GC/MS Determination of Pyrogallol and Gallic Acid in Biological Matrices as Diagnostic Indicators of Oak Exposure

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A selective and sensitive method was developed for the quantitative determination of pyrogallol and detection of gallic acid in biological samples. Pyrogallol was measured as a metabolite of gallotannin in bovine urine and serum samples. Gallic acid was formed by hydrolysis of the gallotannins in blue oak *(Quercus douglasii)* leaves, tannic acid standards, and rumen contents. Acid hydrolyzed or acidified samples were extracted with 5% ethanol in ethyl acetate (v/v), followed by evaporation and derivatization with Deriva-sil. The trimethylsilyl derivatives were analyzed by gas chromatography/mass spectrometry using selected ion monitoring. Method detection limits for gallic acid and pyrogallol were 0.5 ppm in urine and serum samples and 5 ppm in rumen contents. The diagnostic utility of the method was tested by analyzing samples from heifers dosed with blue oak leaves and commercial tannic acid.

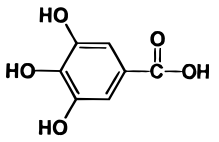
Keywords: *Pyrogallol; gallic acid; gallotannins; GC/MS determination*

INTRODUCTION

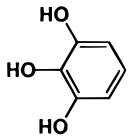
Oak (*Quercus* sp.) toxicosis in livestock has been reported worldwide (Spier et al., 1987; Kasari et al., 1986; Garg et al., 1992; Shi, 1988; Osweiler et al., 1985). Large outbreaks have occurred in California, including an episode during which 2700 cattle died from ingestion of blue oak *(Quercus douglasii)* (Spier et al., 1987). Losses of livestock due to oak poisoning result from gastroenteritis, renal failure, liver damage, death, and deformities in calves (Spier et al., 1987; Zhu and Filippich, 1995; Anderson et al., 1983; Osweiler et al., 1985; Ostrowski, 1989). The toxicity of oak is attributed to the high concentration of hydrolyzable tannins, mainly gallotannins, which are hydrolyzed in the gut to yield gallic acid (Figure 1) and other small phenolic compounds (Murdiati et al., 1992; Basden and Dalvi, 1987; Pigeon et al., 1962; Dollahite et al., 1962; Spier at al., 1987).

Several methods for the analysis of tannins have been developed (Hagerman et al., 1992; Haslam, 1966; Pandey and Makkar, 1991). Analytical methods based on the protein precipitation property of tannins, and a variety of colorimetric methods, including Folin−Ciocalteu, Folin−Denis, and Prussian Blue for total phenols, vanillin−HCl assay for catechins, and butanol−HCl assay for proanthocyanidin-based polyphenolics, have been reviewed by Makkar (1989), Tempel (1982), and Okuda et al. (1989). A quantitative method for gallotannins in plants has been described by Inoue and Hagerman (1988). Gallotannins were hydrolyzed with sulfuric acid, and gallic acid in the hydrolysate was reacted with rhodanine and then assayed spectrophotometrically. The method was the most specific of the spectrophotometric analyses but could not distinguish between gallic acid and pyrogallol.

Chromatographic methods for the analysis of gallotannins are available (Shi, 1988; Galletti and Reeves, 1992; Delahaye and Verzele, 1983; Beasley et al., 1977) The majority of them use high-performance liquid chromatography (HPLC) with UV detection. Although HPLC gives superior separation of the components of gallotannins, it lacks specificity and has high background from other common plant phenolics (Okuda et al., 1989). Additionally, HPLC does not provide sufficient information to confirm the results.



Gallic Acid [149-91-7]



Pyrogallol [87-66-1]

Figure 1. Structures and CAS Registry Numbers of gallic acid and pyrogallol.

Gas chromatography/mass spectrometry (GC/MS) following acid hydrolysis has been employed by Fechtal and Riedl (1991) for the analysis of tannins from the bark of Moroccan eucalyptus and by Arpino et al. (1977) in the analysis of tannins from the constituents of ink. The GC/MS approach offered the specificity and sensitivity necessary to identify the components of tannins; therefore, it was employed in the present study.

