

Determination of the Ergot Alkaloid Ergovaline in Tall Fescue Seed and Straw Using a QuEChERS Extraction Method with High-Performance Liquid Chromatography–Fluorescence Detection

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S Supporting Information

ABSTRACT: Ergovaline is an ergot alkaloid produced by the symbiotic endophyte *Epichloë coenophiala*, which can colonize varieties of the cool-season grass tall fescue (*Festuca arundinacea*). It is the principle toxicant responsible for the vasoconstrictive and reproductive sequelae seen in “fescue toxicosis” in livestock which consume forage exceeding the threshold of toxicity established for this compound. A new method for extraction of ergovaline from tall fescue seed and straw was optimized and validated, on the basis of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, with high-performance liquid chromatography–fluorescence detection. Fourteen extraction solvents were tested; 2.1 mM ammonium carbonate/acetonitrile (50/50, v/v) had the highest and most consistent recovery (91–101%). Linearity, limit of detection, limit of quantitation, accuracy, and intra- and interday precisions for tall fescue seed and straw were 100–3500 µg/kg, 37 and 30 µg/kg, 100 µg/kg, 98%, 3.0 and 1.6%, and 3.8 and 1.0%, respectively. When the currently used solid-phase extraction (SPE) and QuEChERS methods were applied to 17 tall fescue straw samples, there was good agreement (correlation coefficient 0.9978). The QuEChERS method achieved the goals of eliminating chlorinated solvents and developing a fast, efficient, reliable method for quantitating ergovaline in tall fescue forage that can be applied in a high-throughput food safety laboratory.

KEYWORDS: tall fescue, ergovaline, ergot alkaloids, HPLC-fluorescence

INTRODUCTION

The Pacific Northwest, USA, is the world’s center for grass seed production, with most of the acreage devoted to this crop concentrated in the Willamette Valley, Oregon. Tall fescue (*Festuca arundinacea* (Schreb.) seed was harvested from 134680 acres of Oregon farmland in 2013, making it the most productive cool-season grass species, followed closely by annual (*Lolium multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.) (126040 and 104790 harvested acres, respectively).¹ Tall fescue is also grown extensively for pasture, silage, and straw and is used for reducing soil erosion, recycling nutrients from manure and biosolids, and turf. The symbiotic fungal endophyte *Epichloë coenophiala* has been intentionally propagated in tall fescue, as it improves seed survival and growth and enhances stress tolerance and disease resistance in the plant. Some endophyte strains exert these benefits through the production of ergot alkaloids, which unfortunately also cause deleterious effects in cattle and other herbivore species when endophyte-infected grasses are grazed or fed as hay/straw; this is manifested in the diseases “fescue foot” or “summer syndrome.”^{2,3} However, it is possible to utilize tall fescue straw safely in forage/livestock systems if the concentration of the principal ergot alkaloid causing disease, ergovaline (**1**) (Figure 1), is known, and tall fescue is fed below established thresholds of toxicity for the livestock species in question.^{4–6}

Quantitation of **1** can be assessed through qualified service laboratories and typically involves the use of solid-phase extraction (SPE)^{7,8} for sample cleanup, which can be both costly and time consuming. The search for a simpler, more efficient, and

more environmentally friendly extraction procedure led us to evaluate a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)-based method. QuEChERS is an analytical approach that has vastly simplified the analysis of multiple pesticide residues in fruit, vegetables, cereals, and processed products.^{9,10} To date, it has also been successfully employed in the analysis of mycotoxins in a variety of food matrices, including aflatoxins in noodles,¹¹ trichothecenes in wheat flour¹² and cereals,¹³ and multiple mycotoxin detection (coupled with LC-MS/MS) in silage,¹⁴ cereals,¹⁵ and other food matrices.^{16–19} The purpose of this study was to simplify the extraction of **1** from tall fescue seed and straw and eliminate the use of chlorinated solvents through application of the QuEChERS method, coupled with HPLC-fluorescence for detection.

