# Determination of Alkaloid Exposure in a Model Ruminant (Goat) Using a Multiresidue Screening Method

Dirk M. Holstege,*,† Francis D. Galey,† Bill Johnson,† and James N. Seiber‡,§

Toxicology Laboratory, California Veterinary Diagnostic Laboratory System, University of California, Davis, California 95616, and Department of Environmental Toxicology, University of California, Davis, California 95616

A new multiresidue analytical screen for estimating alkaloid exposure in livestock has been evaluated on biological samples from goats dosed with sublethal amounts of five plants known to cause acute poisoning in animals. The plant species selected were *Conium maculatum* (poison hemlock), *Nicotiana glauca* (tree tobacco), *Delphinium barbeyi* (larkspur), *Datura wrightii* (jimsonweed), and *Taxus baccata* (English yew). Animals were euthanized when toxic signs developed or when 3−7 hours had passed after the dose. The liver, kidney, rumen contents, abomasal contents, urine, and serum from each animal were examined for the presence of alkaloids. Alkaloid contents were determined by GC with nitrogen−phosphorus detection, GC/MS, and a modified commercial thin layer chromatography system. Alkaloids from the plants were detected at levels greater than 1 $\mu$g/g in samples of rumen and abomasal contents and in most urine, kidney and liver samples. No alkaloids were detected in serum samples at concentrations greater than 0.5 $\mu$g/g. The multiresidue screening method enabled identification and quantitation of alkaloids in biological samples from goats dosed with sub-lethal amounts of the five plants.

**Keywords:** *Alkaloid; analysis; Delphinium barbeyi; Conium maculatum; Nicotiana glauca; Datura wrightii; Taxus baccata; ruminant; goat*

## INTRODUCTION

Sudden death or nonspecific illness in animals can be caused by ingestion of numerous alkaloid-containing plants, which can cause severe and varied physiological effects (Cheeke and Shull, 1985). In a veterinary diagnostic setting, exposure to alkaloid-containing plants is a possibility that must always be considered in diagnosing sudden death in an animal. Plants of particular interest in the western United States include *Conium maculatum* (poison hemlock), *Nicotiana glauca* (tree tobacco), *Delphinium barbeyi* (tall larkspur), *Datura wrightii* (also *Datura metaloides*, jimsonweed), and *Taxus baccata* (English yew).

*C. maculatum*, which contains piperidine alkaloids such as coniine and $\gamma$-coniceine, has been responsible for bovine losses (Galey et al., 1992; Panter and Keeler, 1989) and can be toxic to cattle at 5−40 g/kg of body weight (Kingsbury, 1964). Repeated doses of 10 g/kg were toxic to sheep (Panter and Keeler, 1989). *N. glauca* contains the toxic pyridine alkaloid nicotine in minor quantities and the pyridylpiperidine teratogen anabasine in major quantities (Plumlee et al., 1993; Bush and Crowe, 1989). *Delphinium* species can be toxic to cattle at 5 g/kg and to sheep at 30 g/kg (Olsen and Manners, 1989; Kingsbury, 1964) and contain numerous diterpenoid alkaloids such as deltaline, a major constituent of *D. barbeyi* (Manners and Ralphs, 1989). *D. wrightii* contains tropane alkaloids, such as hyoscyamine and scopolamine, and can be toxic to cattle at 0.6 to 0.9 g/kg (Kingsbury, 1964). *T. baccata* is toxic to ruminants at 5 g/kg (Kingsbury, 1964; Panter et al., 1993) because of the presence of taxine alkaloids, such as taxine A (Graf et al., 1982).

A new alkaloid multiresidue method (Holstege et al., 1995) can successfully quantitate alkaloid concentrations in biological samples. Tissue, ingesta, and fluid samples from animals with known exposure to alkaloid-containing plants need to be analyzed to determine the most suitable types of samples from which to make a diagnosis and to establish minimum detection limits. The present paper describes the application of the alkaloid multiresidue method (MRM) to samples from goats dosed with five alkaloid-containing plants known to cause sudden death or nonspecific signs of poisoning in animals. The study was conducted in two parts. In the first stage, the animals were dosed at low levels and serum was collected over a 48 h period. The second stage of the study involved dosing at higher levels, after which biological samples from the euthanized animals were collected and tested using the alkaloid MRM. Quantitative results were obtained using gas chromatography with nitrogen−phosphorus detection (GC/NPD), while qualitative results were provided by gas chromatography/mass spectrometry (GC/MS) and modified commercial thin layer chromatography (TLC).

## MATERIALS AND METHODS

**Materials.** Hyoscyamine, scopolamine, coniine, nicotine, and anabasine analytical standards were purchased from Sigma Chemical Co. (St. Louis, MO). Deltaline and methyllycaconitine were provided by Dr. G. D. Manners, Agricultural Research Service, Albany, CA, and $\gamma$-coniceine was provided by Dr. K. E. Panter, Agricultural Research Service, Logan, UT. Neat alkaloid (25 mg) was dissolved in methanol to make a 1 mg/mL standard solution. Subsequent dilutions were made using methanol for spiking standards or ethyl acetate for analytical standards.

Small amounts of delpheline, deltamine, dictyocarpine, lycoctonine, and 14-acetyldictyocarpine were also provided by

† California Veterinary Diagnostic Laboratory System.
‡ Department of Environmental Toxicology.
§ Present address: Center for Environmental Sciences and Engineering, University of Nevada, Reno, NV 89557.