An objective of this study was to develop a specific method to quantitate and confirm pyrogallol in urine and serum samples and to detect gallic acid in stomach contents as an aid in the diagnosis of oak toxicosis in cattle. The method described below is designed for relatively quick turnaround of large sample sets and has been tested on samples presented to the veterinary diagnostic laboratory.

EXPERIMENTAL PROCEDURES

Apparatus and Equipment. A Hewlett-Packard (HP, Palo Alto, CA) Model 5890 gas chromatograph interfaced with an HP Model 5988A mass spectrometer and controlled by an HP ChemStation was used in all analyses. Spectra were obtained at 70 eV, with the mass range scanned from 40 to 500 amu. The analytical column was a 15 m × 0.53 mm × 0.1 um DB-1 (J&W Scientific, Folsom, CA). Helium carrier gas flow rate was 7 mL/min at 60 °C. Operating temperatures were as follows: ion source, 220 °C; transfer line, 280 °C; injector, 240 °C; GC oven program, 60 °C for 0.5 min, 5 °C/min to 110 °C, 10 °C/min to 180 °C, 30 °C/min to 275 °C, 275 °C for 1 min; run time was 21.5 min. A glass, splitless, 2 mm i.d. liner (HP part 18740-80220) with 0.5 cm loosely packed silanized glass wool was used, split/splitless injection valve

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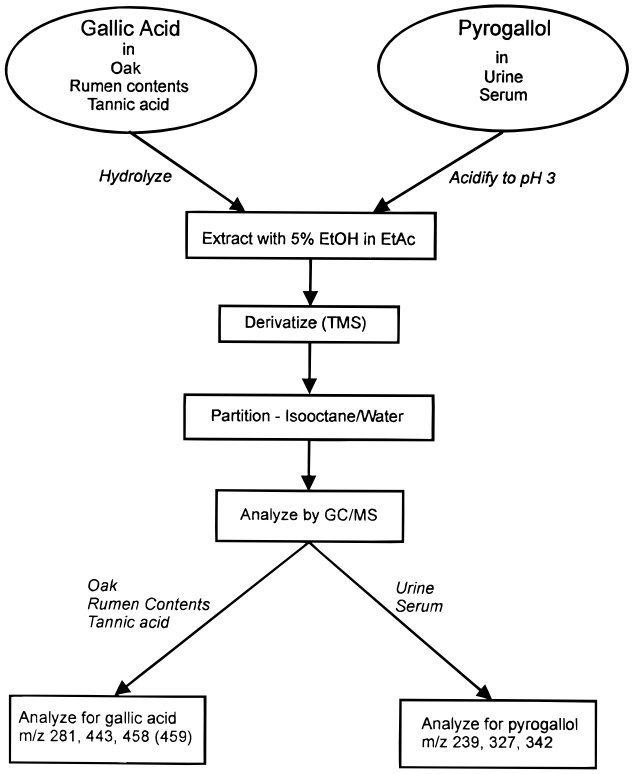

Figure 2. Flow diagram of the overall analytical procedure. EtOH, ethanol; EtAc, ethyl acetate.

off at 0.8 min. An HP Model 7673A autosampler, 2 μL injection volume, was used in all GC analyses. The MS selected ion monitoring (SIM) acquisition program was as follows: group 1 (pyrogallol), start time 5.5 min, dwell 100, ions *m/z* 239, 327, 342; group 2 (gallic acid), start time 11.0 min, dwell 100, ions *m/z* 281, 443, 458. The retention times for pyrogallol and gallic acid were 9.71 and 15.87 min, respectively.

Reagents. Deriva-sil concentrate was from Regis Chemical Co. (Morton Grove, IL). All solvents were of pesticide grade (Fisher Scientific, Fair Lawn, NJ). Sodium sulfate was of ACS reagent grade (Fisher Scientific). Tannic acid, a gallotannin (technical grade), was from Mallinckrodt Chemical Co. (St. Louis, MO).

