Determination of Ergonovine in Bovine Plasma Samples†

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A rapid and sensitive method for the extraction and high-performance liquid chromatographic analysis of ergonovine in bovine plasma is described. Plasma samples underwent extraction into methylene chloride. Chromatography was performed on a Novapak C_{18} column using a mobile phase of acetonitrile–0.01 M ammonium carbonate (pH 7.8) with a flow rate of 1 mL/min. Fluorometric detection was used with an excitation of 328 nm and an emission of 389 nm. The procedure produced a linear curve for the concentration range 1–25 ng/mL. The assay produced rapid, repeatable, and accurate results for detection of ergonovine in bovine plasma.

INTRODUCTION

Livestock grazed on *Acremonium coenophialum*-infested tall fescue (Morgan-Jones and Gams, 1982) annually cost producers millions of dollars in reduced reproductive efficiency, weight gains, and milk production (Bacon et al., 1986; Kester, 1988; Hoveland, 1990; Porter and Thompson, 1992; Lyons et al., 1986; McLaren, 1987). More frequently referred to as the endophyte of infected fescue, *A. coenophialum*'s history and impact on agriculture have been reviewed by Stuedemann and Hoveland (1988), while reviews on the agronomic, biologic, and endophyte–host relationship have been published by Bacon and Battista (1991), and Siegel et al. (1987). In addition, the effects of fescue toxicosis on livestock have been reviewed by Porter and Thompson (1992), Lyons et al. (1986), Ball (1984), Martin and Edwards (1986), and Hammond et al. (1982).

Ergopeptine alkaloids in *A. coenophialum*-infested fescue (Lyons et al., 1986; Yates and Powell, 1988) are thought to be the major cause of fescue toxicosis in cattle. Ergonovine (D-lysergic acid L-2-propanolamide) belongs to the simple lysergic acid amide group of ergot alkaloids (Berde and Schild, 1978) and has been detected in infected fescue seed (Petroski and Powell, 1991; Yates and Powell, 1988). Ergonovine reportedly has pharmacological properties similar to those of the ergopeptine alkaloids (Berde and Schild, 1978) (i.e., vasoconstriction, prolactin inhibition, uterotonic activity). In contrast, some of the alkaloid's activities (i.e., hypothermia, decreased respiration) are different from those of the ergopeptine group (Berde and Schild, 1978). When infused into cattle, ergonovine produced decreased weight gains, respiration rates, and temperatures (Oliver et al., 1991), consistent with this compound's effects in laboratory animals (Berde and Schild, 1978). However, in cattle, ergonovine decreased serum prolactin in the low-dosed animals, whereas it increased serum prolactin in the high-dosed animals (Oliver et al., 1991). These differences may lead to confusing results and/or confused diagnosis of the disease. Therefore, the analysis of ergonovine in cattle warrants investigation.

Ergonovine has not been detected in bovine plasma, and thus this paper describes the development of a rapid and sensitive method for extraction of ergonovine from plasma, with subsequent analysis by high-performance liquid chromatography. The procedure allows analysis of plasma samples that may facilitate the evaluation of ergonovine levels in cattle expressing fescue toxicosis.

EXPERIMENTAL PROCEDURES

Materials. Acetonitrile, hexane, 1-butanol, and methylene chloride were of HPLC grade (Burdick and Jackson Laboratories, Inc., Muskegon, MI). Ergonovine maleate, reagent grade ammonium carbonate, and ammonium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Methysergide maleate (internal standard) was purchased from Research Biochemicals Inc. (Natick, MA). Ergonovine is light sensitive, so all standards and extractions were prepared and analyzed in the dark.

A stock standard solution of ergonovine maleate was prepared by dissolving 5 mg in 50 mL of water, while a stock standard solution of methysergide maleate (5 mg in 50 mL, internal standard) was prepared in methanol. Working standards were made by serial dilutions of the stock standard solutions. Standards were stable in brown bottles at −20 °C for 1 month.

The analytical system consisted of a Model 600E solvent delivery system, a Model 700 WISP autosampler, a RCM 8 mm × 10 cm cartridge holder equipped with a 8 mm × 10 cm Novapak C_{18} cartridge (4-μm particle size) and a C_{18} Guard Pak precolumn insert, a Model 470 scanning fluorescence detector, and a NEC Powermate computer system (Waters, Milford, MA).