S0021-8561(95)00091-4 CCC: $12.00 

**Table 1. Goat Dosing Schedule, Including Plant Type, Dosing Levels, Time of Animal Euthanization following Dose 2, and Plant Moisture Content**

| goat | plant | dose 1 (g/kg) | dose 2 (g/kg) | euthanization time (h) | plant moisture (%) |
|---|---|---|---|---|---|
| 1 | *C. maculatum* | 5 | 20 | 3 | 81 |
| 2 | *C. maculatum* | 5 | 20 | 5 | 81 |
| 3 | *C. maculatum* | 5 | 20 | 7 | 81 |
| 4 | *D. wrightii* | 0.3 | 10 | 4 | 86 |
| 5 | *N. glauca* | 10 | 15 | 5 | 82 |
| 6 | *D. barbeyi* | 1 | 10 | 6 | 6 |
| 7 | *T. baccata* | 4 | 10 | 6 | 61 |
| 8 | alfalfa pellets (control) | | | 3 | |

Dr. G. D. Manners. Neat alkaloid (0.1−1 mg) was dissolved in 100 $\mu$L of methanol for GC/MS qualitative analysis.

All solvents were of pesticide grade (Fisher). Sodium sulfate, sodium chloride, sodium hydroxide, sodium nitrite, and hydrochloric acid were of ACS grade (Fisher).

**Analysis.** The analysis followed that of Holstege et al. (1995). Chopped samples (5 g) were extracted with 100 mL of 5% ethanol in ethyl acetate (v/v) after the addition of 1 mL of 10 N NaOH and 50 g of $Na_2SO_4$. A 40 mL aliquot was extracted with a total volume of 15 mL of 0.5 N HCl after the addition of 100 mL of hexane. The aqueous extract was sparged with a stream of $N_2$, the pH was increased to >10 with 10 N NaOH, and the extract was adsorbed on a polymeric $C_{18}$ SPE column (Interaction Chemicals Inc., Mountain View, CA) under suction. The alkaloids were eluted with 2 mL of ethyl acetate. This extract was analyzed quantitatively using GC/NPD (Autosystem, Perkin-Elmer) with a 5 m × 0.53 mm × 1.0 $\mu$m DB-5 capillary column (J&W Scientific). Semiquantitative analysis was performed by GC/MS (Model HP 5890 with HP 5970 MSD, Hewlett-Packard) using a 12 m × 0.2 mm × 0.33 $\mu$m HP-1 capillary column (Hewlett-Packard). Alkaloids for which standards were not available were quantitated using the response factors of similar compounds. Qualitative analysis was performed by a modified commercial TLC system (Toxi-Lab A, Toxi-Lab Division of Marion Laboratories, Laguna Hills, CA) developed with ethyl acetate−diethylamine (95:5 v/v) and visualized with potassium iodide/iodine/bismuth subnitrate solution (Toxi-Dip A-3 reagent, Dragendorff's reagent) followed by 5% aqueous sodium nitrite (w/v).

**Dosing Study.** Nine female, adult, mixed breed goats were dosed in two stages with plant material (Table 1). Three goats were dosed with *C. maculatum*, and one goat each was dosed with *D. wrightii, N. glauca, D. barbeyi*, and *T. baccata*. One goat was used as a control. The *D. barbeyi* was collected in the Manti La Sal National Forest, Utah, in August 1992, and was dried and milled prior to dosing. The other plants were collected in Yolo County, California, in June 1993. Fresh plants were frozen, ground in a Hobart food processor, and refrozen for storage prior to dosing. Dosing was performed on a wet weight basis (Table 1). Plant material was diluted approximately 1:4 with water and pumped into the rumen of the goat. Food was withheld from the goats for 36 h prior to dosing. The goats were dosed twice. The first dose was at a low level to permit collection of serum samples. Samples were collected at 15 and 30 min and at 1, 2, 4, 8, 12, 24, and 48 h after dosing. The second dose was 1 week later at a higher level, for collection of typical samples used for analytical toxicological testing and pathology.

## RESULTS AND DISCUSSION

The alkaloid multiresidue screen was able to detect alkaloids in the rumen contents and abomasal contents of each goat (Table 2). The primary alkaloids of each plant were also detected in the liver, kidney, and urine of the goats dosed with *C. maculatum*, *N. glauca*, and *D. barbeyi*. Trace amounts of alkaloids were detected in the urine of the goats dosed with *D. wrightii* and *T. baccata*. Rumen contents, abomasal contents, and urine appeared to be the most useful in diagnosing sublethal exposure to these alkaloid-containing plants.

**Dose One.** No alkaloids were found in any of the first-dose serum samples at >0.1 ppm. Spiking of serum at low levels (0.1−0.5 ppm) gave >70% recovery for all alkaloids for which standards were available. Clinical signs following dosing were mild. The goat dosed with *Nicotiana* was able but unwilling to stand for 1 h following dose 1 but subsequently recovered. The goat dosed with *Delphinium* had reduced gastrointestinal motility for 48 h following dosing. Serum appeared to be a poor choice for determination of alkaloid exposure. Conjugated alkaloids, if present, would not be detected without a hydrolysis step, which was not included in the MRM employed.

**Dose Two.** *Pathology*. Postmortem examination revealed no grossly visible abnormalities other than a mild abomasal parasitism. Microscopically, goats dosed with *Conium*, *Taxus*, and *Nicotiana* had mild pulmonary edema, characterized by nonstaining separation of the adventitia surrounding medium-sized arterioles. No alveolar change was noted. The low grade edema in the lungs observed in some animals is difficult to evaluate. It was not present in the control animal. The animals were not *in extremis*, nor were they stressed just prior to euthanasia, suggesting that the edema may have been related to the dosed plant material. The goat receiving *Delphinium* had a very subtle multifocal degenerative myopathy in the heart. The changes were acute, and the morphologic pattern of the degenerative change was not that of an infectious disease process.