Preparation of Standard Solutions. All analytical standards were obtained from Sigma Chemical Co. (St. Louis, MO). Standard solution mix, containing pyrogallol and gallic acid at 100 μg/mL each, was made in methanol. It was stable for 2 months, when stored at 5 °C in dark, 50 mL Qorpak bottles. Subsequent dilutions of the above mixes were made daily in methanol from the stock solution.

Procedure. A flow diagram of the overall method is presented in Figure 2. The details are as follows.

(a) Hydrolysis (Blue Oak Plant, Tannic Acid Standard, Rumen Contents). One gram of sample was weighed into a 15 mL screw-cap, disposable tube. Ten milliliters of aqueous 3% HCl (v/v) was added and the sample vortexed for 10 s. Air in the tube was replaced by blowing nitrogen on the top of the liquid and the side walls of the tube for 1 min. The tube was capped immediately and Teflon tape was wrapped around the cap to ensure a tight seal. The tube was placed in a metal test tube cage in a preheated oven set to 110 °C for 4 h. All control and fortified samples were prepared in the same manner. Rumen contents from cattle not exposed to oak and leaves from an elm tree *(Ulnus parvifolia)* not containing tannins were used for controls.

(b) Extraction (*Blue Oak Plant, Tannic Acid Standard, Rumen Contents).* After the hydrolysis, the tubes were removed from the oven and cooled for 30 min. Using 2 mL portions of 5% ethanol in ethyl acetate (v/v), the contents of the tubes were transferred quantitatively into French, squared homogenization vessels with Teflon-lined caps (Fisher Scientific, Pittsburgh, PA). To each of the hydrolyzed samples were added 100 mL of 5% ethanol in ethyl acetate (v/v) and 50 g of sodium sulfate. Samples were homogenized for 1 min at 9500 rpm using a tissue homogenizer (Model Ultra-Turrax T-25, IKA-Labortechnik/Tekmar Co., Cincinnati, OH). The extracts were centrifuged at 500 rpm (65*g*) for 5 min using an IEC Centra-7 refrigerated centrifuge (International Equipment Co., Needham, MA). Two milliliter aliquots of the extracts were pipetted into screw-cap, disposable test tubes; one drop of 5% decanol in ethyl acetate (v/v) was added, and the samples were evaporated just to dryness using a nitrogen evaporator (N-Evap, Analytical Evaporator, Organomation Associates Inc., Berlin, MA) set at 40 °C. (*Urine, Serum).* Two grams of urine or serum samples was weighed into the homogenization vessels as above. The samples were acidified to pH 3 with dropwise addition of concentrated hydrochloric acid. Thirty milliliters of 5% ethanol in ethyl acetate (v/v) and 15 g of sodium sulfate were used to extract urine and serum samples, following the homogenization and centrifugation step as above. Three milliliter aliquots of urine or serum extracts were evaporated to dryness as above.

(c) Derivatization. Deriva-sil concentrate (100 μL) was added to the dry extracts, and the solutions were vortexed for 10 s to ensure complete dissolution of the residues. The test tubes were capped tightly and placed in a 70 °C water bath for 45 min.

(d) Liquid/Liquid Cleanup. Derivatized samples were dissolved in 1 mL of isooctane. Equal amounts of distilled water were added, and the samples were vortexed for 15 s and centrifuged at 2300 rpm (1200*g*) for 5 min. Approximately 100 μL of the isooctane layer was transferred into an autosampler vial with a disposable pipet.

(e) GC/MS Determination of Gallic Acid and Pyrogallol. Analytical standard mixes containing trimethylsilyl (TMS) derivatives of pyrogallol and gallic acid at concentrations ranging from 0.5 to 7.5 μg/mL of each compound were injected into the GC/MS system described above. Each set of samples analyzed also contained a reagent blank, a control, and a fortified sample. Quantitation was performed using external calibration based on injections of 1−15 ng of standards.

Method Validation. The method was validated by analyzing bovine serum and urine samples ($n = 6$) fortified with pyrogallol and gallic acid at a 5 ppm level. Five replicate fortifications of hydrolyzed elm control at 100 ppm were analyzed for gallic acid using the method described above. Samples of urine, serum, and rumen contents were collected from 10 control animals to examine the background level of these compounds in animals not exposed to any species of oak. The method was also tested by analyzing samples from heifers dosed with blue oak and commercial tannic acid.

