Determination of Acepromazine, Ketamine, Medetomidine, and Xylazine in Serum: Multi-Residue Screening by Liquid Chromatography–Mass Spectrometry

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Abstract

A large variety of drugs are administered to large and small animals by veterinary clinicians for sedation, anesthesia, muscle relaxation, and analgesia. The present paper reports a simple and rapid multi-residue detection and quantitation method for four chemically different drugs: medetomidine, xylazine, ketamine, and acepromazine. Chromatographic separation was carried out on a liquid chromatography–mass spectrometry instrument with a C18-reversed-phase column. Fragmentation patterns were determined with atmospheric pressure chemical ionization mass spectrometry set to operate in a positive selective ion monitoring mode. The method was determined to be linear over the range of concentrations tested (2.0–100.0 ng/mL). Accuracy, precision, and specificity were evaluated and the method was determined to be applicable to detection of medetomidine, xylazine, ketamine, and acepromazine in serum samples of multiple animal species (canine, equine, and bovine). Matrix limits of quantitation were determined to be 5.0 ng/mL for all four analytes, and recoveries ranged between 82.0 and 118%, with a 3.0–18.3% relative standard deviation.

Introduction

A variety of drugs are used in veterinary medicine to restrain animals for diagnostic, clinical, and surgical procedures and to assist in animal transport. Advantages of administering these medications to animals include decreased stress and anxiety, pain control, preanesthesia, and anesthesia.

Medetomidine (MED) and xylazine (XYL) are thiazine derivatives. Thiazine derivatives decrease norepinephrine release in the brain, resulting in sedation and analgesia. Thiazine derivatives also cause muscle relaxation via inhibition of CNS reflexes. XYL is one of the most commonly used sedatives and muscle relaxants in veterinary medicine. Both XYL and MED have the potential to cause severe cardiovascular and respiratory complications, primarily as a result of α1-adrenoreceptor agonist activity. MED is a more potent α2-adrenoreceptor and less potent α1-adrenoreceptor agonist than XYL, which results in greater potency as a sedative and fewer adverse effects. MED has been used primarily in dogs and cats as a sedative and analgesic (1), although its use has been investigated for other animal species as well (2,3). Neither drug is approved for use in humans, although cases of XYL intoxication in people have been reported (4–10).

Ketamine (KET), a congener of phencyclidine (PCP), is a cyclohexamine agent that is used for anesthesia during short surgical procedures and for sedation and pain control in variety of animal species including dogs, cats, birds, horses, exotic species, and humans. It is considered a dissociative anesthetic in that the animal appears to be awake but unaware of its surroundings. It is believed that KET and other dissociative anesthetics disrupt nervous system pathways in the cerebrum and stimulate the reticular activating system of the brain. KET is often administered in combination with other drug agents such as XYL, diazepam, and acepromazine (ACE). Although KET is considered to have a wide margin of safety, it can cause significant respiratory depression when given in excessive doses or too rapidly (11). Because of its pharmacologic similarity to PCP, KET is a human drug of abuse (12), and intoxication is relatively common (13,14).

ACE is a phenothiazine neuroleptic agent commonly used in veterinary medicine for its tranquilizing effect. Although its exact mechanism of pharmacologic action is uncertain, ACE
blocks post-synaptic dopamine receptors in the CNS, inhibits dopamine release, and increases the rate of dopamine turnover (11). ACE is approved for use in dogs, cats, and horses. It is also used in other species such as swine, cattle, rabbits, sheep, and goats. ACE has a wide margin of safety although cardiovascular collapse has been reported in many animal species (11). In human medicine a number of phenothiazines (although not ACE) are used for the management of various psychoneurologic disorders and the control of nausea and vomiting (15). ACE has been used to commit suicide and accidental exposure to the drug has caused human intoxications (16–18).

In the authors’ experience, the need for detection of sedatives, tranquilizers, or anesthetics most often falls into one of three categories. In dogs and cats, requests are made to screen samples in cases where the pet owner suspects that a drug was used without his/her permission. Secondly, in horses, requests for testing are made to ensure that horses are drug-free prior to purchase. Thirdly, drugs such as ACE are illegally used in livestock shows to calm otherwise nervous or intractable animals. Detection of such drugs is critical to ensure the integrity of the shows.