The mobile phase consisted of a 0.01 M ammonium carbonate buffer (pH 7.8) and 30% acetonitrile. The ammonium carbonate buffer pH was adjusted with concentrated nitric acid. The flow rate was 1 mL/min. The column temperature was ambient, and fluorescence was measured at Ex 328 nm and Em 389 nm.

Methods. Plasma samples were obtained from Holstein steer calves (approximately 300 lb) housed in indoor pens and fed alfalfa hay for 1 week before and throughout the 12-day study. Ergonovine was administered via Alzet (Model 2M2L) miniosmotic pumps (Alza Corp., Palo Alto, CA) subcutaneously implanted in the neck region of the animal. One control group received saline, and three treatment groups received ergonovine.

Plasma samples (−20 °C) were thawed and vortexed before use and extracted using the Haering et al. (1985) method. Plasma (1 mL) was pipetted into round-bottom, screw-cap glass tubes with PTFE-lined caps. Samples were spiked with 15 μL of methysergide (1 μg/mL, internal standard), and 1 mL of 1.0 M ammonium chloride buffer (pH 9.2) was added. Samples were then extracted with 7 mL of a 6:1 hexane/1-butanol solution, vortexed, and centrifuged (10 min at 2700 rpm). All of the organic phase was transferred to a second glass tube, and 1 mL of 0.05 M sulfuric acid was added, followed by vortexing and centrifugation (10 min at 2700 rpm). The organic phase was aspirated and discarded and 4 mL methylene chloride plus 1 mL 1.0 M ammonium chloride buffer added. Samples were then vortexed and centrifuged (10 min at 2700 rpm), and the aqueous layer was removed. The organic layer was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 500 μL of the mobile phase, and a 150-μL aliquot of the sample was injected onto the liquid chromatograph for quantification.

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† Supported in part by The Center of Excellence in Livestock Disease and Human Health.

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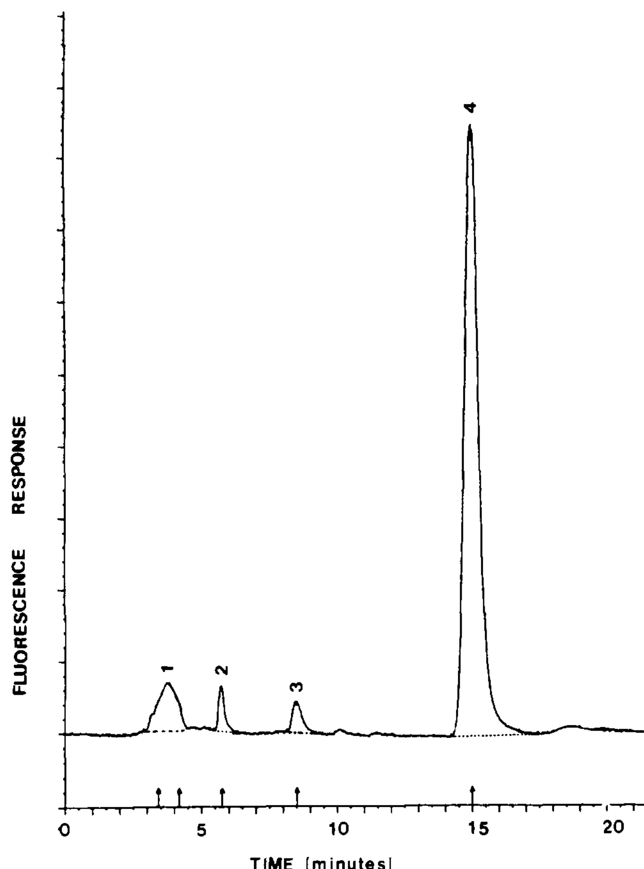

Figure 1. Chromatogram of ergonovine (2) and methysergide (4) extracted from bovine plasma (2.5 ng/mL spike of ergonovine). Peaks 1 and 3 result from endogenous plasma components.