*C. maculatum*. Repeated daily doses of fresh *C. maculatum* at 3.5 g/kg can cause moderate to severe clinical toxicosis in goats. The most severe signs occur 15 min to 2 h after dosing. Clinical signs include prolonged muscular weakness (Panter et al., 1990). Repeated doses of *Conium* in sheep produce prolonged trembling, muscular weakness, ataxia, frequent urination and defecation, and excessive salivation and are lethal at 10 g/kg (Panter et al., 1988). *C. maculatum* has been reported to be lethal to cattle at 5.3 g/kg (Keeler and Balls, 1978). In the present study, three goats were given a single dose of *Conium* at 20 g/kg of body weight. The goats had mild depression and had appeared to have recovered from the dose when euthanized at 3, 5, and 7 h after dosing.

Alkaloids found in *C. maculatum* include coniine, $\gamma$-coniceine, *N*-methyl coniine, conhydrine, conhydrinone, pseudoconhydrine, and *N*-methyl pseudoconhydrine (Panter and Keeler, 1989). Several alkaloids were identified via GC/MS analysis (Table 3). Peak 4 had the same side-chain losses as $\gamma$-coniceine with ions at M − 15 and M − 28, with a molecular ion 14 Da larger than $\gamma$-coniceine. The high relative intensity of the molecular ion and strong M − 28 ion are consistent with a conjugated system, such as in 1′-oxo-$\gamma$-coniceine. Peak 5 had a spectrum consistent with *N*-methyl-$\beta$-coniceine, with a spectral pattern similar to that of *N*-methylconiine, an M − 29 ion, and a large intact ring fragment at *m*/*z* 98. While peak 6 had an apparent molecular ion of *m*/*z* 99, which is consistent with methylpiperidine, it had a GC retention time later than that of coniine. Peak 6 was possibly a fragment ion of a compound with a larger molecular weight. The abundant ion at *m*/*z* 98 is consistent with an *N*-methylconiine derivative. Peak 8 was found only in the urine.

**Table 2. Summary of Qualitative, Semiquantitative, and Quantitative Analyses (in Parts per Million) of Plants and Post-Mortem Samples from Goats following Dosing with Alkaloid-Containing Plants**

| | | | rumen contents | | | abomasal contents | | | kidney | | | liver | | | urine | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| peak | | plant | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| | | | | | | Goats 1−3: *C. maculatum* | | | | | | | | | | | |
| 1 | coniine[a] | 110 | 4.6 | 6.7 | 6.7 | 2.2 | 1.7 | 2.4 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 1.6 |
| 2 | $\gamma$-coniceine[a] | 920 | 37 | 60 | 21 | 27 | 25 | 8.2 | 8.7 | 9.4 | 1.4 | 1.5 | 1.1 | 1.5 | 11 | 14 | 120 |
| 3 | *N*-methylconiine[b] | 550 | 26 | 41 | 23 | 15 | 11 | 9.9 | 1.2 | 0.3 | 0.7 | 1.6 | 1.0 | − | 3.3 | 0.6 | 10 |
| 4 | 1′-oxo-$\gamma$-coniceine[b] | 1.5 | 1.1 | 1.3 | 1.3 | − | 0.6 | 0.6 | − | − | − | 0.6 | − | − | 9.2 | 3.0 | 120 |
| 5 | *N*-methyl-$\beta$-coniceine[b] | 3.0 | 0.2 | − | − | − | − | − | − | − | − | − | − | − | 2.7 | 1.4 | 1.0 |
| 6 | *Conium* alkaloid A[b] | 19 | 1.8 | 1.7 | 0.9 | − | − | − | − | − | 1.7 | − | − | − | − | − | 0.8 |
| 7 | *N*-methylpseudoconhydrine[b] | 5.0 | 0.5 | 0.6 | 0.9 | − | − | − | − | − | − | − | − | − | − | − | − |
| 8 | *Conium* metabolite A[b] | − | − | − | − | − | − | − | − | − | − | − | − | − | 1.8 | 0.7 | 1.0 |
| | | | | | | Goat 4: *D. wrightii* | | | | | | | | | | | |
| 1 | scopine[b] | 1.2 | | − | | | − | | | − | | | − | | | − | |
| 2 | *Datura* alkaloid A[b] | 1.3 | | − | | | − | | | − | | | − | | | − | |
| 3 | 3-OH-6-tigloyloxytropane[b] | 2.6 | | − | | | − | | | − | | | − | | | − | |
| 4 | *Datura* alkaloid B[b] | 8.8 | | 0.8 | | | − | | | − | | | − | | | − | |
| 5 | meteloidine[b] | 16 | | − | | | − | | | − | | | − | | | − | |
| 6 | aponoratropine[b] | 14 | | 2.0 | | | − | | | − | | | − | | | − | |
| 7 | apoatropine[b] | 3.1 | | 1.1 | | | − | | | − | | | − | | | − | |
| 8 | phenylacetoxyscopane[b] | 2.3 | | − | | | − | | | − | | | − | | | − | |
| 9 | aposcopolamine[b] | 13 | | 0.9 | | | 0.1 | | | − | | | 0.1 | | | 11 | |
| 10 | hyoscyamine[a] | 58 | | 7.4 | | | 1.1 | | | − | | | − | | | − | |
| 11 | noratropine[b] | 250 | | 19 | | | 2.4 | | | − | | | − | | | − | |
| 12 | scopolamine[a] | 260 | | 9.6 | | | 2.4 | | | − | | | − | | | 0.7 | |
| 13 | *Datura* metabolite A[b] | − | | − | | | − | | | − | | | − | | | 4.8 | |
| 14 | *Datura* metabolite B[b] | − | | 0.1 | | | − | | | 0.1 | | | 0.1 | | | 23 | |
| | | | | | | Goat 5: *N. glauca* | | | | | | | | | | | |
| 1 | nicotine[a] | − | | − | | | − | | | − | | | − | | | − | |
| 2 | *Nicotiana* alkaloid A[b] | 11 | | − | | | − | | | − | | | − | | | − | |
| 3 | anabasine[a] | 430 | | 20 | | | 10 | | | 2.0 | | | 4.3 | | | 8.3 | |
| 4 | *Nicotiana* alkaloid B[b] | 1.9 | | − | | | − | | | − | | | − | | | − | |
| | | | | | | Goat 6: *D. barbeyi* | | | | | | | | | | | |
| 1 | delpheline[b] | 340 | | 9.7 | | | 2.8 | | | − | | | − | | | − | |
| 2 | deltamine[b] | 420 | | 8.0 | | | 7.8 | | | − | | | − | | | − | |
| 3 | deltaline[a] | 11000 | | 430 | | | 260 | | | 1.7 | | | 2.1 | | | 6.5 | |
| 4 | 14-acetyldictyocarpine[b] | 500 | | 16 | | | 9.2 | | | − | | | − | | | − | |
| 5 | dictyocarpine[b] | 2600 | | 110 | | | 50 | | | − | | | − | | | 2.1 | |
| 6 | lycoctonine[b] | 3600 | | 180 | | | 74 | | | − | | | − | | | − | |
| 7 | dehydrobrowniine[b] | 700 | | 22 | | | 6.9 | | | − | | | − | | | − | |
| 8 | *Delphinium* alkaloid A[b] | 260 | | 4.9 | | | 1.4 | | | − | | | − | | | − | |
| | | | | | | Goat 7: *T. baccata* | | | | | | | | | | | |
| | *Taxus* alkaloid *A*[c] | +++ | | ++ | | | − | | | − | | | − | | | − | |
| | *Taxus* alkaloid B[c] | +++ | | ++ | | | − | | | − | | | − | | | − | |
| | *Taxus* alkaloid C[c] | +++ | | ++ | | | + | | | (+) | | | − | | | − | |
| | *Taxus* alkaloid c[c] | +++ | | ++ | | | − | | | − | | | − | | | − | |