These scenarios involve the detection of the drugs used at therapeutic doses. Exposure to high, potentially toxic doses is also possible. Intoxications of humans have been reported for ACE, KET, and XYL (5, 7–9, 13, 14, 18). Animal intoxications are often suspected but not confirmed because of the unavailability of a simple, multi-residue screening method and a lack of information regarding fluid and tissue drug concentrations associated with intoxication. The availability of a simple screening method for these commonly used veterinary drugs would permit more thorough investigation of suspected animal intoxications.

Methods for the detection and quantitation of each of the drugs individually have been developed. Detection of XYL has been reported in canine urine (19) and equine, sheep, and cattle plasma and blood samples (20–22) by high-performance liquid chromatography (HPLC) and in cattle tissues and milk by gas chromatography–mass spectrometry (GC–MS) (23). MED screening has been established for canine serum by capillary GC–MS (24). Determination of KET in brain and plasma has been performed by GC–MS (25) and HPLC (26). Screening for ACE in blood and urine of racing horses has been established by ELISA and particle fluorescence immunoassay (27).

Determination of individual drugs and their metabolites (28, 29) has been dominated by the development of instrumental screening methodologies involving multi-residue detection of analytes of different chemical nature. A large number of publications deal with the presence of multiple tranquilizers in animal tissues including determination of ACE and XYL in kidney, liver, and muscle samples by HPLC (30, 31) with fluorescence detection (32), with electrochemical detection (33), and with tandem mass spectrometry (LC–MS–MS) (34–36). Reports are available for determination and quantitation of KET in plasma and urine by GC–MS when administered in combination with XYL (37, 38) and by HPLC (39). Reported method detection limits (MDL) range from of 5.0 and 20.0 ng/mL for both compounds in urine and plasma, respectively (38, 39). Screening animal and human plasma for a mix of recreational drugs including KET resulted in publications based on GC–MS (40) with headspace solid-phase microextraction (SPME) procedure for sample purification, HPLC (41, 42), as well as HPLC with MS and SPME (43). Availability of KET in commercial products as a racemic mixture and significantly different pharmacodynamic activities of R(--)- and S(+)–enantiomers of KET resulted in the development of a method for detection and separation of both isomers in plasma by LC–MS (44) with solid-phase extraction (SPE) sample clean-up procedures. Administration of MED with XYL or with KET (45, 46) has been previously reported, however limited publications are available for determination and quantitation of MED as a single drug or in combination with other tranquilizers and/or α2–adrenergic agonists. A radioreceptor assay has been developed for screening of MED and XYL in sheep plasma after alumina purification with MDL levels of 0.24–2.5 ng/mL (45). To the authors’ knowledge, only one report has been published showing multi-residue detection of MED and other anesthetics in canine plasma by LC–MS involving SPE sample purification and a buffer mobile phase system for drug elution (47).

The present report contains the first sensitive, specific, reproducible, and simple multi-residue method for determination and quantitation of XYL, MED, KET, and ACE present in serum. Methodology is based on LC–MS set to operate in a positive atmospheric pressure chemical ionization (APCI) and selective ion monitoring mode (SIM) of MS. Analysis is complete without any requirements for sample purification, derivatization, or buffer use in chromatographic separation.

Material and Methods

Materials

XYL and ACE standards and bovine serum were purchased from Sigma. KET solution was a gift from Dr. Ubah (PA Equine Toxicology and Research Laboratory, West Chester, PA). MED was purchased as Dormitor®. Acetonitrile (ACN) and trifluoroacetic acid (TFA) were obtained from Fisher Scientific (Pittsburgh, PA). All reagents were HPLC grade. Control equine and canine sera were obtained from animals undergoing standard diagnostic screening at the Toxicology Lab of New Bolton Center. Ultra Pure water system from Millipore (Bedford, MA) was used to generate water with resistivity of 18 MΩ·cm. LC autosampler vials (2 mL, wide opening) designed for robotic arm tray were purchased from Agilent Technologies (Wilmington, DE).

