B1) were detected in four different samples tested, as shown in Table 3.

Table 3. Quantification results for analysis of mycotoxins in dairy products.

Sample Type	Detected Compound	Concentration (µg/kg)	RSD (%) (N=9)
Milk	Aflatoxin M1	0.002	0.9
	Ochratoxin A	0.01	2.9
	Ochratoxin-α	0.009	3.1
Milk Beverage	Fumonisin B1	0.11	2.3
Yogurt	Aflatoxin M1	0.002	1.3
Yogurt	Aflatoxin M1	0.005	2.6

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