Dosing Trials. Ten kilograms of fresh blue oak plant (young shoots from mature trees, approximately 20 cm long) was collected in Colusa County, California, in July of 1993. The oak plant was ground ($^3/_{16}$ in. mesh), diluted approximately 1:4 with distilled water, and administered to a 65 kg holstein heifer at 15 g/kg of body weight via intraruminal tube. Tannic acid (122 g) was diluted approximately 1:4 with distilled water and dosed to a 61 kg holstein heifer at 2 g/kg of body weight as above. Samples were collected as follows: urine samples were collected by free catch from both heifers at time 0 (predose), 2.5, 5, 10, 24, and 48 h. Serum samples were obtained from blood collected from the jugular vein with a vacutainer needle and tube at time intervals as above. Rumen contents were collected with nasogastric tube. All samples were kept frozen at −20 °C until analyzed.

RESULTS AND DISCUSSION

Clinical Signs. The heifer dosed with blue oak had no adverse clinical signs, and the animal given tannic acid had mild gastrointestinal upset which resolved 24 h after dosing.

Quantitation of Urinary Metabolites. The analysis of urine samples for gallic acid resulted in the discovery of large quantities of pyrogallol in the majority

Table 1. Pyrogallol (PG) and Gallic Acid (GA) Levels in Urine and Serum Samples of Heifers Dosed with Tannic Acid (TA) and Blue Oak (*Q. douglasii*)

dose (g/kg)	time after dose (h)	urine		serum	
		PG (ppm)	GA (ppm)	PG (ppm)	GA (ppm)
2, TA	0 (pre)	ND[a]	ND	ND	ND
	2.5	360	7.9	8.2	ND
	5	1230	7.8	15.0	2.1
	10	1250	5.7	15.0	ND
	24	350	ND	1.3	ND
	48	0.6	ND	ND	ND
15, oak	0 (pre)	ND	ND	ND	ND
	2.5	270	0.6	1.7	ND
	5	340	ND	2.0	ND
	10	72	ND	ND	ND
	24	ND	ND	ND	ND
	48	ND	ND	ND	ND

[a] ND, not detected, below MDL of 0.5 ppm.

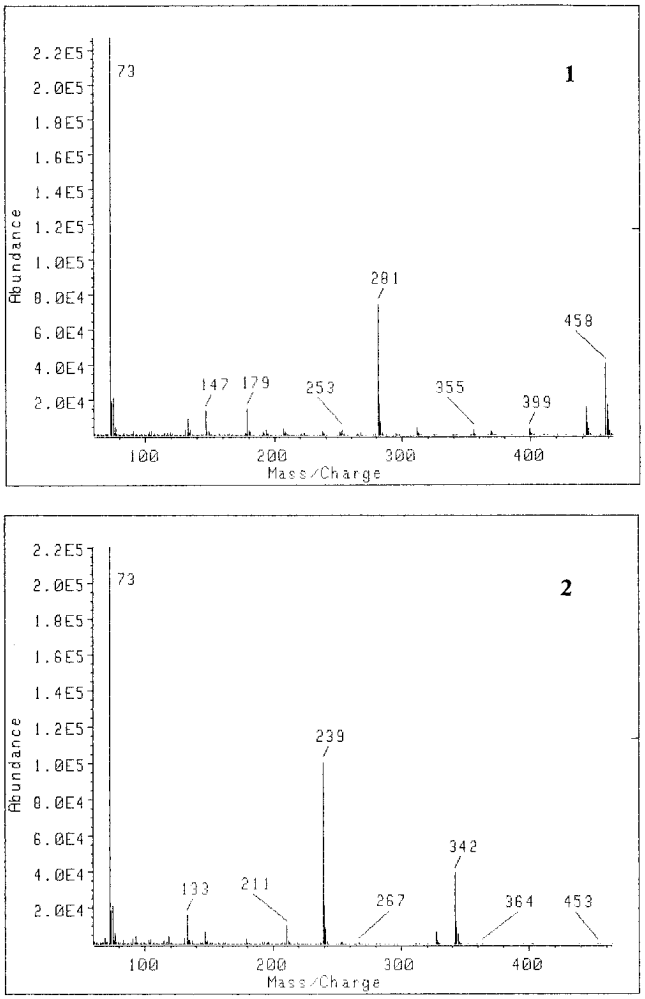

Figure 3. Electron impact ionization mass spectra of TMS derivatives of gallic acid (1) and pyrogallol (2).