Preparation of standards

Individual stock standards of 1000 μg/mL for XYL and ACE were prepared by dissolving 10 mg of each compound in 10 mL of ACN. KET standard of 1000 μg/mL was provided to the lab in methanol. MED was purchased as a solution of 1000 μg/mL. Working standard mixes of all four analytes were made by serial dilution of 1000 μg/mL individual standards with 50% solution of ACN/water. All samples were stored at 4°C when not in use.
Sample extraction
Negative control sera not containing detectable amounts of the investigated analytes was analyzed prior to fortification with anesthetics. Aliquots (0.3 mL) of negative control sera were placed into micro-centrifuge tubes and spiked with anesthetic working standard mix solution at 100, 50, 25, and 5 ng/mL levels. The solutions were then combined with ACN to obtain a final volume of 0.6 mL. All samples were vortex mixed for 5 s, allowed to sit at room temperature for 2 min, and centrifuged for 8 min at 12,000 rpm. Clear supernatants were transferred to HPLC vials for analysis.

LC–MS conditions and analysis of standards
A series 1100 LC–MSD Hewlett Packard system (Wilmington, DE) was equipped with a dual LC pump, a diode-array detector, a degasser, a column thermostat, and an autosampler. A Betasil C18 reversed-phase column from Keystone Scientific (150 x 4.6 mm, 5-µm particle size, Bellefonte, PA) was equilibrated with the mobile phase (50% ACN and 50% of 0.1% TFA in water) at 0.6 mL/min prior to analysis. The LC profiles were developed at 30°C, with UV detection at 254 nm with a spectrum range of 220–310 nm and a range step of 2 nm. The following 7 min program was used: 50% ACN was pumped for 2.5 min, increased to 65% ACN in the next 1.0 min, held this setting for the next 2.0 min, then decreased the gradient back to 50% ACN for 0.5 min, and re-equilibrate the column for 1.0 min. Injected sample volume for each assay was 10 µL. MS conditions were the following: dry gas flow rate was at 4 mL/min and temperature at 250°C, nebulizer pressure was set to 45 psi nitrogen, corona at 4 mA, capillary voltage of 2500V, and vapor temperature was set at 300°C. Fragmentation information for the compounds of interest was acquired with positive mode of API after a 2 min solvent delay with a gain of 5 and fragmentation from 4 to 7 min for ACE. The individual extracted ions eluted at 2.8, 3.3, 3.4, and 4.5 min for KET, XYL, MED, and ACE, respectively. Ion m/z 327 was monitored from 4 to 7 min for ACE. The individual extracted ions eluted at 2.8, 3.3, 3.4, and 4.5 min for KET, XYL, MED, and ACE, respectively.

Linearity, precision, and accuracy
Linearity of instrument standard response was determined for all anesthetics over the whole range of standard concentrations tested (2.0–100 ng/mL). An individual calibration curve was constructed for each analyte based on peak areas at each mass-to-charge ratio value under the corresponding individual extracted current-ion profiles. Linear regression analysis on calibration curves resulted in an average correlation coefficients (R²) between 0.9987 and 0.9999 for all analytes based on replicate analysis (n = 4), indicating that detection was linear up to the highest standard analyzed (100 ng/mL).

Within-day assay precision and accuracy were determined by analyzing replicate standard mixes at 10.0 and 50.0 ng/mL concentrations (n = 5) and fortified serum samples at 50 ng/mL (n = 5). Precision was expressed as the coefficient of variation (% CV) and was based on peak-area response factor for individual analytes at the corresponding mass-to-charge ratio value ions. Accuracy was determined as [average analyte concentration calculated/known concentration] *100%. Precision ranged from 1.32 to 6.55% CV and accuracy varied from 84.4 to 111.0% of known concentrations (Table I). Between-day variability was based on a 3-day minimum design and evaluated for a 50 ng/mL fortified serum samples (n = 3). Precision and accuracy ranged from 5.0 to 9.6% CV and 89 to 110%, respectively (data not shown).

Specificity and extraction efficiency
Specificity was determined from examining MS ion current profiles in negative control serum samples from three different sources (canine, bovine, and equine). Negative control sera and corresponding fortified samples were analyzed to show that all four analytes could be unequivocally identified and quantitated in the presence of any components occurring in the matrix.