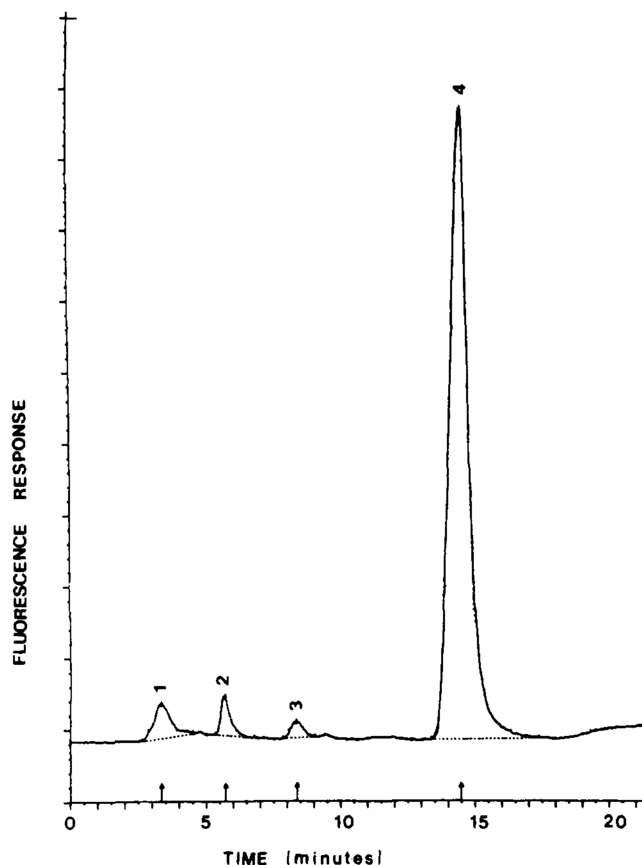

Figure 2. Chromatogram of a plasma sample collected on day 10 after administration of 336 μg/lb ergonovine via osmotic pumps. Peaks 2 and 4 are ergonovine and methysergide, while peaks 1 and 3 are produced by endogenous plasma components.

RESULTS

A representative chromatogram for extracted plasma is shown in Figure 1. The retention times for ergonovine and methysergide were 5.70 and 14.70 min, respectively. Figure 2 is a chromatogram of bovine plasma taken on day 10 after a dose of 336 μg/lb of ergonovine maleate was administered by Alzet miniosmotic pumps. Retention times for ergonovine and methysergide were 5.71 and 14.59 min, respectively. Endogenous peaks produced by the plasma occurred at 3.68 and 8.68 min (Figure 3) and, therefore, did not interfere with the elution of ergonovine.

The method produced a linear curve for the concentration range used in this study, with the correlation coefficients ranging from 0.996 to 0.999. Replicate analyses performed on the same day for plasma spiked with specific concentrations of ergonovine showed coefficients of variation to be 5.7% for 3 ng/mL, 3.6% for 8 ng/mL, and 2.6% for 20 ng/mL (Table I). Interassay variability for plasma replications is shown in Table II. The mean percent recoveries of ergonovine from plasma were 94, 96, 96, 99, and 96% for 1, 2.5, 5, 10, and 25 ng/mL, respectively (Table III). The detection limit for ergonovine in plasma was 0.5 ng/mL. This represents a peak approximately 3 times baseline noise.

DISCUSSION

A quantitative method of analysis for ergonovine is required before pharmacokinetic and pharmacodynamic studies can be conducted. To our knowledge, no quantitative procedures in bovine plasma have been reported. The procedure described here is for the analysis of ergonovine in plasma.

HPLC procedures have been described where ergonovine was extracted from tall fescue seed or flour (Yates and Powell, 1988; Scott and Lawrence, 1980) using either a simple methanol extraction or an extraction involving large amounts (50 mL or more) of methylene chloride, ethyl acetate, methanol, and hexane. The methanol extraction was not adequate for the cleanup of plasma, and our procedure eliminated the use of large volumes of organic solvents.

HPLC procedures have also been developed for the analysis of the ergopeptide alkaloids ergotamine and ergovaline in serum and plasma (Savary et al., 1989; Edlund, 1981), both of which have the ergoline ring common to ergonovine. Edlund's (1981) procedure requires large volumes of organic solvents for the extraction and a large plasma sample (3 mL). Savary et al.'s (1989) procedure requires special siliconized tubes and a large serum sample (6 mL). Our procedure for ergonovine requires only 1 mL of plasma, no specialized tubes, few organic solvents, and smaller amounts of those used. Chromatograms of our extracted plasma contained fewer contaminating peaks than those extracts shown in Savary et al.'s (1989) paper.