of the samples from both dosed animals (Table 1). The preliminary data suggest that gallotannins are eliminated from the body 48 h after dosing. Urine samples collected from 10 control cattle had no detectable levels of pyrogallol or gallic acid. Pyrogallol has been previously identified as one of the metabolites of gallotannins in the urine of sheep dosed with yellow-wood tree *(Terminalia oblongata)* by Murdiati et al. (1992).

Figure 3 shows the mass spectra of the TMS derivatives of pyrogallol and gallic acid standards. The selective ion monitoring program used for all quantitations included the molecular ions *m*/*z* 342 (pyrogallol)

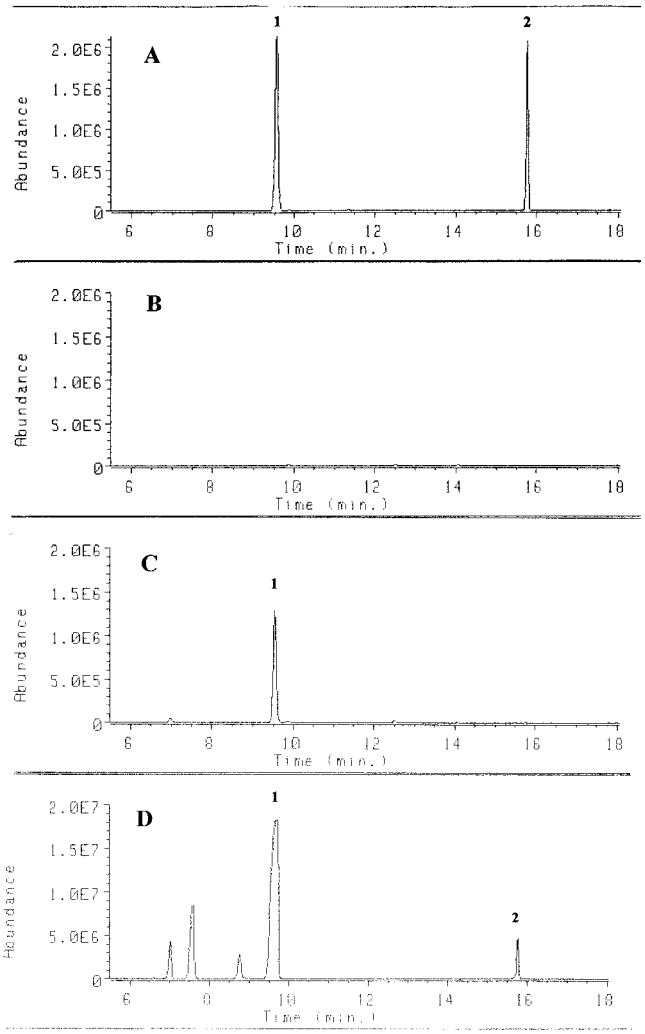

Figure 4. Typical GC/MS-SIM chromatograms of the TMS derivatives of pyrogallol and gallic acid in (A) standard mix, 10 ng each in 0.4 mg of serum matrix; (B) control serum, 0.4 mg; (C) serum from a heifer dosed with blue oak; and (D) urine from a heifer dosed with tannic acid. 1, pyrogallol; 2, gallic acid.

and *m*/*z* 458 (gallic acid). The criteria for choosing other ions were high abundance and no interferences from the matrix components. Figure 4 shows typical chromatograms of TMS derivatives of pyrogallol and gallic acid in serum and urine matrices obtained by running the SIM program. A comparison of the chromatograms clearly shows the presence of a large amount of pyrogallol (1) in serum. Urine samples from dosed animals had a large amount of pyrogallol (1) in comparison with the amount of gallic acid (2). The ion ratios of pyrogallol and gallic acid in samples matched those of the standards and spikes within 5%, thus confirming the presence of these compounds in the samples.