MATERIALS AND METHODS

Chemicals and Reagents. Acetic acid, acetonitrile (MeCN), isopropyl alcohol, and methanol (MeOH) were HPLC grade and were produced by EMD Chemicals (Gibbstown, NJ). Ethyl acetate was HPLC grade and was obtained from Mallinckrodt (Phillipsburg, NJ). Sodium chloride (NaCl) crystalline powder, 99+%, was supplied by Alfa Aesar (Ward Hill, MA). Ammonium carbonate, HPLC grade, and formic acid, ACS reagent, were both supplied by J. T. Baker (Phillipsburg, NJ). The DL-lactic acid sodium salt was purchased from

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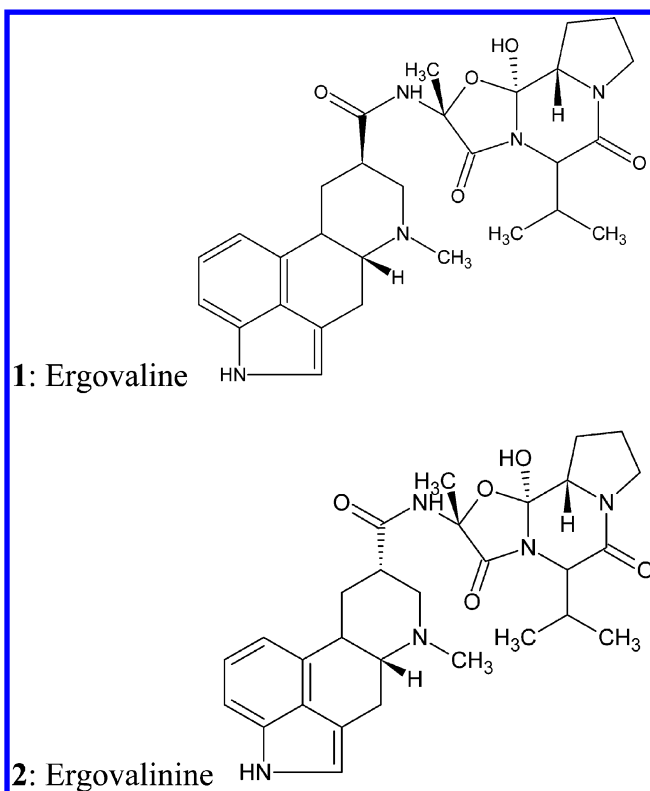


Figure 1. Chemical structures of ergovaline (1) and ergovalinine (2).

Sigma-Aldrich (St. Louis, MO). Anhydrous magnesium sulfate (MgSO_4), 99.5%, was purchased from UCT (Bristol, PA). Ammonium hydroxide, ACS grade, was purchased from VWR BDH Prolabo (West Chester, PA). Ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was purified with a PURELAB Ultra Genetic system (Elga, Marlow, Buckinghamshire, U.K.).

Ergovaline tartrate was prepared by Dr. Forrest Smith, Department of Pharmacal Sciences, Auburn University, Auburn, AL. Chemical characterization and assessment of purity of the crystalline standard was established by nuclear magnetic resonance (NMR) and HPLC/mass spectrometry (HPLC/MS). It was established that the ergovaline tartrate was comprised of ergovaline/tartrate/water in the ratio 2/1/1. The **1** standard was weighed on an analytical balance, diluted in MeOH, dispensed in $10 \mu\text{g}$ aliquots, evaporated under nitrogen, and stored frozen at -20°C . The working standard was prepared by dilution of a $10 \mu\text{g}$ aliquot in MeOH.

Sample Preparation. Tall fescue seed and straw samples were ground in a Cyclotec 1093 sample mill (Foss, Hilleroed, Denmark) to pass through a 0.5 mm screen and stored at -20°C prior to analysis. Details on the collection of a representative straw or seed sample for forage testing can be found online.²⁰

Optimization of Extraction Solvent. The first step of the extraction/partitioning process of the QuEChERS method was evaluated in a tall fescue seed sample with a known high **1** level (1998 $\mu\text{g}/\text{kg}$, $n = 3/\text{solvent}$). The extraction solutions were selected from published procedures that were used to extract mycotoxins via the QuEChERS method or used in the extraction of ergot alkaloids via other techniques and were as follows: extraction solvent 1, 1% acetic acid in MeCN (v/v);¹¹ extraction solvent 2, MeOH/MeCN (15/85, v/v);¹¹ extraction solvent 3, MeOH/MeCN (85/15, v/v);¹¹ extraction solvent 4, MeOH/water (60/40, v/v);¹¹ extraction solvent 5, MeOH/water (80/20, v/v);¹¹ extraction solvent 6, 2.1 mM ammonium carbonate/MeCN (16/84, v/v), modification from a method for the quantitation of ergot alkaloids in cereals and foodstuffs;²⁰ extraction solvent 7, isopropyl alcohol/1% lactic acid (50/50, v/v), method developed to quantitate **1** and peramine in grass tillers;²¹ extraction solvent 8, MeCN/0.1% formic acid (57/43, v/v), modification of a method developed for detection of *Fusarium* mycotoxins in cereals;¹³ extraction solvent 9, ethyl acetate/MeOH/4.1 mM