Relative extraction efficiency for each drug was expressed in terms of concentration of drug recovered versus initial concentration used to spike the negative control serum. Individual standard calibration curves and quantitation was based on the peak areas of the corresponding extracted ions obtained for every analyte present in mix in the matrix match environment as determined by a Hewlett-Packard standard integrator algorithm. Percent drug recovery with corresponding standard deviation (%SD) was determined for each canine, bovine, and equine serum samples fortified with KET, XYL, MED, and ACE fortified at four different concentrations analyzed in replicates of four (n = 4). The data is summarized in Table II.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ketamine</th>
<th>Xylazine</th>
<th>Medetomidine</th>
<th>Acepromazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ng/mL</td>
<td>Precision (%)</td>
<td>1.65</td>
<td>4.29</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>100.2</td>
<td>100.7</td>
<td>100.5</td>
</tr>
<tr>
<td>50 ng/mL</td>
<td>Precision (%)</td>
<td>1.32</td>
<td>3.29</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>100.6</td>
<td>101.0</td>
<td>99.1</td>
</tr>
<tr>
<td>50 ng/mL spike</td>
<td>Precision (%)</td>
<td>3.57</td>
<td>5.95</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>84.4</td>
<td>94.0</td>
<td>104.6</td>
</tr>
</tbody>
</table>

* All data were based on n = 5 replicate analysis.
* Concentration of working standard mixes of all anesthetics analyzed.
* Precision is expressed as the coefficient of variation (%CV) based on current ion profile peak areas.
* Accuracy is expressed in terms of % as described in Methods section.
* Data corresponds to a 50 ng/mL fortified equine serum sample.
Limits of detection (LOD) and quantitation (LOQ)

Instrument LOD (ILOD) was defined as the lowest concentration of each analyte present in a standard mix that could be detected and expressed as a concentration at signal-to-noise (S/N) ratio of 3:1 based on the individual extracted-ion chromatograms. ILOD was determined to be 2.0 ng/mL for all analytes of interest.

Sample LODs were defined as the lowest concentration of each analyte that can be detected in a fortified serum at S/N of 3:1. LODs fell at 2.5 ng/mL for KET and ACE, and at 5 ng/mL for XYL and MED. The lower sample LOQ was defined as the lowest measured concentration (based on ILOD) of each analyte that can be recovered from the fortified serum sample within (±) 20% of true value based on an individual ion of quantitation. LOQ for all analytes was determined to be 5 ng/mL.

Results

Chromatographic separation and mass spectral behavior

Initial studies were performed to establish the chromatographic profiles and mass spectral fragmentation behavior of KET, XYL, MED, and ACE under the present method's conditions with MS set to operate in scan mode. 100 ng/mL individual analyte standards showed no detectable UV signals with achieved chromatographic elution at 2.8, 3.2, 3.4, and 4.5 min for KET, XYL, MED, and ACE in the total MS profile, respectively. Fortification of these analytes was not complete (Rs > 1.25 is necessary for clear compound separation), but because of the observed differences in their mass spectral fragmentation patterns, the full separation was found to be nonessential for analyte detection and quantitation.

Limits of detection (LOD) and quantitation (LOQ)

The (tr)b and (tr)a correspond to the retention times of MED and XYL obtained from the MS profiles. The corresponding peak widths for the two analytes are expressed as the Ws and Wb values (48). The calculations showed that separation of these two anesthetics was not compete (Rs > 1.25 is necessary for clear compound separation), but because of the observed differences in their mass spectral fragmentation patterns, the full separation was found to be nonessential for analyte detection and quantitation.

Degree of fragmentation of each anesthetic was determined for 100 ng/mL individual standards in the full-scan mode of positive APCI-MS. Protonated molecular ions ([M+H]+) were observed for KET, XYL, MED, and ACE at m/z 238, 221, 201, and 327 (Figure 1) at maximum abundance similar to the previously published findings (47). As shown in Figure 1, additional fragmentation of analytes was minimal with the generation of an ion at m/z 191 for all drugs. Independent of the absence of additional fragmentation of each of the anesthetics, unambiguous detection of analytes present in a standard mix was achieved in SIM mode of MS because of their chromatographic separation and nonoverlapping of protonated molecular ions. Quantitation was based on extracted ion current profiles of [M+H]+ ions. Detection in SIM mode was possible at 2 ng/mL level (ILOD) for all analytes as described in Methods section.