Determination of Gallic Acid in Blue Oak, Tannic Acid Standard, and Rumen Contents. The levels of gallic acid liberated during acid hydrolysis of samples containing gallotannins are listed in Table 2. Blue oak collected in Colusa County, California, used for the dosing experiment described above, had a background level before hydrolysis of 64 ppm of gallic acid (GA). After 4 h of hydrolysis at 110 °C in 3% aqueous HCl, the level of GA increased to 1421 ppm ($n = 3$, CV = 3.8%). Further hydrolysis carried out for up to 25 h resulted in a linear increase of the GA up to 20 h, at which time the hydrolysis was complete (2533 ppm,

Table 2. Gallic Acid (GA) Levels in Blue Oak Plant (*Q. douglasii*), Tannic Acid (TA) Standard, and Rumen Contents from Dosed Heifers before (Fresh) and after Hydrolysis in 3% HCl, 110 °C for 4 h

matrix type	GA (ppm) fresh	GA (ppm) hydrolyzed
blue oak, Colusa County, CA	64	1421
rumen content controls ($n = 10$)	ND[a]	ND
rumen fluid A[b]	ND	7.6
rumen fluid B[c]	ND	85.3
TA, Mallinckrodt, lot 1674 KJTS	1500	233,000

[a] ND, below detection limit of 5 ppm. [b] Rumen fluid collected from a heifer 10 h after dosing with blue oak at 15 g/kg. [c] Rumen fluid collected from a heifer 10 h after dosing with tannic acid at 2 g/kg.

Table 3. Average Recoveries ($n = 6$) and Coefficients of Variation (% CV) of Gallic Acid (GA) and Pyrogallol (PG) from Bovine Urine and Serum Samples Fortified at 5 ppm Level

compound	urine % recovery (% CV)	serum % recovery (% CV)
gallic acid[a]	92 (11)	91 (13)
pyrogallol[b]	84 (3)	90 (5)

[a] Results collected over 6 month period from quality control of diagnostic samples. [b] Results obtained during the validation of the method.

$n = 3$, CV = 4.2%). From the practical standpoint of the diagnostic laboratory 4 h of hydrolysis time was chosen due to the need for fast turn around times. A shorter hydrolysis time (4 h) was chosen for the GC/MS analysis of hydrolyzable tannins by Fechtal and Riedl (1991) and Arpino et al. (1977). Four hours of hydrolysis time was sufficient to detect gallic acid in the rumen contents from the heifers dosed with blue oak and tannic acid standard in this trial. The stability of gallic acid during the hydrolysis was measured by spike recovery studies of fortified plant material analyzed along with the samples. Five replicates of plant matrix fortifications at 100 ppm of gallic acid gave an average recovery of 95% with 4% CV (relative standard deviation) after 4 h of hydrolysis. During hydrolysis elm tree control spiked with 500 ppm of tannic acid released 116 ppm of gallic acid ($n = 3$, 3.5% CV). Rumen content samples collected from 10 cows not exposed to oak (controls) had no detectable gallic acid.

Method Performance. Several extraction techniques were investigated during the course of method development. Solvent extractions with acetone and 5% ethanol in ethyl acetate resulted in the best spike recoveries of pyrogallol and gallic acid. A mixture of 5% ethanol in ethyl acetate was chosen for the final method because it provided the cleanest extract, with minimum coextractives from hydrolyzed plant material. It was important to adjust the pH with hydrochloric acid as the use of sulfuric acid resulted in large chromatographic interferences. Table 3 summarizes the overall spike recoveries from control bovine urine and serum samples fortified at 5 ppm with pyrogallol and gallic acid. Addition of the keeper (decanol) was necessary for acceptable spike recoveries of pyrogallol from urine and serum.