ammonium carbonate (62.5/25/12.5, v/v), modification of a method for ergot alkaloid extraction from food and feed sources;²² extraction solvent 10, water/0.5% acetic acid in MeCN (50/50, v/v), modification of a QuEChERS method developed for the analysis of 17 mycotoxins in cereals;¹⁵ extraction solvent 11, ethyl acetate/MeOH/25% ammonium hydroxide solution (75/5/7, v/v), modification of a method developed for analysis of ergot alkaloids in rye and rye products;²³ extraction solvent 12, MeCN/1.0% acetic acid (80/20, v/v); extraction solvent 13, MeCN/1 mM ammonium carbonate (80/20, v/v); extraction solvent 14, MeOH/1% acetic acid (80/20, v/v).

For the initial survey of solvent systems, 1.0 g samples of ground tall fescue seed were placed into $16 \times 125 \text{ mm}$ glass screw top tubes in triplicate. A 5 mL portion of the solvent mixture was added; the tubes were capped and vortexed for 10 s. The tubes were placed on an end-over-end rotary mixer for 30 min, after which 0.5 g of MgSO_4 and NaCl (4/1, w/w)⁹ was added. The tubes were capped and vortexed immediately to prevent agglomeration of the salts for 10 s, four times during a 10 min period. The samples were then centrifuged at $913g$ for 10 min. A 1 mL portion of the supernatant was evaporated to dryness at 50°C under a gentle stream of nitrogen. The samples were reconstituted in 0.5 mL of MeOH and, after vortexing and sonication for 20 s each, transferred to a centrifugal filter tube with a $0.45 \mu\text{m}$ modified nylon membrane (VWR, Radnor, PA). The tubes were centrifuged at $8161g$ (10000 rpm) for 5 min; the filtrate was then transferred to an amber HPLC vial for analysis.

Final Extraction Procedure. A 5 mL portion of 2.1 mM ammonium carbonate/MeCN (50/50, v/v) extraction solvent was added to 0.5 g of ground plant material, mixed by vortexing for 30 s, and then placed on the rotary mixer for 30 min. Subsequently, 0.4 g of preweighed MgSO_4 and 0.1 g of NaCl was added and the tubes were capped and vortexed immediately for 10 s, four times during a 10 min period. Samples were centrifuged at $913g$ for 10 min. A 2.0 mL aliquot of the separated MeCN phase was evaporated to dryness at 50°C under a gentle stream of nitrogen. The samples were reconstituted in 0.5 mL of MeOH and, after vortexing and sonication for 20 s each, were transferred to a centrifugal filter tube. The tubes were centrifuged at $8161g$ for 5 min, and the filtrate was transferred to an amber HPLC vial for analysis.

HPLC Analysis. A Series 200 HPLC system (PerkinElmer, Waltham, MA) was used throughout the experiment. This system consisted of an autosampler, binary pump, column oven, and fluorescence detector ($\lambda_{\text{ex}} 250 \text{ nm}$, $\lambda_{\text{em}} 420 \text{ nm}$). Chromatographic data were managed using TotalChrom Workstation Software version 6.3.2 (PerkinElmer). HPLC separations were performed with a $100 \text{ mm} \times 4.6 \text{ mm}$ i.d., $2.7 \mu\text{m}$ Brownlee SPP C18 HPLC column (PerkinElmer), with a 4.6 mm i.d. SecurityGuard ULTRA C18 guard column (Phenomenex (Torrance, CA)) maintained at 35°C . The mobile phase consisted of 30% MeCN in 200 mg/L ammonium carbonate in purified water (solvent A) and 100% MeCN (solvent B), run at a flow rate of 1.8 mL/min. Initial gradient conditions were 5% B held for 1.0 min, increasing linearly to 65% B over 0.8 min and held for 0.8 min. Finally, there was a linear return to initial conditions over 0.3 min, which was then held for 1.3 min for a total run time of 4.2 min. The injection volume was $10 \mu\text{L}$.

Method Validation. Performance characteristics were evaluated using guidelines developed by the Food and Drug Administration for bioanalytical analysis.²⁴ Validation of the optimized extraction method included linearity, selectivity, recovery, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, and stability. Linearity was assessed using matrix-matched calibration curves established using extracts of endophyte-free (E-) tall fescue seed and straw fortified with the working **1** standard at 50, 100, 500, 1000, 2500, and 3500 $\mu\text{g}/\text{kg}$. Selectivity was established using comparison of noninfected seed and straw sample chromatograms with **1**-fortified extracts. Interference (visual examination of the chromatogram for coelution of other peaks) at the retention times of **1** (1.75 min) and ergovalinine (**2**) (3.60 min) was checked.