[a] Quantitative analysis using GC/NPD analysis with GC/MS confirmation. [b] Semiquantitative analysis using GC/MS analysis. [c] TLC analysis (+++, highly positive; ++, positive; +, slightly positive; (+), trace amounts; −, not detected).

**Table 3. Summary of Compounds Found in *C. maculatum* with Mass Spectral and Column Retention Data**

| peak | compound | RT (min) | note | characteristic ions (abundance %) |
|---|---|---|---|---|
| 1 | coniine | 3.3 | *a* | 127 ($M^+$, 2), 126 (2), 98 (2), 84 (100), 70 (7), 56 (30) |
| 2 | $\gamma$-coniceine | 3.7 | *a* | 125 ($M^+$, 11), 124 (8), 110 (29), 97 (100), 82 (12), 70 (24) |
| 3 | *N*-methylconiine | 3.8 | *b* | 141 ($M^+$, 1), 112 (1), 99 (7), 98 (100), 70 (21) |
| 4 | 1′-oxo-$\gamma$-coniceine | 4.2 | *c* | 139 ($M^+$, 42), 124 (66), 110 (30), 111 (100), 96 (41), 68 (68) |
| 5 | *N*-methyl-$\beta$-coniceine | 5.1 | *c* | 139 ($M^+$, 8), 124 (7), 110 (9), 98 (100), 83 (23), 82 (22), 70 (16) |
| 6 | alkaloid A | 5.5 | | 99 (4), 98 (100), 84 (63), 70 (37), 56 (27) |
| 7 | *N*-methylpseudoconhydrine | 5.9 | *b* | 157 ($M^+$, 3), 142 (2), 128 (2), 114 (100), 98 (13), 86 (8), 70 (10), 57 (37) |
| 8 | metabolite A | 6.8 | | 153 (37), 152 (13), 138 (25), 125 (16), 124 (25), 110 (12), 107 (13), 97 (18), 96 (31), 68 (100) |

[a] Identified by comparison of retention data and mass spectral data with reference material. [b] Identified by comparison to known spectra (M. F. Roberts, unpublished results). [c] Identified by interpretation of spectral data.

The molecular ion is consistent with a coniine derivative containing a methyl and a hydroxyl group with two sites of unsaturation.

$\gamma$-Coniceine was the primary alkaloid found in the plant, with high concentrations of *N*-methylconiine and coniine (Table 2). These were also the primary alkaloids in the postmortem samples, along with a large amount of 1′-oxo-$\gamma$-coniceine (peak 4) and metabolite A (peak 8). No alkaloids were detected in the serum samples, with the exception of 0.3 ppm of $\gamma$-coniceine detected in the serum of goat 3. The goats were euthanized at 3, 5, and 7 h after dosing. The *Conium* alkaloid concentrations decreased over this time period, while alkaloid concentrations in the urine increased over the same time period. Detection of >10 ppm of $\gamma$-coniceine in the rumen contents and 1 ppm in the other postmortem samples was sufficient to determine sublethal exposure to *C. maculatum* 3−7 h following dosing.