Detection of anesthetics in fortified serum

Negative control bovine, canine, and equine serum samples not containing detectable amounts of the investigated analytes were analyzed prior to any drug fortifications. Results (data not shown) indicated that the proposed method can be used accurately and specifically for a number of different serum sample matrices. Total MS ion chromatograms obtained for all drug-free samples by positive APCI-MS in a full-scan mode (m/z 180–340) were clear of matrix interferences, where none of the analyte's characteristic ions were produced at the corresponding retention times (data not shown). Fortification of an individual serum matrix was performed at four different concentrations of a standard mix (5.0, 25.0, 50.0, and 100.0 ng/mL) and repeated for n = 4 replicates. Total ion chromatogram of a 25 ng/mL fortified bovine serum obtained in a SIM mode of MS is shown in Figure 2. The absence of matrix interferences, the analyte's peak behavior, and the elution patterns were consistent with that of a standard mixture and suggests the

Table II. Average Extraction Efficiencies* of Anesthetics in Serum

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Ketamine</th>
<th>Xylazine</th>
<th>Medetomidine</th>
<th>Acepromazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ng/mL</td>
<td>82.0 ± 3.4</td>
<td>100 ± 16.1</td>
<td>95.0 ± 16.2</td>
<td>118 ± 7.2</td>
</tr>
<tr>
<td>25 ng/mL</td>
<td>90.4 ± 12.8</td>
<td>88.0 ± 6.4</td>
<td>103 ± 13.6</td>
<td>109 ± 11.0</td>
</tr>
<tr>
<td>50 ng/mL</td>
<td>97.0 ± 5.2</td>
<td>88.5 ± 5.9</td>
<td>113 ± 3.4</td>
<td>113 ± 11.3</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>96.0 ± 7.1</td>
<td>88.0 ± 5.3</td>
<td>108 ± 8.3</td>
<td>109 ± 10.9</td>
</tr>
<tr>
<td>Canine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ng/mL</td>
<td>83.0 ± 18.3</td>
<td>99.5 ± 12.2</td>
<td>83.3 ± 27.9</td>
<td>103 ± 10.7</td>
</tr>
<tr>
<td>25 ng/mL</td>
<td>86.0 ± 8.1</td>
<td>97.0 ± 10.8</td>
<td>99.0 ± 13.3</td>
<td>108 ± 8.0</td>
</tr>
<tr>
<td>50 ng/mL</td>
<td>89.0 ± 5.4</td>
<td>99.0 ± 6.9</td>
<td>106 ± 4.2</td>
<td>105 ± 5.7</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>90.0 ± 5.0</td>
<td>91.0 ± 4.0</td>
<td>98.0 ± 3.5</td>
<td>99.0 ± 6.0</td>
</tr>
<tr>
<td>Equine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ng/mL</td>
<td>84.5 ± 3.0</td>
<td>103 ± 7.2</td>
<td>82.5 ± 12.1</td>
<td>115 ± 9.5</td>
</tr>
<tr>
<td>25 ng/mL</td>
<td>92.1 ± 12.9</td>
<td>101 ± 9.4</td>
<td>103 ± 1.2</td>
<td>103 ± 5.3</td>
</tr>
<tr>
<td>50 ng/mL</td>
<td>94.5 ± 9.2</td>
<td>111 ± 11.0</td>
<td>106 ± 3.2</td>
<td>109 ± 0.9</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>96.0 ± 10.7</td>
<td>107 ± 11.7</td>
<td>103 ± 3.0</td>
<td>109 ± 4.9</td>
</tr>
</tbody>
</table>

* Quantitation performed based on individual extracted ion current profiles at m/z 201, 221, 238, and 327 for KET, XYL, MED, and ACE, respectively.
achievement of sufficient sample purification for drug analysis. The same data was obtained for the other serum matrices (data not shown).