For recovery investigations, the **1** standard was added to E- tall fescue seed and straw at fortification levels of 100, 1000, and 2500 $\mu\text{g}/\text{kg}$ ($n = 10/\text{level}$). To avoid epimerization of **1**, the MeOH in the spiked sample was evaporated under a gentle steam of nitrogen, and then the

tube was capped and frozen at $-20\text{ }^{\circ}\text{C}$ until extraction. The LOD and LOQ were estimated by measurement of the peak-to-peak signal-to-noise ratio (S/N) and calculated at $S/N > 3$ and $S/N > 10$, respectively. LOQ values must meet an accuracy of $\pm 20\%$ and a precision of $< 20\%$ relative standard deviation (RSD).

The accuracy and precision of the method was determined using in-house tall fescue seed and straw reference material. The **1** content of the reference material was established using the Ergosil silica gel solid phase extraction (SPE) method.^{5,8} The intraday precision was established using five replicates at four levels of **1**. Interday precision and accuracy were evaluated over three separate days of analysis of five replicates at the four concentration levels. The stability of the sample extracts was investigated by repeat analysis of the samples ($n = 48$) after 24 h at room temperature.

Statistical Analysis. Statistical analyses (averages, standard deviations (SD), relative standard deviations (RSD), t tests (two-tailed, two-sample equal variance) and ANOVA) were performed in Excel (Microsoft Office 2010, Redmond WA) or GraphPad Prism (Version 5.01, La Jolla, CA). Results were considered significant when the p value was less than 0.05.

RESULTS

Optimization of Extraction Procedure. The 14 solvent mixtures were significantly different from each other in their ability to extract **1** (Kruskall-Wallis test, $P = 0.001$) (Figure 2).

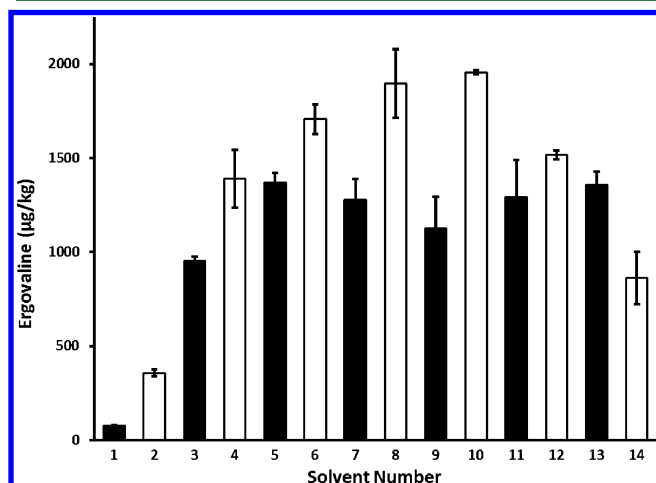


Figure 2. Evaluation of different solvent mixtures for extraction of **1** from endophyte-infected tall fescue seed containing $1998\text{ }\mu\text{g}$ of **1**/kg using the QuEChERS method ($n = 3$ /solvent). For solvents tested refer to Materials and Methods.

The maximum amount of extracted **1** was obtained with water/0.5% acetic acid in MeCN (50/50), which extracted 98% of the $1998\text{ }\mu\text{g}$ of **1**/kg in the seed that was being used, with a low standard deviation of $12\text{ }\mu\text{g}/\text{kg}$ (RSD 0.6%). The MeCN/0.1% formic acid (57/43) mixture showed a similar ability to extract **1**, extracting 95% of the $1998\text{ }\mu\text{g}$ of **1**/kg in seed, but had a higher standard deviation of $182\text{ }\mu\text{g}/\text{kg}$ (RSD 9.6%). While the two solvent combinations were not statistically different from each other ($p = 0.611$), the water/0.5% acetic acid in MeCN (50/50) mixture was chosen for its lower standard deviation, which would ensure greater throughput under the rigorous quality control standards in a diagnostic laboratory setting. Once this was established, different types of aqueous solution, namely 0.5% and 1% acetic acid, 1% formic acid, 2.1 mM ammonium carbonate, and 1 mM ammonium carbonate, were studied in both seed ($1998\text{ }\mu\text{g}$ of **1**/kg) and high **1** content straw ($850\text{ }\mu\text{g}/\text{kg}$). It was found that the highest and most consistent recoveries were achieved with 2.1 mM ammonium carbonate (Figure 3). This