**Table 4. Summary of Alkaloids Found in *D. wrightii* with Mass Spectral and GC/MS Retention Time Data**

| peak | compound | RT (min) | note | characteristic ions (abundance %) |
|---|---|---|---|---|
| 1 | scopine | 7.4 | *e* | 155 ($M^+$, 50), 138 (5), 126 (7), 112 (36), 110 (42), 94 (63), 84 (94), 57 (100) |
| 2 | alkaloid A | 12.8 | | 241 (11), 140 (27), 113 (10), 95 (70), 94 (100), 69 (13), 57 (18) |
| 3 | 3-OH-6-tigloyloxytropane | 14.0 | *d* | 239 (19), 156 (5), 138 (5), 122 (7), 113 (100), 96 (42), 95 (30), 94 (54), 84 (14) |
| 4 | alkaloid B | 15.0 | | 311 (8), 296 (4), 212 (9), 129 (5), 110 (18), 95 (60), 94 (100) |
| 5 | meteloidine | 15.6 | *d* | 255 ($M^+$, 5), 195 (5), 138 (2), 126 (4), 96 (8), 95 (59), 94 (100), 55 (16) |
| 6 | aponoratropine | 15.9 | *e* | 257 ($M^+$, 14), 126 (2), 111 (9), 110 (100), 103 (19), 80 (26), 68 (25) |
| 7 | apoatropine | 16.0 | *b* | 271 ($M^+$, 35), 237 (2), 140 (10), 138 (28), 124 (100), 103 (22), 96 (46), 94 (83) |
| 8 | phenylacetoxyscopane | 16.1 | *e* | 273 ($M^+$, 36), 183 (3), 154 (44), 138 (72), 110 (46), 108 (75), 94 (100), 91 (76) |
| 9 | aposcopolamine | 16.6 | *b* | 285 ($M^+$, 49), 275 (2), 183 (3), 154 (36), 138 (51), 110 (80), 103 (64), 94 (100) |
| 10 | hyoscyamine | 16.9 | *a* | 289 ($M^+$, 13), 125 (9), 124 (100), 103 (7), 96 (10), 95 (9), 94 (22), 83 (24), 82 (27) |
| 11 | noratropine | 16.9 | *c* | 275 ($M^+$, 3), 111 (8), 110 (100), 80 (19), 68 (19) |
| 12 | scopolamine | 17.5 | *a* | 303 ($M^+$, 21), 154 (31), 138 (72), 108 (46), 103 (21), 97 (27), 94 (100), 81 (23) |
| 13 | metabolite A | 17.4 | | 331 (13), 154 (19), 138 (41), 136 (18), 108 (19), 94 (47), 58 (100) |
| 14 | metabolite B | 18.3 | | 331 (71), 194 (4), 154 (30), 138 (83), 136 (30), 108 (45), 94 (100) |

[a] Identified by comparison of retention data and mass spectral data with reference material. [b] Identified by comparison to known spectra (Kagei et al., 1980). [c] Identified by comparison to known spectra (Hartmann et al., 1986). [d] Identified by comparison to known spectra (Witte et al., 1987). [e] Identified by interpretation of spectral data.

**Table 5. Summary of Alkaloids Found in *N. glauca* with Mass Spectral and GC/MS Retention Time Data**

| peak | compound | RT (min) | note | characteristic ions (abundance %) |
|---|---|---|---|---|
| 1 | nicotine | 7.8 | *a* | 162 ($M^+$, 15), 161 (15), 133 (29), 119 (8), 84 (100) |
| 2 | alkaloid A | 8.5 | | 160 ($M^+$, 4), 159 (5), 148 (14), 147 (26), 120 (20), 119 (100), 118 (16), 106 (10), 105 (12), 93 (11), 92 (9), 92 (11), 80 (23), 70 (66), 63 (10), 51 (19) |
| 3 | anabasine | 9.5 | *a* | 162 ($M^+$, 28), 160 (124), 147 (3), 133 (47), 119 (40), 106 (48), 105 (61), 84 (100), 80 (28) |
| 4 | alkaloid B | 9.7 | | 160 ($M^+$, 85), 158 (72), 145 (19), 131 (20), 130 (19), 120 (52), 119 (11), 118 (44), 117 (90), 106 (56), 105 (80),82 (78), 79 (61), 77 (63), 55 (100) |

[a] Identified by comparison of retention data and mass spectral data with reference material.

*D. wrightii.* *Datura* is toxic to cattle with clinical signs of exposure including thirst, impaired vision, pupillary dilation, delirium, rapid and weak heartbeat, and convulsions (Keeler, 1983). One goat was dosed with *Datura* at 10 g/kg of body weight. The goat was mildly disoriented within 1 h of dosing but had no pupillary dilation.

Alkaloids found in the leaves of *D. wrightii* include hyoscyamine, scopolamine, meteloidine, norscopolamine, and apoatropine. Numerous other tropane alkaloids have been identified in *D. wrightii* and related species (Evans and Woolley, 1965; Lounasmaa and Tamminen, 1993; Witte et al., 1987; Hartmann et al., 1986).

Table 4 shows the alkaloids detected in the *D. wrightii* used in this study. Peak 1 had the molecular ion of scopine and the fragment ions of 94 Da and (M − OH) 138 Da, typical of the scopane structure. Peak 4 had a molecular ion of 311 Da, which has not been reported as the molecular weight for any tropane alkaloid from Solanaceous plants (Lounasmaa and Tamminen, 1993). Peak 6 had a spectrum consistent with aponoratropine and was similar to noratropine (Hartmann, 1986) with a molecular ion of 257 Da (noratropine minus $H_2O$). Peak 7 had all of the ions reported for apoatropine (Kagei et al., 1980), with an additional ion at $m/z$ 138, which was perhaps due to a small amount of a coeluting scopane alkaloid. Peak 8, phenylacetoxyscopane, showed a fragmentation pattern similar to that of 3α-phenylacetoxytropane (Hartmann, 1986) with the addition of 16 Da to the higher $m/z$ ions. The peaks at 16.9 min (10, 11) were the sum of hyoscyamine and noratropine. When the hyoscyamine spectrum was subtracted, the resulting spectrum was identical to those reported for noratropine (Hartmann, 1986).