A challenging aspect of this work was the development of a derivatization method that would provide good reproducibility, linearity, and stability of derivatives. Several TMS derivatization agents were evaluated. Matrix, different solvents, and the addition of pyridine as a catalyst created great variability in derivatization yields (10−75% CV between batches) and nonlinearity. Derivatization of gallic acid and pyrogallol in various extracts with concentrated Deriva-sil gave the best reproducibility among all conditions examined in this study. During a 6 month period batch to batch derivatization reproducibility was 5% for pyrogallol and less than 15% CV for gallic acid. Three batches of gallic acid standards derivatized within the same day gave a CV of 1.3%. The standard curves were linear, with r^2 ranging from 0.9990 to 0.9999 for pyrogallol and from 0.9940 to 0.9998 for gallic acid. Matrix can enhance the GC/MS response up to 10%. For optimum accuracy, samples should be quantitated against standards derivatized in a similar matrix. The pyrogallol−TMS derivatives were stable for 72 h and the gallic acid−TMS derivatives for 24 h. Injections of derivatized samples did not produce adverse effects on the GC/MS system. After 440 injections of sample extracts, there was no sign of a general decrease in column or instrument performance. Retention times over the course of the study varied by no more than 1%, and the peak areas for standard injections varied by less than 10%.

The sensitivity and specificity of the method compare favorably with those of existing methods for hydrolyzable gallotannins. Injections of 0.2 ng each of pyrogallol and gallic acid standards gave instrument response of signal/noise 1:100. The limits of detection in all sample types were sufficiently low to indicate the exposure of the cattle to blue oak even when the clinical signs were mild. The chromatographic resolution of the standard mix from the sample components by the megabore GC column was superior to those reported previously for packed columns (Shi, 1988). Gallic acid and pyrogallol were easily detected, quantitated and confirmed in 20 samples per day. The drawback of the method is the lack of commercially available, pure, hydrolyzable gallotannin standard to test the accuracy and precision of the gallotannin hydrolysis step. This problem is encountered in all methods for quantitation of gallotannins in plants.

A further feeding study is needed to better interpret the quantitative results from diagnostic samples. Understanding of the toxicokinetics of pyrogallol in the urine and serum will shed light on the distribution and elimination of tannin and perhaps a greater understanding of the mechanism of oak toxicosis.

Conclusions. The method proved to be suitable for the quantitative analysis of pyrogallol and the detection of gallic acid in a variety of matrices. It was validated by spiking experiments and also by application to analysis of samples from heifers dosed with tannic acid and blue oak. Both the sensitivity (MDL = 0.5 ppm) and specificity (GC/MS) of the method enable it to be applied to the diagnosis of oak exposure in animals.

LITERATURE CITED

Anderson, G. A.; Mount, M. E.; Vrins, A. A.; Ziemer, E. L. Fatal acorn poisoning in a horse: pathologic findings and diagnostic considerations. *J. Am. Vet. Med. Assoc.* **1983,** *182,* 1105−1110.

Arpino, P.; Moreau, J. P.; Oruezabal, C.; Flieder, F. Gas chromatographic-mass spectrometric analysis of tannin hydrolysates from the ink of ancient manuscripts (XIth to XVIth century). *J. Chromatogr.* **1977,** *134,* 433−439.

Basden, K. W.; Dalvi, R. R. Determination of total phenolics in acorns from different species of oak trees in conjunction with acorn poisoning in cattle. *Vet. Hum. Toxicol.* **1987,** *29,* 305−306.

Beasley, T. H.; Ziegler, H. W.; Bell, A. D. Determination and characterization of gallotannin by high performance liquid chromatography. *Anal. Chem.* **1977,** *49,* 238−243.

Delahaye, P.; Verzele, M. Analysis of gallic, digallic and trigallic acids in tannic acid by high-performance liquid chromatography. *J. Chromatogr.* **1983,** *265,* 363−367.

Dollahite, J. W.; Pigeon, R. F.; Camp, B. J. The toxicity of gallic acid, pyrogallol, tannic acid, and *Quercus havardi* in the rabbit. *Am. J. Vet. Res.* **1962,** *23,* 1264−1267.