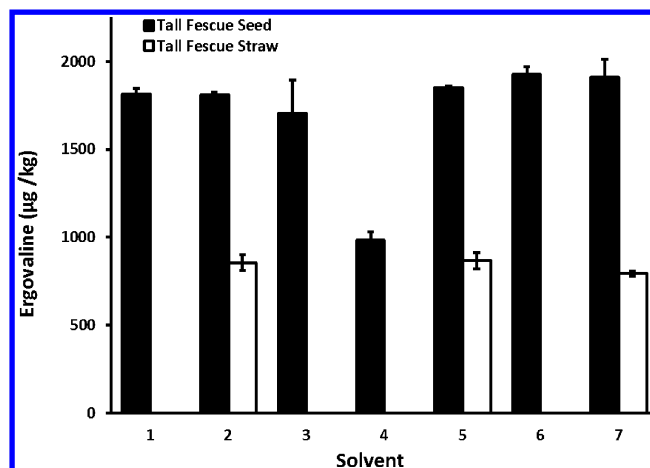


Figure 3. Additions to the aqueous component of the extraction solution evaluated for recovery of **1** from tall fescue seed ($1998\text{ }\mu\text{g}$ of **1**/kg) and tall fescue straw ($850\text{ }\mu\text{g}$ of **1**/kg) ($n = 3$ /sample type/solvent mixture): (1) 1% acetic acid/MeCN (50/50); (2) 2.1 mM ammonium carbonate/MeCN (50/50); (3) 1 mM ammonium carbonate/MeCN (50/50); (4) 1% formic acid/MeCN (50/50); (5) 0.5% acetic acid/MeCN (50/50); (6) water/1% acetic acid in MeCN (50/50); (7) water/MeCN (50/50).

solvent mixture extracted $90.5 \pm 1.0\%$ of the **1** in the tall fescue seed ($1998\text{ }\mu\text{g}/\text{kg}$) and $100.5 \pm 5.3\%$ in the tall fescue straw ($850\text{ }\mu\text{g}/\text{kg}$) that were used. The addition of the 2.1 mM ammonium carbonate/MeCN extraction solution added as a mixture or separately as two components to tall fescue seed containing $1998\text{ }\mu\text{g}$ of **1**/kg was investigated; no statistical difference was found between either method ($n = 3$ replicates/method, t test $p = 0.656$). Thus, combining the 2.1 mM ammonium carbonate/MeCN (50:50, v/v) as one addition to the sample was chosen.

The optimum mixing time for the sample and extraction solvent was evaluated at 30, 60, 90, and 120 min in both seed and straw samples (Table 1). Thirty minutes was found to be a

Table 1. Comparison of Mixing Times for Solutions of 2.1 mM Ammonium Carbonate/MeCN (50/50, v/v) from Tall Fescue Seed and Straw for Extraction of Ergovaline (1) Using the QuEChERS Method

	amt of ergovaline (1), $\mu\text{g}/\text{kg}^a$			
	30 min	60 min	90 min	120 min
tall fescue seed ($1998\text{ }\mu\text{g}/\text{kg}$)	1901(3)	1771(4)	1637(4)	1644(3)
tall fescue straw ($776\text{ }\mu\text{g}/\text{kg}$)	712(8)	675(8)	568(11)	620(9)

^aRSD values are given in parentheses ($n = 5$).

sufficient amount of time for the sample and extraction solvent to mix to maximize the amount of **1** extracted, with low RSD values. Following addition of the 0.4 g of anhydrous MgSO_4 and 0.1 g NaCl, immediate vortexing and mixing four times during a 10 min period was required to give the most consistent mixing and to promote homogeneity.

Figure 4 shows representative HPLC-fluorescence chromatograms of the final, optimized QuEChERS procedure for tall fescue seed and straw, in comparison to the Ergosil SPE method currently used in our laboratory.⁵

Method Validation. Linearity. A calibration curve was constructed by plotting the peak area of **1** against the nominal concentrations of **1** at 50, 100, 500, 1000, 2500, and 3500 $\mu\text{g}/\text{kg}$. The correlation coefficient (R^2) generated from the calibration