For unambiguous identification and quantitation of each compound present in a mixture, extracted ion current profiles for KET (m/z 238), XYL (m/z 221), MED (m/z 201), and ACE (m/z 327) were determined and compared for drug-free and fortified serum samples. Figure 3 corresponds to the extracted ion chromatograms of a 25 ng/mL canine fortified serum. All peaks were free of matrix interferences and detected at 3:1 S/N ratio. Poor chromatographic separation of XYL and MED observed in the total MS profile of a 25 ng/mL fortified sample (Figure 2) could be resolved based on the two non-shared individual extracted protonated molecular ions as described in the previous section. Thus, extracted ion profiles obtained for fortified sample as compared to the drug-free serum demonstrated the specificity and application of the multi-residue detection of four different anesthetics in animal serum.

**Extraction efficiencies, LOD, and LOQ**

Quantitation of all fortified samples was based on the analyte's extracted ion peak areas at protonated molecular ion mass-to-charge ratio values and the individual standard calibration curves. Analysis of three different serum matrices fortified at four different concentrations (n = 4) resulted in overall range of average (SD) recoveries of 82.0-97.0% for KET, 88.0-111% for XYL, 82.5-113% for MED, and 99.0-118% for ACE as summarized in Table II. All data was evaluated for %SD based on an average recoveries for n = 4 replicates. Results showed less than 15% SD for an equine fortified matrix over the whole range of concentrations tested for all analytes and less than 20% SD for bovine and canine fortified serum. One exception was seen for an MED-spiked canine serum with a 27.9% SD at 5.0 ng/mL (Table II).

Matrix LOD values were determined for all anesthetics expressed in terms of concentration and defined as those values at which quantitation mass-to-charge ratio ions could be observed in fortified drug-free serum (irrespective of the animal species) at a S/N ratio of 3:1. The values of LODs were determined to be 2.5 ng/mL for KET and ACE and 5.0 ng/mL for XYL and MED. LOQ values were defined as that concentration at which %drug recovery could be observed at (±) 20% of the target concentration. Thus, LOQ of 5.0 ng/mL was determined for all drugs under SIM monitoring mode of positive APCI.

**Discussion**

A simple and rapid multi-residue detection and quantitation method for four chemically different compounds used in veterinary medicine for animal restraint, preanesthesia, anesthesia, or pain control is reported. Serum deproteination is achieved in a one-step sample treatment with ACN, omitting the need for any extensive SPE purification techniques that could lead to the loss of one or more analytes. Analysis was completed on a standard C18 reversed-phase column with LC–MS set to operate in a positive mode of APCI. A full-scan MS spectrum was determined for each drug, resulting in the detection of all protonated molecular ions. Independent of HPLC separation, quantitation ions for KET, XYL, MED, and ACE showed no overlap, allowing for clear identification of individual components present in a mixture under SIM mode of MS. Application of this method was evaluated based on fortified serum experiments and submitted clinical cases investigated by the authors. Specificity of the procedure was based on examining negative and fortified serum samples from large and small animals (canine, bovine, and equine). Independent of the type of serum used, matrix LOQ was determined to be 5.0 ng/mL for all analytes and extraction efficiencies ranged between 82.0 and 118% for all compounds over a range of concentrations (5.0-100.0 ng/mL).

Therapeutic doses of MED, XYL, KET, and ACE and resulting plasma concentrations vary greatly. Unfortunately, exact concentrations are not provided for many pharmacokinetic studies. Intramuscular (IM) injections of MED at 30 μg/kg in sheep resulted in a mean peak plasma concentration of 4.98 ppb (49). In dogs given a bolus of MED at 40 μg/kg, peak sedative and analgesic effects were observed at a mean plasma concentration of 18.5 ng/mL (50). Maximal sedation was observed in cats given a constant IV infusion of 1 mg/kg/min of MED with a corresponding plasma concentration of approximately 66 ng/mL. Mean peak plasma concentrations of ACE maleate in non-exercised horses following a single 25-mg IM injection and five

![Figure 1](https://academic.oup.com/jat/article-abstract/29/6/544/825197)
daily 5-mg IM injections were 10.5 and 3.3 ng/mL, respectively. Values were slightly higher for exercised horses treated in an identical manner (51).