The chromatograms confirm the presence of two peaks due to endogenous plasma components. They, however, did not interfere with the elution of ergonovine. The use of methysergide as an internal standard allows compensation for any intra- or interassay variability in the extraction and chromatography steps.

The procedure was instrumental in a recent study where cattle were administered different doses of ergonovine by osmotic pumps surgically implanted (subcutaneously) in the neck region (Oliver et al., 1991). The purpose was to demonstrate that ergonovine (and possibly other purified ergot alkaloids) could be recovered in plasma after administration over time via Alzet osmotic pumps. Mean

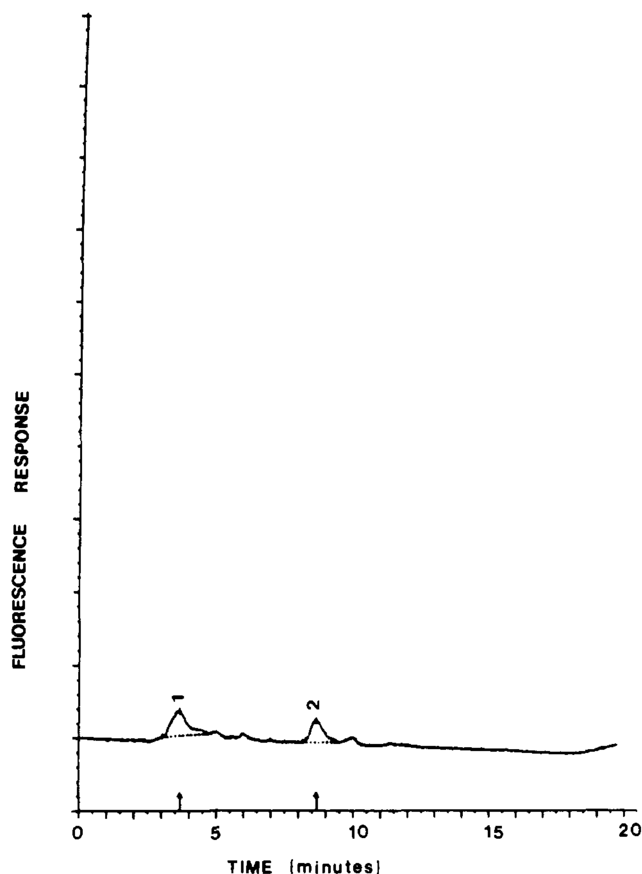

Figure 3. Chromatogram of blank bovine plasma. Peaks 1 and 2 are endogenous plasma components.

Table I. Assay Precision of Ergonovine in Bovine Plasma (n = 4)

concn added, ng/mL	concn found, ng/mL (mean ± SD)	coeff of variation, %
3.0	3.1 ± 0.2	5.7
8.0	7.8 ± 0.3	3.6
20.0	20.2 ± 0.6	2.6

Table II. Interassay Precision of Ergonovine in Bovine Plasma (n = 4)

concn added, ng/mL	concn measured, ng/mL	area ratios[a] (mean ± SD)	coeff of variation, %
1.0	1.0	0.056 ± 0.004	6.6
2.5	2.4	0.096 ± 0.006	6.1
5.0	5.1	0.149 ± 0.016	10.1
10.0	9.9	0.317 ± 0.016	5.0
25.0	22.0	0.829 ± 0.045	5.0

[a] Mean area ratio of ergonovine/internal standard.

Table III. Recovery of Ergonovine from Bovine Plasma (n = 4)

concn added, ng/mL	recovery, % (mean ± SD)	coeff of variation, %
1.0	94 ± 11	11.0
2.5	96 ± 5	5.2
5.0	96 ± 5	6.2
10.0	99 ± 5	5.0
25.0	96 ± 5	5.2

levels of plasma ergonovine detected in this study ranged from 0.5 to 2.7 ng/mL over a 12-day period. Thus, plasma levels of ergonovine in cattle can be studied by this method. Further studies are underway to determine whether ergonovine can be detected in cattle grazing on *A. coenophialum*-infested fescue grass.

In conclusion a rapid, sensitive, and useful HPLC procedure has been developed for analysis of ergonovine in bovine plasma. Further studies are needed to determine if this technique can be used to quantitate other alkaloids.

ACKNOWLEDGMENT

We thank Ms. Betsy Cagle for preparing the manuscript.

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Received for review April 28, 1992. Revised manuscript received August 14, 1992. Accepted September 9, 1992.