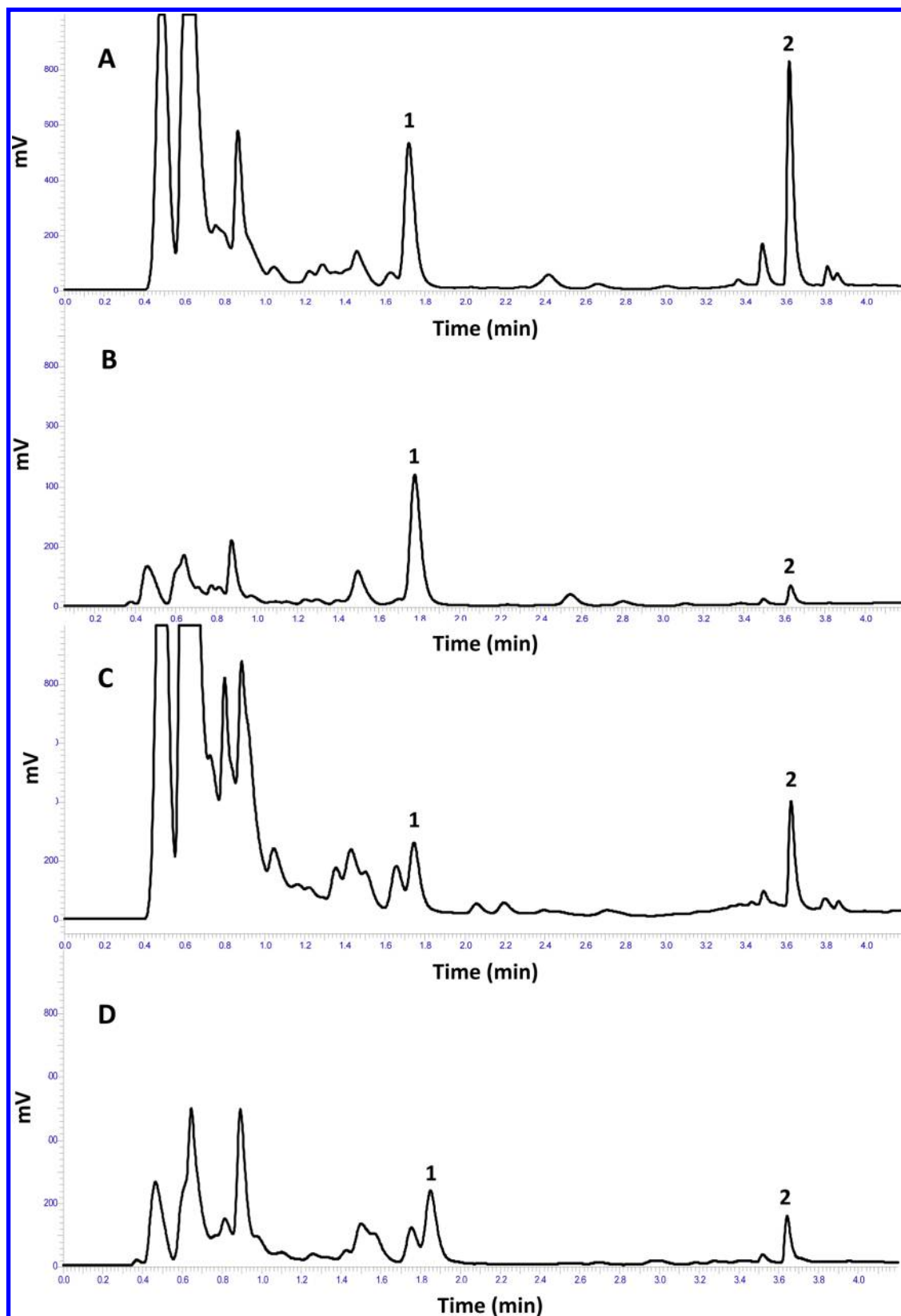


Figure 4. Representative HPLC-fluorescence chromatograms comparing extraction of tall fescue seed and straw between the optimized QuEChERS method and an Ergosil SPE method for **1** and its epimer **2**: (A) QuEChERS extract of tall fescue seed; (B) Ergosil SPE extract of tall fescue seed; (C) QuEChERS extract of tall fescue straw; (D) Ergosil SPE extract of tall fescue straw.

curve was >0.999 in both seed and straw from six replicate standard sets. The linear dynamic range was found to be $100\text{--}3500\ \mu\text{g}/\text{kg}$.

Selectivity. Noninfected seed and straw samples were extracted to verify that there were no peaks interfering with the fluorescent detection of **1** or its epimer **2** at

their expected retention times (1.75 and 3.60 min, respectively).

Recovery. **1** was spiked into E- seed and straw samples at three levels: 100, 1000, and 2500 $\mu\text{g}/\text{kg}$ ($n = 10/\text{level}$). The mean recoveries varied from 91.3 to 97.2% in tall fescue seed and 89.6–98.1% in tall fescue straw.

LOD and LOQ. The LOD values were determined to be 37 and 30 $\mu\text{g}/\text{kg}$ in tall fescue seed and straw, respectively. An LOQ level of 100 $\mu\text{g}/\text{kg}$ was established in both seed and straw, with demonstrated accuracies of 98% and 91% and precision of 9% and 6%, respectively.