Psychedelic effects of KET were strongly correlated with plasma KET concentrations between 50 and 200 ng/mL (52). In humans given KET intravenously (IV) at an average dose of 41 μg/kg/min and supplemented with nitrous oxide, a stable mean plasma KET concentrations between 50 and 200 ng/mL (52). In swine, the lowest plasma KET concentrations associated with immobilization were approximately 2 μg/mL (55).

There is a need for a simple, multi-residue screening method for these commonly used veterinary drugs to detect their presence in animals ante and postmortem following surreptitious or illegal use at therapeutic doses. In addition, intoxications have been reported for ACE, KET, and XYL in a number of animal species including humans (5,7-9,13,14,18). Unfortunately, the detection and quantification of the drugs in suspected animal poisonings is rare because of the unavailability of testing.

Tissue and fluid data are available from several human poisonings associated with exposure to KET, XYL, and ACE. In a suicide involving ACE, a postmortem chest-cavity blood concentration was 0.6 μg/mL (18). Postmortem analysis of heart and subclavian blood from another suicide involving XYL detected concentrations of 2.3 μg/mL and 2.9 μg/mL, respectively (9). Following an attempted suicide from self-injection of XYL, a plasma concentration of 4.6 μg/mL was detected (8). Another successful suicide attempt following the injection of XYL resulted in a postmortem blood concentration of 0.20 μg/mL (5). Similar concentration data is not available for the investigation and confirmation of suspected animal intoxications.

The established method's LOQ values for the quantitation of KET, XYL, MED, and ACE (5.0 ng/mL) fell well below the established therapeutic drug concentrations thus allowing clear diagnosis between therapeutic and intoxication cases.

Diagnostic samples submitted for testing originated from horses, dogs and cats. All submitted samples were analyzed for the four drugs of interest as described. Two equine cases involved MED administration IV at 4 μg/kg doses and submitted samples were those collected prior to and 2 min postadministration. Data showed that prior to treatment, serum samples did not contain any detectable analyte of interest. No matrix interferences at the retention time of MED and mass-to-charge ratio quantitation ion were observed for the pretreatment samples. MED was detected at 2 min postadministration in both animals at 28.8 and 83.5 ng/mL (data not shown). Serum samples were also submitted from a horse where XYL was administered 24 h prior to the described MED treatment. The extracted ion chromatograms for all four analytes in the third equine case are shown in
Figure 3A. XYL (m/z 221) and MED (m/z 201) eluted at 3.3 and 3.4 min (Figure 3A, peaks 1 and 2), respectively. XYL was quantified at 23.6 ng/mL 24 h postadministration. MED was quantitated at 884 ng/mL after an additional 1:10 sample dilution performed in order to place the extracted ion peak area on the calibration curve.

The same methodology was applied to submitted canine (n = 3) and feline (n = 1) sera and blood samples (1 canine and 2 felines) from animals known to have been treated with ACE or KET. Except for ACE (canine serum) or KET (feline serum), no other anesthetics were detected in available sera samples (n = 4). The extracted ion profiles resulted in analyte quantitations between 12.1 and 22.5 ng/mL and 14.3 ng/mL for ACE and KET, respectively. The four blood samples were processed under identical conditions to the serum samples. Extracted ion chromatograms observed for all four anesthetic agents of interest obtained for the blood sample from the dog treated with ACE are shown in Figure 3B. Matrix effects were seen at the elution time of MED. Quantitation of ACE in blood serum and blood samples of different animal species for detection and quantitation of several drugs frequently used in veterinary medicine for sedation, tranquilization, analgesia, or anesthesia. The methodology is rapid and requires a minimum amount of sample (0.3 mL) which makes this a valuable screening procedure in the diagnostic laboratory.

The described procedure has been shown to be applicable to serum and blood samples of different animal species for detection and quantitation of several drugs frequently used in the veterinary medicine for sedation, tranquilization, analgesia, or anesthesia. The methodology is rapid and requires a minimum amount of sample (0.3 mL) which makes this a valuable screening procedure in the diagnostic laboratory.

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