Fechtal, P. M.; Riedl, B. Analyse des Extraits Tannants des Ecorces des Eucalyptus apres Hydrolyse Acide par la Chromatographie en Phase Gazeuse Couplee avec la Spectrometrie de Masse (GC-MS) (Analysis of tannin extracts after acid hydrolysis from Eucalyptus bark by gas chromatography coupled with mass spectrometry (GC-MS)). *Holzforschung* **1991,** *45,* 269−273.

Galletti, G. C.; Reeves, J. B. Pyrolysis/gas chromatography/ion-trap detection of polyphenols (vegetable tannins): preliminary results. *Org. Mass Spectrom.* **1992,** *27,* 226−230.

Garg, S. K.; Makkar, H. P.; Nagal, K. B.; Sharma, S. K.; Wadhwa, D. R.; Singh, B. Oak (*Quercus incana*) leaf poisoning in cattle. *Vet. Hum. Toxicol.* **1992,** *34,* 161−163.

Hagerman, A. E.; Robbins, C. T.; Weerasuriya, Y.; Wilson, T. C.; McArthur, C. Tannin chemistry in relation to digestion. *J. Range Manage.* **1992,** *45,* 57−62.

Haslam, E. *The Scope of Vegetable Tannin Chemistry. Chemistry of Vegetable Tannin*; Academic Press: London, 1966; pp 1−14.

Inoue, K. H.; Hagerman, A. E. Determination of gallotannin with rhodanine. *Anal. Biochem.* **1988,** *169,* 363−369.

Kasari, T. R.; Pearson, E. G.; Hultgren, B. D. Oak (*Quercus garryana*) poisoning of range cattle in southern Oregon. *Compend. Cont. Ed. Pract. Vet.: Food Anim.* **1986,** *8,* F17−F24.

Makkar, H. P. Protein precipitation methods for quantitation of tannins: a review. *J. Agric. Food Chem.* **1989,** *37,* 1197−1202.

Murdiati, T. B.; McSweeney, C. S.; Lowry, J. B. Metabolism in sheep of gallic acid, tannic acid and hydrolysable tannin from *Terminalia oblongata. Aust. J. Agric. Res.* **1992,** *43,* 1307−1319.

Okuda, T.; Yoshida, T.; Hatano, T. New methods of analyzing tannins. *J. Nat. Prod.* **1989,** *52,* 1−31.

Ostrowski, S. R.; Smith, B. P.; Spier, S. J.; Norman, B. B.; Oliver, M. N. Compensatory weight gain in steers recovered from oak bud toxicosis. *J. Am. Vet. Med. Assoc.* **1989,** *195,* 481−484.

Osweiler, G. D.; Carson, T. L.; Buck, W. B.; Van Gelder, G. A. *Clinical and Diagnostic Veterinary Toxicology*; Kendall Hunt Publishing: Dubuque, IA, 1985; pp 495.

Pandey, R. K.; Makkar, H. P. Variation of tannins in oak leaves. *Biochem. Physiol. Pflanzen.* **1991,** *187,* 392−394.

Pigeon, R. F.; Camp, B. J.; Dollahite, J. W. Oral toxicity and polyhydroxyphenol moiety of tannin isolated from *Quercus havardi* (shin oak). *Am. J. Vet. Res.* **1962,** *23,* 1268−1270.

Shi, Z. C. Identification of the phenolic substances in bovine urine associated with oak leaf poisoning. *Res. Vet. Sci.* **1988,** *45,* 152−155.

Spier, S. J.; Smith, B. P.; Seawright, A. A.; Norman, B. B.; Ostrowski, S. R.; Oliver, M. N. Oak toxicosis in cattle in northern California: clinical and pathologic findings. *J. Am. Vet. Med. Assoc.* **1987,** *191,* 958−964.

Tempel, A. S. Tannin-measuring techniques: a review. *J. Chem. Ecol.* **1982**, *8,* 1289−1298.

Zhu, J.; Filippich, J. Acute intra-abomasal toxicity of tannic acid in sheep. *Vet. Human Toxicol.* **1995,** *37,* 50−54.

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