Reported urinary metabolites of scopolamine include aposcopolamine, aponoratropine, *p*-hydroxyscopolamine, *m*-hydroxyscopolamine, *p*-hydroxy-*m*-methoxyscopolamine, and 6-hydroxyatropine (Wada et al., 1991). Postmortem urine contained aposcopolamine and two unidentified alkaloid metabolites (peaks 13 and 14), both with a molecular ion of $m/z$ 331, consistent with a phenolic metabolite of aposcopolamine, such as methoxyhydroxylaposcopolamine. Acetylatropine (molecular weight 331) coeluted with scopolamine, as did peak 13, but had a different mass spectrum. Acetylatropine had a spectrum of $m/z$ 311 ($M^+$, 18%), 140 (7%), 125 (10%), 124 (100%), and 94 (20%).

The primary alkaloids in the plant material and postmortem samples were scopolamine, noratropine, and hyoscyamine (Table 2). Detection of 1 ppm of scopolamine or hyoscyamine was sufficient to determine exposure at this dosing level in ingesta. Routine screening should include detection of aposcopolamine in urine samples. Scopolamine was only present at 0.7 ppm in urine and was not detected in the tissue or serum samples. A hydrolysis step, not included in this method, perhaps would have increased the amount of alkaloids detected in these samples.

*N. glauca.* Goats dosed twice daily with *N. glauca* at 6.25 g/kg have moderate to severe clinical signs. Clinical signs include muscular weakness, uncoordination, sternal recumbency, and cervical muscular weakness, causing the goats to rest their heads on the ground The most severe clinical signs occur 1−2 h following dosing (Panter et al., 1990). In the present study one goat was dosed at 15 g/kg with ground *N. glauca*. The goat was mildly depressed and weak but appeared to be recovering from the effects of the dose when euthanized at 5 h.

The primary alkaloid found in *N. glauca* is anabasine, accounting for 80−90% of the total alkaloid content (Bush and Crow, 1989). Other alkaloids found in *N. glauca* include nicotine, nornicotine, and anatabine. Anabasine concentration in *N. glauca* can be as high as 8800 ppm in dry plant material (Saitoh et al., 1985). Only anabasine (peak 3) was identified in the *N. glauca* used in this study (Table 5), which contained 430 ppm of anabasine on the basis of the wet weight of plant used (Table 2). Peak 2 had a molecular ion of $m/z$ 160, consistent with that of anatabine; however, the litera-

**Table 6. Summary of Alkaloids Found in *D. barbeyi* Mass Spectral and GC/MS Retention Time Data**

| peak | compound | RT (min) | note | characteristic ions (abundance %) |
|---|---|---|---|---|
| 1 | delpheline | 19.4 | *a* | 449 ($M^+$, 2), 434 (2), 419 (26), 418 (100), 390 (17), 388 (10), 360 (5) |
| 2 | deltamine | 19.6 | *a* | 465 ($M^+$, 2), 450 (1), 435 (33), 434 (100), 406 (7) |
| 3 | deltaline | 19.9 | *a* | 507 ($M^+$, 1), 492 (1), 477 (28), 276 (100), 448 (7) |
| 4 | 14-Ac-dictyocarpine | 20.2 | *a* | 535 ($M^+$, 1), 520 (1), 505 (29), 504 (100), 476 (10), 446 (5) |
| 5 | dictyocarpine | 20.6 | *a* | 493 ($M^+$, 2), 463 (29), 462 (100), 434 (17), 421 (12),420 (25), 392 (13), 122 (3) |
| 6 | lycoctonine | 20.7 | *a* | 467 ($M^+$, 4), 453 (7), 452 (26), 437 (26), 436 (100), 434 (16), 404 (3) |
| 7 | dehydrobrowniine | 21.4 | *b* | 465 ($M^+$, 4), 451 (9), 450 (33), 435 (27), 434 (100), 432 (11), 416 (5), 402 (4) |
| 8 | *Delphinium* alkaloid A | 21.6 | | 453 ($M^+$, 4), 439 (10), 438 (35), 424 (5), 423 (27), 422 (100), 404 (4), 390 (7) |

[a] Identified by comparison of retention data and mass spectral data with reference material. [b] Identified by comparison to known spectra (Yunusov et al., 1985).

ture indicates a longer retention time for anatabine than for anabasine on a nonpolar GC column (Saitoh et al., 1985). A small peak (4) with a molecular ion of $m/z$ 160 also appeared after anabasine. Peak 4 is possibly anatabine, as the spectrum is similar to that of anabasine with a molecular weight 2 Da less. Anabasine was detected in all of the postmortem samples tested, including 0.1 ppm in the serum. Detection of greater than 1 ppm anabasine in ingesta, tissue, or urine via GC/MS was sufficient to determine exposure in the goat at this sublethal dose.

*D. barbeyi*. One goat was dosed at 10 g/kg with dried D. barbeyi. Reported clinical signs include weakness, followed by generalized paralysis. Increased salivation and constipation may also occur (Olsen and Manners, 1989). The goat had mild depression and weakness and was killed 6 h after dosing.

Several norditerenoid alkaloids are found in *D. barbeyi*. Lycoctonine alkaloids include lycoctonine, browiine, 14-dehydrobrowniine, and anthranoyllycoctonine. Methylsuccinimidoanthroyllycoctonine (MSAL) alkaloids include methyllycaconitine (MLA). 7,8-Methylenedioxylycoctonine (MDL) alkaloids include deltaline, deltamine, 6-dehydrodeltamine, dictyocarpine, 14-acetyldictyocarpine, and delpheline (Manners et al., 1993).

The GC/MS analysis of the *Delphinium* samples revealed numerous late-eluting alkaloids (Table 6). The order of elution was consistent with the literature (Manners and Ralphs, 1989) on a nonpolar column. Peak 6 may be a mixture of lycoctonine and browniine, as both have similar spectra (Yunusov et al., 1985). The retention time of browniine was not determined on the GC column used. The large abundance of the $(M - 31)^+$ ion in peak 8 is consistent with an alkaloid with molecular weight 453 and a $C_1$ methoxy group, such as found in desmethyleneeldelidine (Yunusov et al., 1985).