Precision and Accuracy. Precision and accuracy were determined by the extraction of **1** from tall fescue seed (143–1998 μg of **1**/kg) and straw (181–1061 $\mu\text{g}/\text{kg}$) reference material. The intraday precision, expressed in RSD, ranged from 1.8 to 5.8% in tall fescue seed and 0.5–3.4% in tall fescue straw. The interday precision ranged from 1.9 to 6.4% in seed and 0.7–4.8% in straw. The results from the experiments to establish accuracy ranged from 97.9 to 98.6% in tall fescue seed and 97.3–99.2% in tall fescue straw.

Stability. The extracted samples demonstrated stability comparable with that of the Ergosil SPE method upon repeat analysis after storage at room temperature for 24 h.

Application of the Method to Real Samples. The validated method was applied to 17 tall fescue straw samples which were previously analyzed using the Ergosil SPE method.^{5,8} Including the standard reference material, controls, and samples run in duplicate, the run size was 48 individual extractions, which is a typical sample run in our laboratory. There was good agreement between the two different methods. Both methods found that 7 of the samples had less than a quantitative amount of **1** (<100 $\mu\text{g}/\text{kg}$). The results from the remaining 10 samples had a RSD of 10% or better agreement. **1** values for the QuEChERS/Ergosil SPE methods were 119/119, 156/173, 203/224, 237/244, 238/240, 339/318, 499/469, 523/530, 526/518, 535/482, and 632/603 $\mu\text{g}/\text{kg}$, respectively. Comparative statistics showed that application of the two extraction methods to the 17 samples gave results that were not different from each other (t test, $p = 0.258$) and that the methods were very well correlated (correlation coefficient 0.9978).

DISCUSSION

Endophyte (*Epichloë coenophiala*)-infected tall fescue (*Festuca arundinacea*) is used as forage for livestock in the form of pasture, straw, silage, and pellets. Some endophyte strains produce ergot alkaloids as a protective mechanism against herbivory which cause vasoconstrictive and reproductive abnormalities that present as the diseases known as “summer syndrome” and “fescue foot” in livestock.^{2,3} **1** is the ergopeptide alkaloid produced in highest concentration in tall fescue²⁵ and is a potent vasoconstrictor with the potential to bioaccumulate in vascular tissue;^{26,27} as such, it has been used to establish thresholds of toxicity for these diseases in relevant livestock species.⁵ When it is used safely with these thresholds in mind, tall fescue can be fed as an important nutritional component in livestock feeding programs. To achieve this, accurate testing of **1** concentration in tall fescue material is vital. While we currently employ a robust SPE method for **1** quantitation,^{5,8} we were interested in application of a QuEChERS method to make the assay more efficient and eliminate the use of chlorinated solvents.

Fourteen extraction solvents were examined, and 2.1 mM ammonium carbonate/MeCN (50/50, v/v) was found to have the highest and most consistent recovery ($90.5 \pm 1.0\%$ and

$100.5 \pm 5.3\%$ for tall fescue seed and straw, respectively) (Figures 2 and 3). Thirty minutes was found to be an adequate amount of time for mixing and produced the best recovery of **1** from tall fescue seed and straw (Table 1). Total extraction time was improved to 4.4 h, in comparison to 5.9 h using traditional SPE, giving a time savings of 1.5 h.

On validation of the method, performance characteristics were similar to existing extraction procedures using HPLC-fluorescence for detection. The QuEChERS procedure had an LOD of 37/30 $\mu\text{g}/\text{kg}$ in tall fescue seed/straw, respectively, a LOQ level of 100 $\mu\text{g}/\text{kg}$ in both seed and straw, a linear dynamic range of 100–3500 $\mu\text{g}/\text{kg}$, and recoveries of spiked **1** from 91.3 to 97.2% for seed and 89.6–98.1% for straw. The Ergosil SPE method^{5,7} has an LOD value of 31 $\mu\text{g}/\text{kg}$, an LOQ value of 100 $\mu\text{g}/\text{kg}$, a linear dynamic range of 100–2000 $\mu\text{g}/\text{kg}$, and a recovery of 91% and is the method currently in use by the Endophyte Service Laboratory at Oregon State University. Previous extraction methods upon which the Ergosil SPE method was based also showed similar values with an LOD value of 50 $\mu\text{g}/\text{kg}$ and a recovery of 85%²⁸ or 93%.⁸ These parameters are in line with the ability to determine clinically relevant concentrations of **1** in feed, on the basis of the established thresholds of toxicity (300–800 μg of **1**/kg, depending on species and climatic conditions).⁵ Another method using 2-propanol/lactic acid as an extraction solvent to examine **1** distribution throughout the plant established an LOQ value of 100 $\mu\text{g}/\text{kg}$, a linear dynamic range of 400–10000 $\mu\text{g}/\text{kg}$, and recoveries of 93–105% and had the advantage that small (2–5 mg) sample amounts could be used for quantitation.²¹ More recently, an SPE method using sodium-neutralized strong cation exchange columns was developed which accounted for both the native toxicologically active *R* epimer of ergot alkaloids as well as their biologically inactive *-inine S* epimer forms in rye flour and wheat germ oil samples, while minimizing epimer formation during the extraction itself.²⁹ They achieved LOD values of 0.3–0.8 $\mu\text{g}/\text{kg}$, LOQs of 0.7–2.0 $\mu\text{g}/\text{kg}$, linear dynamic ranges of 8–200 $\mu\text{g}/\text{kg}$, and recoveries of 71–120%.

Advancements in LC-MS have made it increasingly common in diagnostic laboratories. Application of the QuEChERS extraction method to cleanup samples, particularly for those destined for screening of multiple ergot alkaloids via LC-MS/MS, is a logical next step, as this mode of detection will likely allow the limits of detection and quantitation to be lowered. Two methods using electrospray ionization (ESI) (+) for detection of **1** and other ergot alkaloids analyzed by selected reaction monitoring (SRM) laid out a robust framework which also allowed for elucidation of “unusual” ergot alkaloid compounds and metabolites.^{30,31} Another LC-MS method, using extraction procedures developed by Spiering et al.,²¹ employed SRM for the transition m/z 534–268 and found concentrations of 240–3480 μg of **1**/kg in five populations of tall fescue from Iran.³² While **1** was not specifically included, LC-MS/MS determination of multiple ergot alkaloids in the following methods had performance characteristics of LOQs of 0.17–2.78 $\mu\text{g}/\text{kg}$ (with LODs similar to LOQs), recoveries of 69–105%, concentration ranges of 0.5–200 $\mu\text{g}/\text{kg}$,³³ LODs of 0.04–0.29 $\mu\text{g}/\text{kg}$, LOQs of 0.1–1 $\mu\text{g}/\text{kg}$, and recoveries of 88–110%.³⁴ Finally, a method using saphenous vein tissue as a matrix gave an LOD value of 1 nM, an LOQ value of 2 nM, a linear dynamic range of 0.1–50 pmol, and a recovery of 86–90% for spiked **1**.³⁵

We have observed epimerization of **1** to **2** in SPE-extracted samples at room temperature after approximately 12 h. On this basis, a complete batch of samples, validated reference materials, and controls are either always run immediately after extraction

via HPLC-fluorescence or stored in a freezer, which halts epimerization until samples can be analyzed.³⁶ For quantitation, only **1** and not **2** is analyzed for total concentration in this method, as the Ergosil silica material in the SPE appears limited in its ability to bind **2**. Further, the thresholds of toxicity for fescue toxicosis in livestock⁵ were established using analysis of feed samples for **1** (and not its epimer) via the SPE method; thus, measurement of **1** in feed samples which will be used in livestock feeding programs to avert fescue toxicosis remains relevant. On the other hand, the QuEChERS method had a significant peak for **2** in both seed and straw samples (Figure 4A,C); this technique appears to be able to extract more of this stereoisomer than the SPE method and/or epimerization from the *R* to the *S* isomer configuration is occurring at a greater rate over the course of performing this method. However, quantitation was again performed using only **1**, to compare extractability of **1** alone between the two methods. When the QuEChERS extraction procedure was applied to 17 tall fescue straw samples that had also been quantitated for **1** using the Ergosil SPE method, a correlation coefficient of 0.9978 was obtained on comparison of the two methods. The concentrations tested ranged from 7 that were below the LOQ value (100 $\mu\text{g}/\text{kg}$) to an additional 10 from 119 to 632 $\mu\text{g}/\text{kg}$, giving a good representation of the **1** concentrations that are typically seen in samples submitted to the Endophyte Service Laboratory.

In summary, a method following the QuEChERS extraction procedure was developed for determination of **1** in tall fescue seed and straw material using HPLC-fluorescence for detection. The method has eliminated the need for halogenated/chlorinated solvents, is as fast and efficient as the Ergosil SPE method currently in use in our laboratory, and was validated to perform across a linear range of 100–3500 μg of **1**/kg in tall fescue seed and straw material. These features make it ideal for application in a high-throughput food safety laboratory.

■ ASSOCIATED CONTENT

📄 Supporting Information

A figure giving additional chromatographic data and a table giving validation data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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