The pattern of alkaloids in the postmortem samples shows that deltaline is the primary alkaloid in this sample of *D. barbeyi*. Deltaline is a good marker compound for exposure to this plant. Deltaline has an oral $LD_{50}$ of 720 mg/kg in mice, compared to 32 mg/kg of MLA (Manners et al., 1993). While deltaline is indicative of exposure to *Delphinium*, MLA is more toxic and therefore more significant. The large amount of lycoctonine in the plant extract is probably indicative of large amounts of MLA, which is readily hydrolyzed to lycoctonine at high pH (Majak, 1993). The presence of lycoctonine in samples using this method was also indicative of exposure. Both deltaline and lycoctonine were detected via TLC, and deltaline was also detected by GC/MS, in all postmortem samples except serum at $>1$ ppm.

*T. baccata*. *Taxus baccata* is toxic to ruminants, with clinical signs including cardiac failure and sudden death (Vohora, 1972; Kingsbury, 1964). The one goat dosed with *T. baccata* had no noticeable signs and was euthanized 6 h after dosing. Several taxine alkaloids, such as taxine A, have been identified in *T. baccata* (Graf et al., 1982). Taxines are not commercially available. The presence of numerous alkaloids in the plant material was determined via TLC analysis. There were four principal spots via TLC analysis following the $NaNO_2$ visualization dip. These had $R_f$ values of 0.90 (alkaloid A), 0.79 (B), 0.61 (C), and 0.47 (D). There were additional spots with less intense color at $R_f$ 0.85 and 0.29 after the first visualizing dip (Dragendorff's reagent), which faded following the $NaNO_2$ dip. This pattern of alkaloids was also detected in the rumen contents of the goat, with alkaloid C detected in the abomasal contents and urine. No peaks were observed via GC/MS, indicating that these compounds had insufficient volatility or thermal stability for GC analysis on a 0.2 mm i.d. capillary column. Two peaks were observed in the plant via GC/NPD using a 5 m DB-17 megabore column. These peaks were also observed in the rumen contents. Large baseline disturbances characteristic of thermal breakdown products were present in the plant and rumen samples. The pattern of alkaloids on the TLC plate from the *T. baccata* clearly matched that of the rumen contents and could be used to determine exposure to *T. baccata* at this sublethal dose.

**Method Performance.** The method performed well on the compounds tested, demonstrating the utility of the method as a general screen for alkaloids. The hydrolysis of MLA was surprising, as other esters, such as in the tropane alkaloids, were not hydrolyzed using this analytical method. This could possibly be remedied by minimizing the time that extracts are exposed to extreme pH values, or by the use of lower pH buffers.

Detection limits using the GC/NPD were 0.1 ppm for alkaloids for which analytical standards were available. While the 5 m megabore column yielded rapid GC analysis and allowed chromatography of thermally labile compounds such as *Taxus* alkaloids, several important alkaloids were not resolved. *N*-Methylconiine and $\gamma$-coniceine had identical retention times, as did hyoscyamine and noratropine. Detection limits using the GC/MS in the scan mode were 1 ppm for the compounds tested. The major alkaloids in each alkaloid-containing plant were identifiable from peak spectra. Only semiquantitative results were obtained from the GC/MS analyses, as only the primary alkaloids from each plant type were available and the GC/MS response is affected by matrix interactions (Holstege et al., 1995). The 12 m HP-1 column partially resolved *N*-methylconiine and $\gamma$-coniceine, but not hyoscyamine and noratropine, thus complicating quantitation. TLC analysis provided a rapid method for determining the presence of alkaloids in the samples. Alkaloids were detected via TLC in all samples indicated to be positive by GC/MS.

The GC/NPD provided a lower detection limit than the GC/MS in the scan mode, with a linear response for all of the alkaloids examined. However, all GC/NPD results had to be confirmed via GC/MS. The GC/NPD was useful in quantitating low levels of well-resolved alkaloids but had limited utility screening samples containing complex mixtures of alkaloids, as would be expected in samples from animals experiencing multiple plant exposures.

**Conclusion.** The alkaloid MRM method of Holstege et al. (1995) enabled quantitation of numerous alkaloid concentrations in plant and biological samples. The analysis of rumen contents demonstrated that a 1 ppm method detection limit would be sufficient to detect exposure to these alkaloid-containing plants in goats dosed once at sublethal amounts 3−7 h previously. This detection limit is readily obtained with the TLC and GC/MS screening methods used. Urine was also a good substance for testing, with levels of the primary alkaloids of these plants ranging from 0.7 to 120 ppm. Kidney and liver offered some utility for testing, while serum was a poor sample for analysis. The addition of a step to hydrolyze alkaloid conjugates would perhaps increase the alkaloid contents detected in these samples. Further work will examine incorporating a hydrolysis step and the construction of GC/MS and TLC libraries of plant extracts for rapid diagnosis of animal exposure to alkaloid-containing plants.

ACKNOWLEDGMENT

We thank Dr. K. E. Panter, Dr. G. D. Manners, Dr. R. J. Molyneux, and Dr. A. D. Jones for technical assistance, M. C. Booth for help in plant collection, and F. C. Brown for help in animal dosing.

LITERATURE CITED

Bush, L. P.; Crowe, M. W. *Nicotiana* alkaloids. In *Toxicants of Plant Origin, Vol. I, Alkaloids*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; pp 87−107.

Cheeke, P. R.; Shull, L. R. *Natural Toxicants in Feeds and Poisonous Plants*; AVI Publishing: Westport, CT, 1985; pp 1−8, 92−172.

Evans, W. C.; Woolley, J. G. The alkaloids of *Datura meteloides* D. C. *J. Chem. Soc.* **1965**, 4936−4939.

Galey, F. D.; Holstege, D. M.; Fisher, E. G. Toxicosis in dairy cattle exposed to poison hemlock (*Conium maculatum*) in hay: isolation of *Conium* alkaloids in plants, hay, and urine. *J. Vet. Diagn. Invest.* **1992**, *4*, 60−64.

Graf, E.; Kirfel, A.; Wolff, G. J.; Breitmeier, E. Die Aufklärung von taxin A aus *Taxus baccata* L. *Liebigs Ann. Chem.* **1982**, 376−381.

Hartmann, T.; White, L.; Oprach, F.; Toppel, G. Reinvestigation of the alkaloid composition of *Atropa belladonna* plants, root cultures, and cell suspension cultures. *Planta Med.* **1986**, *52*, 390−395.

Holstege, D. M.; Seiber, J. N.; Galey, F. D. A rapid multiresidue screen for alkaloids in plant material and biological samples. *J. Agric. Food Chem.* **1995**, *43*, 691−699.

Kagei, K.; Ikeda, M.; Sato, T.; Ogata, Y.; Kunii, T.; Toyoshima, S.; Matsumura, S. Studies on *Duboisia* species IV. Minor alkaloids in leaves of *Duboisia leichhardtii*. *Yakugaka Zasshi* **1980**, *100* (2), 216−220.

Keeler, R. F. Naturally occurring teratogens from plants. In *Handbook of Natural Toxins;* Keeler, R. F., Tu, A. Eds.; Dekker: New York, 1983; Vol. 1, 178 pp.

Keeler, R. F.; Balls, L. D. Teratogenic effects in cattle of *Conium maculatum* and conium alkaloids and analogs. *Clin. Toxicol.* **1978**, *12* (1), 49−64.

Kingsbury, J. M. *Poisonous Plants of the United States and Canada*; Prentice-Hall, Englewood Cliffs, NJ, 1964. pp 121−123, 131−140, 278−282, 284−287.

Lounasmaa, M.; Tamminen, T. The tropane alkaloids. In *The Alkaloids, Chemistry and Pharmacology*; Cordell, G. A., Ed.; Academic Press: New York, 1993; pp 1−114.

Majak, W. Alkaloid levels in a species of low larkspur and their stability in rumen fluid. *J. Range Manage.* **1993**, *46*, 100−104.

Manners, G. D.; Ralphs, M. H. Capillary gas chromatography of *Delphinium* diterpenoid alkaloids. *J. Chromatogr.* **1989**, *466*, 427−432.

Manners, G. D.; Panter, K. E.; Ralphs, M. H.; Pfister, J. A.; Olsen, J. D.; James, L. F. Toxicity and chemical phenology of norditerpenoid alkaloids in the tall larkspurs (*Delphinium* species). *J. Agric. Food Chem.* **1993**, *41*, 96−100.

Olsen, J. D.; Manners, G. D. Toxicology of diterpenoid alkaloids in rangeland larkspur (*Delphinium* spp.). In *Toxicants of Plant Origin, Vol. I, Alkaloids*, Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; pp 291−326.

Panter, K. E.; Keeler, R. F. Piperidine alkaloids of poison hemlock (*Conium maculatum*). In *Toxicants of Plant Origin, Vol. I, Alkaloids*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; pp 109−132.

Panter, K. E.; Bunch, T. D.; Keeler, R. F. Maternal and fetal toxicity of poison hemlock (*Conium maculatum*) in sheep. *Am. J. Vet. Res.* **1988**, *49* (2), 281−283.

Panter, K. E.; Bunch, T. D.; Keeler, R. F.; Sisson, D. V.; Callan, R. J. Multiple congentital contractures (MCC) and cleft palate induced in goats by ingestion of piperidine alkaloid-containing plants: reduction in fetal movement as the probable cause. *Clin. Toxicol.* **1990**, *28* (1), 69−83.

Panter, K. E.; Molyneux, R. J.; Smart, R. A.; Mitchell, L.; Hansen, S. English yew poisoning in 43 cattle. *J. Am. Vet. Med. Assoc.* **1993**, *202* (9), 1476−1477.

Plumlee, K. H.; Holstege, D. M.; Blanchard, P. C.; Fisher, K. M.; Galey, F. D. *Nicotiana glauca* toxicosis of cattle. *J. Vet. Diagn. Invest.* **1993**, *5*, 498−499.

Saitoh, F.; Noma, M.; Kawashima, N. The alkaloid content of sixty *Nicotiana* species. *Phytochemistry* **1985**, *24* (3), 477−480.

Vohora, S. B. Studies on *Taxus baccata*. II. Pharmacological investigation of the total extract of leaves. *Plant Med.* **1972**, *22* (1), 59−65.

Wada, S.; Yoshimitsu, T.; Koga, N.; Yamada, H.; Oguri, K.; Yoshimura, H. Metabolism *in vivo* of the tropane alkaloid, scopolamine, in several mammalian species. *Xenobiotica* **1991**, *21* (10), 1289−1300.

Witte, L.; Müller, K.; Afrmann, H. Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid-chromatography-mass spectrometry. *Planta Med.* **1987**, *53*, 192−197.

Yunusov, M. S.; Rashkes, Y. V.; Salimov, B. T.; Ametova, E. F.; Fridlyanskii, G. V. Features of the splitting of a methyl radical from lycoctonine alkaloids under electron impact. *Khim. Prir. Soedin.* **1985**, *4*, 525−536.

Received for review February 10, 1995. Accepted May 15, 1996.[⊗] This work was supported in part by Livestock Disease Research Laboratory funds, University of California, Davis.

JF950091X

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1996.